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Design and Synthesis of Novel Tetrahydoquinolines/1,2,3 Triazole Compounds Derivatives and Their Anticholinergic Activity as Potential Anti-Alzheimer agents †

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Abstract

This research focuses on synthesizing novel tetrahydroquinoline-1,2,3-triazole hybrids as potential agents for neurodegenerative diseases, particularly Alzheimer's disease (AD). The series of structurally distinct hybrid compounds synthesized in this study are previously unreported in the literature. The synthetic strategy involved a diastereoselective imino Diels-Alder reaction (Povarov reaction) to construct the tetrahydroquinoline (THQ) core, where various catalysts, including phthalic acid, lewis acids, KSF (montmorillonite), and ceric ammonium nitrate (CAN), were screened. Phthalic acid was selected as the most efficient catalyst for this crucial step. Following this, efficient click chemistry was employed to introduce the triazole moiety, adhering to green chemistry principles throughout the process. The chemical structure of the synthetized compounds was assigned using analysis of Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS) and Infrared (IR) spectroscopy. Furthermore, in silico analyses performed with Swiss ADME and OSIRIS Property Explorer indicated that most compounds exhibited excellent drug-like characteristics and favorable pharmacokinetic profiles. The synthesized compounds were evaluated as inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using the modified Ellman's methodology. The inhibitory activity is presented as IC_{50} values for each enzyme and compared to galantamine as a reference standard. These findings offer promising directions for the development of new therapeutic agents for AD based on organic synthesis.

Keywords: THQ; triazole; Alzheimer disease; AChE; BChE

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1. Introduction

Alzheimer's disease (AD) is the leading cause of dementia, accounting for roughly 70% of cases and affecting tens of millions worldwide [1], and its multifactorial pathology produces progressive loss of memory and other cognitive functions [2]. Among pathogenic hypotheses, the cholinergic theory remains therapeutically tractable: degeneration of basal forebrain cholinergic neurons and decreased acetylcholine (ACh) levels in hippocampus and cortex contribute directly to deficits in attention, learning and memory [3]. In pharmacological view, the inhibition of acetylcholinesterase (AChE) and Butirylcholinesterase (BChE) increases the bioavailability of acetylcholine (ACh) and can partially restore cholinergic signaling in the central nervous system (CNV) [4,5]. The actual authorized drugs from the FDA are not completely useful, because that not very selective and have secondary adverse effects. Here we report the continuation of our last advance [6], now involves design, synthesis, in-vitro evaluation of potential inhibitors and their ADME/Tox profiles predicted in-silico.

2. Results and Discussion

2.1. Synthesis

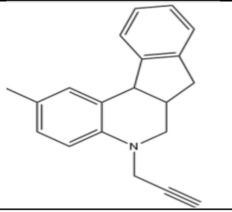
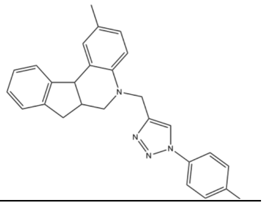
2.1.1. Povarov Reaction to THQ

The Povarov reaction was not explored with this combination of reactives, and the results show a great advance in our last report [6] and other alternative methods to obtain THQ core [7,8]. The test of 4 different catalysts probes the importance of molecular conversion and product distribution. Phthalic acid demonstrates to be the most effective catalyst, reaching yields of 85% (Table 2). On the other hand, the Lewis acid ($InCl_3$) produced lower yields and slower conversions; notably, KSF and CAN, though effective in some Povarov variants proved less suitable for our substrate set and did not improve yields, which suggests that their modes of activation are suboptimal for the electron-density patterns present in our anilines and dienophiles. These results highlight the importance of matching an optimal catalyst accord to the specific electronic and steric demands of the chemical systems and their reaction patterns. In this case Phthalic acid rises as the better choice.

2.1.2. Click Chemistry to Triazole Hybrids

The copper-catalyzed azide-alkine cycloaddition (Click Chemistry) was adapted to conjugate aryl azides onto the THQ core to produce the new 3 THQ-triazole hybrids, that synthetized compounds were not reported in the literature. This reaction results very efficiently in the isolated yields (up to 99%) and in the reaction times (around 1 h). The click chemistry was able to merge 2 pharmacophore chemical skeletons, expanding molecular diversity. It's important to note the stereoselectivity of click chemistry [9], was a relevant tool to make just 1 molecule and facilitate the purification and the posterior evaluation. Table 1 show the results of the synthesis procedure including yield, molecular weight and IUPAC name, since the THQ (A) and the 3 hybrids compounds (B, C, D).

Table 1. Compound abbreviation, nomenclature, reaction yield and structure of synthesized tetrahydroquinoline and tetrahydroquinoline/1,2,3 triazole hybrids compounds.

Compound Abbreviation	Nomenclature	Molecular Weight (g/mol)	Reaction Yield (%)	Chemical Structure
A	1-methyl-2-(2,3-dihydroindene)-1,2,3,4-tetrahydroquinoline	273.15	85%	
B	2-methyl-5-((1-(p-tolyl)-1*H*-1,2,3-triazol-4-yl)methyl)-6,6a,7,11b-tetrahydro-5*H*-indeno[2,1-c]quinoline	406.22	80%	

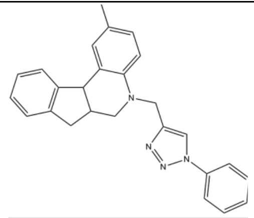
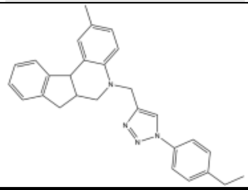
C	2-methyl-5-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-6,6a,7,11b-tetrahydro-5*H*-indeno[2,1-c]quinoline	392.20	99%	
D	5-((1-(4-ethylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-methyl-6,6a,7,11b-tetrahydro-5*H*-indeno[2,1-c]quinoline	420.56	72%	

Table 2. Summary of yields of every tested catalyst.

Catalyst	Yield (%)
Phthalic acid	85%
Cesium and nitrate ammonium (CAN)	69%
Montmorillonite (KSF)	44%
Indium III chloride ($InCl_3$)	45%

2.2. In Silico Predicction

In the first step the ADME properties were evaluated for the four compounds, the pharmacological target (AChE and BChE) are in the SNC, with this focus, we emphasized properties that enable central exposure [10]: Predicted blood-brain barrier (BBB) permeability. For this case every compound were able to cross that barrier according to the prediction, and it's relevant to note, the compound A was evaluated before, but this result showed the THQ-triazole core was able to cross too [11,12]. On the other hand, the efflux flow show, was evaluated according to the P-glycoprotein (P-gp) substrate criteria [13], the result shows for all compound a positive for P-gp substrate, demonstrating that the fusion of THQ with triazole group doesn't change this property of the Indene-THQ (compound A) previously reported. From a translational perspective, the P-gp liability implies that measured in vitro potency may overestimate in vivo CNS efficacy unless efflux is mitigated by structural modification, higher free fraction, or co-administration strategies; it also highlights the need to include P-gp assays and brain-penetration studies early in the screening cascade.

The evaluation of toxicity risk outcomes across mutagenicity, tumorigenicity, reproductive toxicity and irritancy (Table 3), strengthening the case for advancing several series members to enzymatic and cellular assays [14]. This result indicates an outlier in the mutagenicity of compound D, this value means a moderate risk of mutagenicity in this case and is due to the ethyl bond to benzyl group near the triazole. That information is key to avoiding toxicity for similar structures in the formation of hybrids and warrants immediate experimental follow-up (e.g., Ames test, micronucleus assay) before further resource investment.

Table 3. Summary of pharmacological and toxicity profiles of every compound. (🟢) nontoxic; (🔴) high toxicity.

Compound[1]	PGP Substrate [1]	BBB Penetration [2]	PAINS [3]	MUT [4]	TUM [5]	IRRI [6]	REP [7]
A	Yes	Yes	0	🟢	🟢	🟢	🟢
B	Yes	Yes	0	🟢	🟢	🟢	🟢
C	Yes	Yes	0	🟢	🟢	🟢	🟢
D	Yes	Yes	0	🟠	🟢	🟢	🟢

[1] P-Glycoprotein (P-gp) substrate; [2] BBB: Blood barrier penetration; [3] PAINS: Interference compounds in assays; [4] MUT: Mutagenic; [5] TUM: Tumorigenic; [6] IRRI: irritant; [7] REP: Reproductive effects.

2.3. Biological Activity

The activity profile reveals a clear and tunable effect of the triazole substituent on both potency and enzyme selectivity. The parent THQ (compound A) (IC_{50}AChE = 5.5 μM; IC_{50} BChE = 316 μM) is very AChE-preferring (selectivity index AChE vs BChE ≈ 57), while the three THQ–triazole hybrids (compounds B, C and D) shift potency by more than an order of magnitude and split into distinct selectivity patterns (Table 4). For AChE the hybrids measured: B = 1.03 μM, C = 0.18 μM and D = 4.02 μM. For BChE they measured: B = 0.26 μM, C = 27.81 μM and D > 500 μM. Calculated selectivity (IC_{50}BChE/IC_{50}AChE) therefore shows that the B is BChE-biased (SI ≈ 0.25), D is strongly AChE-selective (SI ≈ 155), and C is strongly AChE-selective (SI > 124).

These results carry two important implications. First, incorporation of the triazole does not simply increase potency uniformly but reprograms target preference depending on the aryl substituent (Table 4), indicating that small electronic or steric changes at the triazole handle substantially alter interactions within the catalytic gorge of each cholinesterase, that result is expected because the wide spectrum of benefits for triazole in drug-related molecules [15]. Second, the D compound (IC_{50} AChE = 0.18 μM; SI = 155) emerges as a highly selective AChE inhibitor that matches the programmatic aim of central AChE augmentation with minimal BChE engagement, a profile that can reduce off-target peripheral cholinergic effects and simplify interpretation of in vivo pharmacology [16,17]. Conversely, C compound displays sub micromolar BChE potency (IC_{50} = 0.26 μM) is notable: selective BChE inhibition has therapeutic relevance in later AD stages where BChE activity increases and may complement or offer an alternative symptomatic strategy. [18] The mixed profile of the compound B (high AChE selectivity) suggests the same mode of action of compound C, with less potency and selectivity. But bring valuable data for understanding the interactions between the series compounds and the biological targets.

Mechanistically, the divergent selectivity suggests that the substituents modulate key interactions for example, by altering fit in the peripheral anionic site, hydrogen-bonding networks, or orientation toward catalytic residues rather than merely changing overall lipophilicity. These differential outcomes validate the hybridization approach to tune both potency and selectivity and identify distinct leads for either AChE-focused or BChE-focused therapeutic strategies.

Table 4. Enzymatic inhibition of the synthesized compounds.

Compound	IC_{50} AChE (μM)	IC_{50} BChE (μM)	Selectivity (AChE/BChE)
A	5.58	316.88	56.8
B	1.03	0.26	0.25
C	4.02	> 500	> 124
D	0.18	27.81	154.5
Galantamine	0.10	8.00	80.0

3. Conclusions and Perspectives

Phthalic acid proved to be an efficient and practical promoter of the Povarov reaction, affording the THQ core in yields up to 85%, while the subsequent click chemistry step delivered THQ–triazole hybrids in excellent yields (up to 99%).

Biological evaluation demonstrated that triazole hybridization markedly enhanced AChE inhibitory potency compared with the parent THQ, with substituents dictating the selectivity profile. Among the series, compound D emerged as the most potent and selective AChE inhibitor (IC_{50} = 0.18 μM), approaching galantamine potency and showing no major predicted toxicity alerts, while the remaining derivatives also displayed favorable safety predictions. In silico ADME analysis supported blood–brain barrier permeability across the series but consistently flagged all compounds as P-gp substrates, highlighting a potential limitation for in vivo brain exposure.

Taken together, these findings identify THQ–triazole hybrids as a promising scaffold for cholinesterase inhibition in AD. Future work should prioritize compound D and selected analogues for confirmatory enzymatic and genotoxicity assays, while leveraging focused SAR around the aryl-triazole substituent to retain potency, mitigate mutagenicity risks, and reduce P-gp recognition. Docking and molecular modeling studies will be essential to rationalize substituent effects and guide optimization toward candidates with improved CNS pharmacological profiles.

4. Materials and Methods

4.1. General Information

All reagents were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and used without further purification. The obtained products were characterized by spectroscopic and spectrometric methods (IR, MS, ^1H-NMR, ^{13}C-NMR). The progress of the reactions was monitored by thin layer chromatography on aluminum TLC plates. Column chromatography was performed using silica gel (60–120 mesh) and the solvents used were of analytical grade.

4.2. Synthesis

4.2.1. Synthesis of N-Propargyl Toluidine

In a round bottom flask, 3 g of toluidine (28 mmol), 1.5 and 0.3 equivalents of K_2CO_3 and KI respectively were added in 10 mL of DMF and left in agitation for 15 min in ice bath (0 °C), additionally, a solution of 0.8 equivalents of propargyl bromide in 5 mL of DMF was prepared. After 15 min the solution was added drop by drop in the flask, finished the dripping, the reaction was maintained at room temperature for 2 h. Monitoring by thin layer chromatography. The solution was extracted with 20 mL of Brine solution and 20 mL of ethyl acetate (20 mL × 3). The organic phase was separated and filtered in 5% approximately of Sodium Sulfate. Finally, the product of interest was purified by liquid chromatography on silica gel, eluting with a mixture of ethyl acetate and petroleum ether.

4.2.2. Synthesis of Tetrahydroquinolines

In a round bottom flask, the resulting propargyl-Toluidine was added in 10 mL of Acetonitrile, formaldehyde (70%) (5–6 mL) was added in excess, kept in agitation and between 30 and 35 °C for 15 min. After this time the Indene was added dropwise, slightly in excess. It kept shaking for 24 h. The solution was extracted with 20 mL of Brine Solution and 20 mL of ethyl acetate (20 mL × 3). It was filtered in 5% approximately of Sodium Sulfate. Finally, the product of interest was purified by liquid chromatography on silica gel, eluting with a mixture of ethyl acetate and petroleum ether.

4.2.3. Synthesis of Triazole Hybrids

In a round bottom flask, 100 mg of the THQ and 1 equivalent of each aryl azide was added in 11 mL of a mixture 5:3:3 of THF: MeOH: H_2O solvent. After 15 min 40% of sodium ascorbate was added, after another 15 min 8% of $CuSO_4 * 5H_2O$ was added as catalyst and the reaction was maintained at room temperature for 1–4 h. The solution was extracted with 20 mL of Brine solution and 20 mL of ethyl acetate (20 mL × 3). The organic phase was separated and filtered in 5% approximately of Sodium Sulfate. Finally, the product of interest was purified by liquid chromatography on silica gel, eluting with a mixture of ethyl acetate and petroleum ether.

4.3. Biological Activity

The Ellman method was used to determine the inhibition of cholinesterases; this photometric method has been widely used to determine AChE and BChE activity through changes in color intensity produced by fast and sensitive coupling reactions [19].

To evaluate the inhibitory activity of compounds A-D, the samples were prepared using 96-well plates (50 µL) with a phosphate buffer (K_2HPO_4 8 mM, NaH_2PO_4 2.3 mM, NaCl 150 mM and 0.5% Tween 20, pH 7.6). AChE or BChE solution (50 µL, 0.30 units/mL) from *Electrophorus electricus* and equine serum respectively, was added to the same buffer. Then, the solutions to be tested were pre-incubated with the enzymes at room temperature for 30 min. Subsequently, the substrate solution (Na_2HPO_4 (40 mM), acetylthiocholine (ATC)/butyrylthiocholine (BTC) (0.24 mM) and 5,5-dithio-bis(2-nitrobenzoic acid)(0.2 mM, DTNB, Ellman's reagent)), was added, incubated for 5 min and measured absorbance at 405 nm. Enzyme activity was calculated as a percentage of inhibition and compared with the control. Compounds were tested in a dilution range of 500 to 0.002 µg/mL in triplicate. IC_{50} values were determined by regression analysis. Galantamine was used as the reference compound.

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References

1. Long, S.; Weidner, W.; Benoist, C. Informe Mundial sobre el Alzheimer 2023. 2023.
2. Scheltens, P.; De Strooper, B.; Kivipelto, M.; Holstege, H.; Chételat, G.; Teunissen, C.E.; Cummings, J.; van der Flier, W.M. Alzheimer's disease. *Lancet* **2021**, *397*, 1577–1590. https://doi.org/10.1016/S0140-6736(20)32205-4.
3. Delozier, S.J.; Davalos, D. A Systematic Review of Metacognitive Differences between Alzheimer's Disease and Frontotemporal Dementia. *Am. J. Alzheimer's Dis. Other Dement.* **2016**, *31*, 381–388. https://doi.org/10.1177/1533317515618899.
4. Bekdash, R.A. The cholinergic system, the adrenergic system and the neuropathology of alzheimer's disease. *Int. J. Mol. Sci.* **2021**, *22*, 1273. https://doi.org/10.3390/ijms22031273.
5. Shome, A.; Jha, K.T.C.; Chawla, V.; Chawla, P.A. An Insight into Medicinal Chemistry and SAR Studies of Cholinesterase and BACE-1 Inhibitors for Alzheimer's Disease. *CNS Neurol. Disord.—Drug Targets* **2024**, *24*. https://doi.org/10.2174/0118715273315191241002115155.
6. Dinamarca, C.; Pinochet, M.; Gutierrez, M. Synthesis and In-Silico Analysis of Novel Tetrahydroquinolines and Their Antioxidant Activity. *ECSOC* **2024**, *2024*, 99. https://doi.org/10.3390/ecsoc-28-20135.
7. Kant, K.; Naik, P.; Patel, C.K.; Some, S.; Banerjee, S.; Aljaar, N.; Atta, A.K.; Malakar, C.C. Organocatalytic Approaches Towards the Synthesis of Asymmetric Tetrahydroquinoline (THQ) Derivatives. *Synthesis* **2025**, *57*, 296–330. https://doi.org/10.1055/a-2376-3524.
8. Muthukrishnan, I.; Sridharan, V.; Menéndez, J.C. Progress in the Chemistry of Tetrahydroquinolines. *Chem. Rev.* **2019**, *119*, 5057–5191. https://doi.org/10.1021/acs.chemrev.8b00567.
9. Kumar, V.; Lal, K.; Naveen; Tittal, R.K. The fate of heterogeneous catalysis & click chemistry for 1,2,3-triazoles: Nobel prize in chemistry 2022. *Catal. Commun.* **2023**, *176*, 106629. https://doi.org/10.1016/j.catcom.2023.106629.
10. Sun, Y.; Zabihi, M.; Li, Q.; Li, X.; Kim, B.J.; Ubogu, E.E.; Raja, S.N.; Wesselmann, U.; Zhao, C. Drug Permeability: From the Blood–Brain Barrier to the Peripheral Nerve Barriers. *Adv. Ther.* **2023**, *6*, 2200150. https://doi.org/10.1002/adtp.202200150.
11. Daina, A.; Michielin, O.; Zoete, V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.* **2017**, *7*, 42717. https://doi.org/10.1038/srep42717.
12. Komura, H.; Watanabe, R.; Mizuguchi, K. The Trends and Future Prospective of In Silico Models from the Viewpoint of ADME Evaluation in Drug Discovery. *Pharmaceutics* **2023**, *15*, 2619. https://doi.org/10.3390/pharmaceutics15112619.
13. Elmeliegy, M.; Vourvahis, M.; Guo, C.; Wang, D.D. Effect of P-glycoprotein (P-gp) Inducers on Exposure of P-gp Substrates: Review of Clinical Drug–Drug Interaction Studies. *Clin. Pharmacokinet.* **2020**, *59*, 699–714. https://doi.org/10.1007/s40262-020-00867-1.
14. Ajay Kumar, T.V.; Kabilan, S.; Parthasarathy, V. Screening and Toxicity Risk Assessment of Selected Compounds to Target Cancer using QSAR and Pharmacophore Modelling. *Int. J. PharmTech Res.* **2017**, *10*, 219–224. https://doi.org/10.20902/IJPTR.2017.10428.
15. Rohila, Y.; Sebastian, S.; Ansari, A.; Kumar, D.; Mishra, D.K.; Gupta, M.K. A Comprehensive Review of the Diverse Spectrum Activity of 1,2,3-Triazole-linked Isatin Hybrids. *Chem. Biodivers.* **2024**, *21*, e202301612. https://doi.org/10.1002/cbdv.202301612.
16. Gambardella, M.D.; Wang, Y.; Pang, J. The Cholinergic Selectivity of FDA-Approved and Metabolite Compounds Examined with Molecular-Docking-Based Virtual Screening. *Molecules* **2024**, *29*, 2333. https://doi.org/10.3390/molecules29102333.
17. Liston, D.R.; Nielsen, J.A.; Villalobos, A.; Chapin, D.; Jones, S.B.; Hubbard, S.T.; Shalaby, I.A.; Ramirez, A.; Nason, D.; White, W.F. Pharmacology of selective acetylcholinesterase inhibitors: Implications for use in Alzheimer's disease. *Eur. J. Pharmacol.* **2004**, *486*, 9–17. https://doi.org/10.1016/j.ejphar.2003.11.080.
18. Fernández-Bolaños, J.G.; López, Ó. Butyrylcholinesterase inhibitors as potential anti-Alzheimer's agents: An updated patent review (2018–present). *Expert Opin. Ther. Pat.* **2022**, *32*, 913–932. https://doi.org/10.1080/13543776.2022.2083956.
19. Ellman, G.L.; Courtney, K.D.; Andres, V.; Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95. https://doi.org/10.1016/0006-2952(61)90145-9.