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Synthesis and In Silico Analysis of Novel Tetrahydroquinolines and Their Antioxidant Activity †

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Abstract: Within the area of study of neurodegenerative diseases, particularly Alzheimer's disease (AD), this research focused on the synthesis and evaluation of novel tetrahydroquinoline (THQ) derivatives with potential antioxidant activity. The toluidine N-propargylation synthesis protocol was optimized, achieving a significant increase in yield by using sodium carbonate and reaction temperature variation. Subsequently, four THQs compounds with alkene variation were successfully synthesized, including some not previously reported in the literature. The synthesized compounds were characterized by nuclear magnetic resonance (NMR), mass spectrometry, and infrared spectroscopy (IR), which confirmed their structures and purity. In silico analyses performed with SwissADME and OSIRIS Property Explorer indicated that most of the compounds exhibited excellent drug-like characteristics and favorable pharmacokinetic profiles. Antioxidant evaluation was performed using DPPH and ABTS assays where all compounds demonstrate excellent antioxidant capacity, with EC50 values below 10 μg/mL in the ABTS assay, significantly outperforming the ascorbic acid control (EC50 = 35 μg/mL). The results suggest that the predominant radical scavenging mechanism is single electron transfer (SET). This study provides a solid foundation for further investigations into the potential of THQs derivatives as antioxidants and potential cholinesterase inhibitors in the context of neurodegenerative diseases such as Alzheimer's. As a future projection, an enzymatic evaluation, including mechanism of action and the exploration of a hybrid synthesis of THQ/triazole is proposed based on these promising results.

Keywords: THQ; synthesis; povarov reaction; antioxidant

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1. Introduction

Tetrahydroquinolines (THQ) are nitrogen-containing heterocyclic organic compounds that have a wide range of biological activities, including antioxidant and cholinesterase inhibitory effects [1]. Furthermore, research suggests that these compounds may play a role in cancer treatment [2,3] and exhibit antibacterial activity [4].

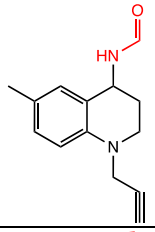
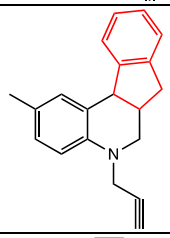
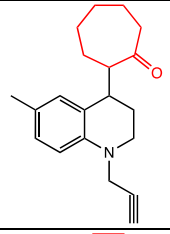
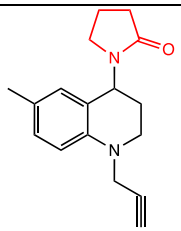
One practical way to obtain THQs is by organic synthesis. Different methods have been described for obtaining these compounds [5], with the Povarov or hetero Dields-Alder reaction being one of the most widely [6]. This reaction has evolved, generating different options based on the reagents and catalyst used [7]. In general terms, the Povarov reaction involves the condensation of three components: an aniline, an aldehyde and an electron-rich alkene. The reaction requires a Lewis acid as a catalyst and result in a THQ [8].

Because THQs are compounds with bioactive properties, there is a growing interest in further improving such capabilities. Organic synthesis allows the design and obtaining of specific organic compounds, in this case THQs. Consequently, a rational design of these

compounds can be carried out seeking to improve their properties, whether antioxidant, inhibitory, antibacterial, among others.

As a objective of this study, it proposed to synthetize novel THQs with the variation of the alkene in the reaction, trying to synthetize novel THQ with alkenes like indene and vinyl caprolactam, who doesn't are reported in the literature. The compounds are shown in Table 1.

Table 1. Compound abbreviation, nomenclature, reaction yield and structure of synthesized tetrahydroquinolines.

Compound Abbreviation	Nomenclature	Molecular Weight (g/mol)	Reaction Yield %	Chemical Structure
A	1-methyl-2-(N-formylamino)-1,2,3,4-tetrahydroquinoline	218.29	47.84	
B	1-methyl-2-(2,3-dihydroindene)-1,2,3,4-tetrahydroquinoline	259.34	43.75	
C	1-methyl-2-(N-caprolactamyl)-1,2,3,4-tetrahydroquinoline	254.33	44.52	
D	1-methyl-2-(N-pyrrolidonyl)-1,2,3,4-tetrahydroquinoline	282.38	41.38	

2. Methods

2.1. 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 spectral data (IR, MS, ^{1}H-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.

2.2. Synthesis

2.2.1. Synthesis of N-Propargyl Toluidine

In a round bottom flask, 3 g of toluidine (28 mmol), 5.9756 g of Potassium Carbonate (K_2CO_3) (43 mmol), 1.1719 g of potassium iodide (KI) (7 mmol), 10 mL of dimethylformamide were added and left in agitation for 15 min in ice bath (0 °C), additionally, a solution

of 2.4 mL of propargyl bromide (80 wt.%) in 5 mL of Dimethylformamide was prepared (21.5 mmol). After 15 min the just mentioned solution was added drop by drop in the flask maintaining the agitation and ice bath, finished the dripping, the ice bath was removed, and the reaction was maintained at room temperature for 2 h. Monitoring in thin layer chromatography. The solution was extracted with 20 mL of Brinz 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.

2.2.2. Synthesis of Tetrahydroquinolines

In a round bottom flask, the resulting propargyl-Toluidine (approx. 1 g) 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 corresponding alkene (vinyl formamide, Vinyl-pyrrolidine, Indeno, Vinyl-caprolactam) was added dropwise, slightly in excess, (1,1 molar equivalents of propargyl-toluidine obtained). It was kept between 30 and 35 °C and shaking for 24 h. The solution was extracted with 20 mL of solution Brinz 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.

2.3. Biological Activity

2.3.1. Measurement of DPPH Radical Scavenging Activity

The synthesized compounds were evaluated for their antioxidant activity by scavenging DPPH+ free radicals, following the procedure described previously [9]. Ascorbic acid was used as a reference standard with an SC_{50} value of 1.5 mg/mL.

2.3.2. Measurement of ABTS Radical Scavenging Activity

The synthesized compounds were evaluated for their antioxidant activity against ABTS+ radicals, following the previously reported procedure [10]. Ascorbic acid was used as a reference standard with an SC_{50} value of 35 μg/mL.

2.3.3. In Silico Prediction of Pharmacokinetic Properties

To study the potential toxicological risks, tools such as SwissADME and OSIRIS were used [11,12]. SwissADME was used to perform in silico analysis of the pharmacokinetic and pharmacodynamic properties, assessing the absorption, distribution, metabolism and excretion of the THQs. OSIRIS, on the other hand, allowed the prediction of potential adverse effects by analyzing the chemical structure of the compounds and identifying fragments associated with toxicity.

3. Results and Discussion

3.1. Synthesis

In the case of the synthesis of THQ by the Povarov reaction, the results obtained were consistent with what was expected, showing uniform yields in all compounds, as observed in Table 1. On the other hand, the results of the optimization of the synthesis of N-propargyl-toluidine indicated that, when allowed to react for 2 h at room temperature, crystals are obtained, in contrast to the previous method described previously [13], in which the reaction was carried out on ice and oils were obtained. Regarding the yields, the substitution of potassium carbonate for sodium carbonate was evaluated, which resulted in a yield of 68,23%, maintaining the reaction at room temperature, which represented a significant increase in yield.

All the new synthesized THQs were structurally characterized using NMR spectroscopic techniques, mass spectrometry and IR spectroscopy. In the IR spectra, typical

vibration bands of the propargyl fragment (3209–3302 cm^{-1}) were observed. All ^{1}H and ^{13}C NMR spectra of the synthesized THQs were very similar and were characterized by the presence of three groups of signals: aromatic, aliphatic and close to the heteroatom. The mass spectra correspond to the expected masses for the proposed structures, which constitutes evidence that the Diels-Alder reaction was carried out successfully.

3.2. Biological Activity

All the obtained compounds were evaluated as antioxidant agents in the presence of the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) at a concentration ranging from 10 to 100 μL and compared with ascorbic acid. The DPPH scavenging activity was poor with EC_{50} values higher than 100 μg/mL. On the other hand, Table 2 showed that the compounds have good activity in scavenging the ABTS radical, presenting better EC_{50} values than those found for ascorbic acid, used as a reference.

This result suggest that the radical trapping mechanism used by the compounds in each assay is different. Both assays are mixed, being able to use either the HAT (hydrogen atom transfer) or SET (single electron transfer) mechanism [16].

The incubation time was different for each assay: 5 min for DPPH and 30 min for ABTS. It is proposed that the radical trapping mechanism for every compounds is mainly SET, since this mechanism is slower as it occurs in 2 steps. Moreover, the solvents used (methanol for DPPH and ethanol:water (1:1) for ABTS) are protic polar solvents, which favor the SET mechanism [16].

It is important to note that the proposed SET mechanism for both tests is an approximate based on the results obtained. To ensure the radical trapping mechanism, it would be ideal to use a third assay specific to one type of mechanism rather than one of a mixed nature.

Table 2. Antioxidant activity in in vitro DPPH and ABTS assays of the new THQs compounds.

Compound	EC50 DPPH (μg/mL)	EC50 ABTS (μg/mL)
A	>100	1,09
B	>100	1,52
C	>100	< 10
D	>100	< 10
Ascorbic acid	1500	35

3.3. Analysis In Silico of the Compounds

Pharmacokinetic analysis is crucial, as it provides important information on the potential use of these compounds as drugs. The compounds are required to cross the blood-brain barrier (BBB) and have high gastrointestinal absorption (HIA). All of the proposed compounds meet these requirements (Table 3).

Another important parameter is that the compounds are not substrates of permeability glycoprotein (P-gp) and that they are inhibitors of the cytochrome P450 family. P-gp is responsible for eliminating molecules unknown to the human body by regulating the outflow [14]. On the other hand, the P450 family of cytochromes is responsible for eliminating molecules such as drugs by metabolic biotransformation [15], so their inhibition is important. Of the 4 synthetized compounds, are not substrates for P-gp, the exception being compound B. In general, the proposed THQs are good CYP inhibitors, inhibiting 3 CYPs with the compound A as a exception (Table 3)

Drug-likeness evaluates different approaches, including Lipinski, Ghose, Veber, Egan, and Muegge. These are filters for molecules with potential use as oral drugs, based on chemical properties such as molecular weight, lipophilicity, hydrogen bonding, flexibility, polarity, and bioavailability [12]. Three of four compounds meet all of these standards except Compound C, which has more than 2 heteroatoms and high lipophilicity (Table 3).

The "boiled egg" graph provided by SwissADME indicates the absorption of the compounds in the intestine (HIA) and their ability to cross the blood-brain barrier (BBB). The proposed compounds exhibit high gastrointestinal absorption and blood-brain barrier permeability, except for compound B (THQ indeno), which is a P-gp substrate (Supplementary material)

OSIRIS Property Explorer evaluates biological risks associated with the compounds studied, such as mutagenesis, tumorigenesis, irritancy, and reproductive effects. None of the 4 compounds tested present these risks. Also gives a resume of Drug-likeliness of the compounds.

Table 3. Swiss ADME pharmacokinetic predictions and Osiris toxicological risks for new THQs. (): Non-toxic; (): High toxicity.

Compound	Pharmacokinetics	PGP Substrate [1]	BBB [2] Penetration	PAINS [3]	MUT [4]	TUM [5]	IRRI [6]	REP [7]	DL [8]
A	-	No	Yes	0					
B	Inhibitor of CYP1A2, CYP2C9, CYP2D6	Yes	Yes	0					
C	Inhibitor of CYP2C19, CYP2C9, CYP2D6	No	Yes	0					
D	Inhibitor of CYP2C19, CYP2D6	No	Yes	0					

[1] P-glycoprotein (P-gp) substrate; [2] BBB: Blood Brain Barrier; [3] PAINS: Interference compounds in assays. [4] MUT: mutagenic; [5] TUM: tumorigenic; [6] IRRI: irritant; [7] REP: Reproductive effects; [8] DL: Drug-likeliness.

4. Conclusions

This study successfully optimized the synthesis of N-propargyl toluidine and developed two novel tetrahydroquinoline (THQ) derivatives. Key findings include:

- Improved synthesis yields using Na_2CO_3 at room temperature.
- Favorable in silico drug-like properties for most compounds, with no significant predicted toxicological risks.
- Exceptional antioxidant activity in the ABTS assay, particularly for every compound (EC_{50} < 10 µg/mL), outperforming ascorbic acid (EC_{50} = 35 µg/mL).
- Evidence suggesting a primary single electron transfer (SET) mechanism for radical scavenging.

These results provide a foundation for further exploration of THQ derivatives as potential antioxidant agents, especially in the context of neurodegenerative diseases. Future work should focus on enzymatic assays, detailed bioinformatic analyses, and investigation of the compounds' mechanism of action. The promising antioxidant activity and favorable predicted properties position these THQs as intriguing candidates for further development in neurodegenerative disease research.

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References

1. Nammalwar, B.; Bunce, R. Recent Syntheses of 1,2,3,4-Tetrahydroquinolines, 2,3-Dihydro-4(1H)-quinolinones and 4(1H)-Quinolinones using Domino Reactions. *Molecules* **2013**, *19*, 204–232. https://doi.org/10.3390/molecules19010204.
2. Chaube, U.; Dey, R.; Shaw, S.; Patel, B.D.; Bhatt, H.G. Tetrahydroquinoline: An Efficient Scaffold As mTOR Inhibitor for the Treatment of Lung Cancer. *Future Med. Chem.* **2022**, *14*, 1789–1809. https://doi.org/10.4155/fmc-2022-0204.
3. Maksoud, S.; Mayora, A.; Colma, L.; Sojo, F.; Pimentel, A.; Kouznetsov, V.; Merchán-Arena, D.; Romero, Á.; Arvelo, F.; de Sanctis, J.B.; Benaim, G. Effect of tetrahydroquinoline derivatives on the intracellular Ca^{2+} homeostasis in breast cancer cells (MCF-7) and its relationship with apoptosis. *Investig. Clínica* **2022**, *63*, 243–261. https://doi.org/10.54817/IC.v63n3a04.
4. Zablotskaya, A.; Segal, I.; Geronikaki, A.; Shestakova, I.; Nikolajeva, V.; Makarenkova, G. N-Heterocyclic choline analogues based on 1,2,3,4-tetrahydro(iso)quinoline scaffold with anticancer and anti-infective dual action. *Pharmacol. Rep.* **2017**, *69*, 575–581. https://doi.org/10.1016/j.pharep.2017.01.028.
5. Sridharan, V.; Suryavanshi, P.A.; Menéndez, J.C. Advances in the Chemistry of Tetrahydroquinolines. *Chem. Rev.* **2011**, *111*, 7157–7259. https://doi.org/10.1021/cr100307m.
6. 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.
7. Bello Forero, J.S.; Jones Junior, J.; da Silva, F.M. The Povarov Reaction as a Versatile Strategy for the Preparation of 1, 2, 3, 4-Tetrahydroquinoline Derivatives: An Overview. *Curr. Org. Synth.* **2015**, *13*, 157–175. https://doi.org/10.2174/1570179412666150706183906.
8. Eschenbrenner-Lux, V.; Kumar, K.; Waldmann, H. The Asymmetric Hetero-Diels–Alder Reaction in the Syntheses of Biologically Relevant Compounds. *Angew. Chem. Int. Ed.* **2014**, *53*, 11146–11157. https://doi.org/10.1002/anie.201404094.
9. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT—Food Sci. Technol.* **1995**, *28*, 25–30. https://doi.org/10.1016/S0023-643880008-5.
10. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free. Radic. Biol. Med.* **1999**, *26*, 1231–1237. https://doi.org/10.1016/S0891-584900315-3.
11. Molecular Properties Prediction—Osiris Property Explorer. Organic-Chemistry.org. 2024. Available online: https://www.organic-chemistry.org/prog/peo/ (accessed on).
12. 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.
13. Rodriguez Núñez, Y.A.; Norambuena, M.; Romero Bohorquez, A.R.; Morales-Bayuelo, A.; Gutíerrez, M. Efficient synthesis and antioxidant activity of novel N-propargyl tetrahydroquinoline derivatives through the cationic Povarov reaction. *Heliyon* **2019**, *5*, e02174. https://doi.org/10.1016/j.heliyon.2019.e0217410.
14. 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.
15. Rendic, S.P.; Guengerich, F.P. Human Family 1–4 cytochrome P450 enzymes involved in the metabolic activation of xenobiotic and physiological chemicals: An update. *Arch. Toxicol.* **2021**, *95*, 395–472. https://doi.org/10.1007/s00204-020-02971-4.
16. Munteanu, I.G.; Apetrei, C. Analytical Methods Used in Determining Antioxidant Activity: A Review. *Int. J. Mol. Sci.* **2021**, *22*, 3380. https://doi.org/10.3390/ijms22073380.