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Synthesis of *N*-Allyl and *N*-Propargyl Tetrahydroquinolines: Evaluation of Antioxidant Activity and Cholinesterase Inhibition in the Context of Neurodegenerative Diseases such as Alzheimer's †

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Abstract: In the context of the study of neurodegenerative diseases, particularly Alzheimer's disease (AD), this work focuses on the synthesis of tetrahydroquinolines (THQ) using a previously reported method, with *N*-allyl and *N*-propargyl structural variants. The antioxidant activity was evaluated through ABTS and DPPH assays, contributing to the calculation of SC_{50} (free radical scavenging) through dose-response curves. Similarly, the enzymatic inhibition of AChE (acetyl cholinesterase) and BChE (butyryl cholinesterase) was determined to obtain the IC_{50} values, framed within the cholinergic hypothesis for AD treatment. A toxicity analysis was carried out through the in vitro hemolysis assay, demonstrating low toxicity of the evaluated compounds. Additionally, in silico ADME analysis (Swiss ADME tool) predicted a high probability of penetration through the blood-brain barrier (BBB), along with the estimation of relevant parameters regarding their biotransformation and involvement in xenobiotic metabolism. It is important to highlight that the *N*-allyl/propargyl-THQs with a methyl substituent showed the highest activity in the different assays, where *N*-allyl exhibited better efficiency in inhibiting BChE and *N*-propargyl in AChE. Furthermore, ABTS seems to be more suitable than DPPH for THQ compounds, as DPPH showed low reactivity overall. These findings represent a significant advancement in the development of compounds with potential palliative therapeutic effects for AD, proposing THQs as promising candidates. The future research projection aims to elucidate mechanisms of action and complement with various bioactivity and cytotoxicity assays.

Keywords: tetrahydroquinoline; cholinesterase inhibitor; scavenging activity; AChE; BChE; DPPH; ABTS; ADME; IC_{50}; SC_{50}

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1. Introduction

Alzheimer's disease (AD) is an irreversible and progressive neurodegenerative illness that emerges in middle or late age and eventually leads to death. AD is a prominent type of dementia associated with cognitive and behavioral disabilities such as memory loss and speaking and learning difficulties. Although the primary cause of this disease is still unknown, some factors are found to be involved in its progression, including acetylcholine (ACh) deficiency in cholinergic synapses, amyloid-beta (Aβ) depositions, hyperphosphorylated tau (τ) aggregations, oxidative stress, and biometals dyshomeostasis [1].

The lack of effective treatment and the particularity of AD as a multifactorial condition make new research and the search for new potential drugs that help in its control

necessary. Nitrogen heterocycles are a series of compounds widely studied due to their pharmacological potential, among them, tetrahydroquinolines (THQs) are of interest in medicinal chemistry due to their wide spectrum of reported activities and the effectiveness shown against certain targets. We developed the synthesis, purification, characterization and bioactivity of a new series of THQs against therapeutic targets that point to the cholinergic theory and free radical scavenging activity focused on the control of AD.

2. Methods

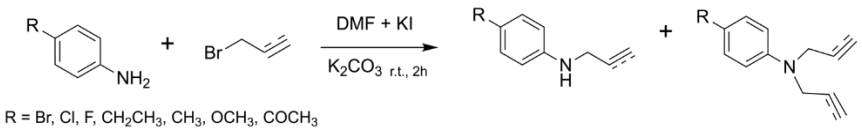
2.1. General Information

All reagents were purchased from either Merck (Darmstadt, Germany) or Sigma and Aldrich Chemical Co (St. Louis, MO, USA) and used without further purification. All products were characterized by spectral data (IR, MS, ^{1}H-NMR, ^{13}C-NMR). NMR spectra (^{1}H and ^{13}C) were measured on a Bruker Ultrashield-400 spectrometer (Rheinstetten, Germany), using $CDCl_3$ as solvent and reference. J values are reported in Hz. The reaction progress was monitored using thin layer chromatography on PF254 TLC aluminum sheets from Merck. Column chromatography was performed using Silica gel (60–120 mesh) and Solvents employed were of analytical grade.

2.2. Chemistry

THQ derivatives were efficiently synthesized according to methodology reported previously [2], the protocol outlined in Figure 1.

(**A**)



(**B**)

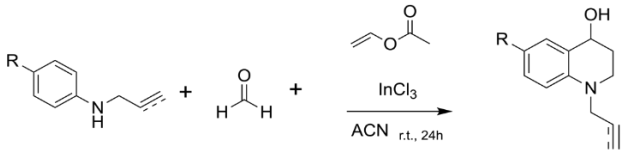


Figure 1. Synthesis scheme for THQ in two steps. (**A**) preparation of *N*-allyl/propargylamine, and (**B**) the obtained product is used in the cationic imino Diels-Alder reaction.

2.3. Biological Activity

2.3.1. Anticholinesterase Assay

The enzymatic inhibition turned into finished through making use of the spectrophotometric technique of Ellman AChE (from *Electrophorus electricus*) and BChE (from equine serum) [3]. The compounds were assayed in the dilution interval of 15–500 μg/mL and the alkaloid galantamine was used as the reference compound. Each assay was run in triplicate and each reaction was repeated at least three independent times. The IC_{50} values were calculated by means of regression analysis.

2.3.2. Measurement of DPPH Radical Scavenging Activity

The newly synthesized compounds were evaluated for $DPPH^{+}$ free radical scavenging activity. According to the method previously described and adapted [4]. Ascorbic acid was used as the reference compound with an SC_{50} value of 1.5 mg mL^{-1}.

2.3.3. Measurement of ABTS Radical Scavenging Activity

The newly synthesized compounds were evaluated for the radical scavenging activity of $ABTS^{+}$ according to a published test [5]. Ascorbic acid, with an SC_{50} value of 35 μg mL^{-1}, was used as a positive control.

2.3.4. Cytotoxicity Activity

Based on the previous assays, the compounds with the highest bioactivity were selected and evaluated for hemolysis assay, according to a previously described method with modifications.

2.3.5. In Silico Prediction of Pharmacokinetic Properties

Pharmacokinetic properties of THQ compounds were calculated an in silico way through ADME descriptors using Swiss ADME tool. Based on the Lipinski's rule of 5, some of the descriptors predicted were molecular weight, Van der Waals, surface areas of polar nitrogen and oxygen atoms (TPSA), H bond acceptors (HBA), H bond donors (HBD), Log P (octanol/water) and aqueous solubility [7], proposing a first analysis of the newly synthesized compounds as drug-likeness.

3. Results and Discussion

3.1. Chemistry

The synthesis of target *N*-allyl/propargyl 4-substituted 1,2,3,4-THQ derivatives (allyl and propargyl series) was carried out using a straightforward and efficient synthesis based on the acid-catalyzed one-pot multicomponent cationic imino Diels-Alder reaction, outlined in Figure 1. In the case of the synthesis of *N*-allyl/propargyl THQ series, the reaction occurs in anhydrous MeCN between preformed *N*-allyl/propargyl-anilines, formaldehyde, and vinyl acetate in presence of $InCl_3$ as acid-catalyst at room temperature. These THQs derivatives were obtained as solids with 30–60% yield after their chromatography purification.

All new *N*-allyl/propargyl-THQs derivatives were structurally characterized using NMR spectroscopic techniques, electrospray ionization-mass spectrometry (ESI-MS) and IR spectroscopy. In the IR spectra, bands for C-OH (3500 cm^{-1}) vibrations were observed, In the particular case of *N*-allyl THQ, IR spectra showed typical bands from allyl fragment (910–923 cm^{-1}) vibrations, while that in the IR spectra for *N*-propargyl-THQ was easily identified as bands for propargyl fragment (3209–3302 cm^{-1}) vibrations. All ^{1}H and ^{13}C NMR spectra of the synthesized *N*-allyl/propargyl THQs were very similar and characterized by the presence of three groups of signals: aromatic, aliphatic and near of heteroatom. The mass spectra correspond to the expected masses for the proposed structures. This set of signals constitutes evidence that the cationic imino Diels-Alder reaction took place successfully.

3.2. Biological Activity

All synthesized compounds were evaluated in vitro as dual AChE/BChE inhibitors. The compound's concentration required for 50% of enzyme inhibition (IC_{50}) was calculated by means of regression analysis. The news THQs obtained showed high selectivity for AChE, except for A-Me (Table 1). The most active compounds were P-Me with IC_{50} of 1.27 μM for AChE and A-Me with IC_{50} of 11.83 μM for BChE. All compounds obtained were evaluated as antioxidant agents in 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 were poor with IC_{50} values greater than 100 μg/mL. On the other hand, Table 1 showed that compounds P-Me, P-OMe, P-Et, and A-OMe have good activity in scavenging ABTS radical, presenting better SC_{50} values than those found for ascorbic acid, used as reference. This is consistent with the results reported previously where described compounds having an electron withdrawing group like

fluorine, and electron donating groups, like methoxy on the phenyl rings, exhibit good antioxidant activity [8,9]. Table 2 summarizes the determined biological activities for hemolysis assay and different estimations by Swiss ADME analysis.

Table 1. Simplified names and nomenclature of the new THQ compounds, and biological activities from in vitro assays.

Compound (Simplified)	Abbreviated Nomenclature	IC_{50} AChE (μM)	IC_{50} BChE (μM)	SC_{50} ABTS (μg mL^{-1})
P-Br	6-bromo-*N*-propargyl-THQ-4-ol	25.84	342.24	23,74
A-Br	*N*-allyl-6-bromo-THQ-4-ol	81.12	104.22	82.91
P-Cl	6-chloro-*N*-propargyl-THQ-4-ol	21.95	446.98	14.72
A-Cl	*N*-allyl-6-chloro-THQ-4-ol	20.80	14.88	76.05
P-Et	6-ethyl-*N*-propargyl-THQ-4-ol	6.62	26.68	1.25
A-Et	*N*-allyl-6-ethyl-THQ-4-ol	30.69	32.09	1.72
P-Me	6-methyl-*N*-propargyl-THQ-4-ol	1.27	22.45	0.86
A-Me	*N*-allyl-6-methyl-THQ-4-ol	36.13	11-84	1.71
P-OMe	6-methoxy-*N*-propargyl-THQ-4-ol	5.06	110.51	1.16
A-OMe	*N*-allyl-6-methoxy-THQ-4-ol	6.82	60.07	1.67
P-F	6-fluor-*N*-propargyl-THQ-4-ol	60.45	964.97	3.02
A-F	*N*-allyl-6-fluor-THQ-4-ol	29.17	245.04	3.70
P-(CO)Me	1-(4-hidroxy-*N*-propargyl-THQ)-etan-1-ona	21.27	403.53	21.25
Galantamine		0.54	8.8	
Ascorbic acid				35

Results from DPPH assays are not presented due to overall low reactivity of the compounds (SC_{50} > 100 μg mL^{-1}).

Table 2. Hemolysis assay and theoretical predictions of new THQs.

Compound (Simplified)	Hemolysis % <100 μg mL^{-1}	Pharmacokinetics	Bioavailability Score [1]	BBB Penetration [2]	PAINS
P-Et	0	CYP2D6 inhibitor	0.55	Yes	0
A-Et	0	CYP2D6 inhibitor	0.55	Yes	0
P-Me	0	-	0.55	Yes	0
A-Me	0	CYP1A2 and CYP2D6 inhibitor	0.55	Yes	0
P-OMe	0	CYP1A2 inhibitor	0.55	Yes	1
A-OMe	0	-	0.55	Yes	1
A-F	0	CYP1A2 and CYP2D6 inhibitor	0.55	Yes	0

[1] All the synthesized compounds were given a bioavailability score of 0.55 in a scale from 0 to 1. [2] BBB: Blood Brain Barrier.

3.3. Cytotoxicity and Computational Estimation of Biological Parameters

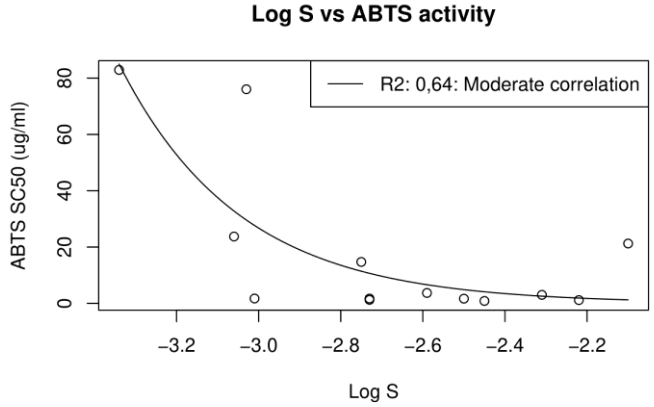
Computational methods are interesting tools to predict some compound properties with good experimental biological activities, e.g., QikProp is a quick, accurate, and easy to use absorption, distribution, metabolism, and excretion (ADME) prediction program. The main parameters are shown in Tables 2 and 3. Considering the Lipinski's rule of five (molecular weight below 500 Da, hydrogen bond donor less than 5, acceptor less than 10, and Log P (octanol/water partition coefficient) for the ligand less than five), the synthesized compounds did not present violations to this rule, being within of permissible range of each descriptor [7]. Likewise, the synthesized THQ series satisfies other parameters involved in the absorption, distribution, and membrane penetration as water solubility (Log S) and polar surface area (TPSA). Finally, the predicted qualitative oral absorption was

calculated. This prediction is made through the analysis of the appropriate values of different descriptors. The analysis showed that the compounds could present good oral absorption. In general, the new *N*-propargyl THQs presented permissible values in the different descriptors calculated.

Table 3. ADME properties of new THQs.

Compound	MW g mol^{-1}	Log P	Log S	TPSA Å^2	RB	HBA	HBD
P-Br	266.13	2.34	−3.06	23.47	1	1	1
A-Br	268.15	2.59	−3.34	23.47	2	1	1
P-Cl	221.68	2.23	−2.75	23.47	1	1	1
A-Cl	223.70	2.44	−3.03	23.47	2	1	1
P-Et	215.29	2.30	−2.73	23.47	2	1	1
A-Et	217.31	2.59	−3.01	23.47	3	1	1
P-Me	201.26	1.99	−2.45	23.47	1	1	1
A-Me	203.28	2.22	−2.73	23.47	2	1	1
P-OMe	217.26	1.70	−2.22	32.70	2	2	1
A-OMe	219.28	1.90	−2.50	32.70	3	2	1
P-F	205.23	1.98	−2.31	23.47	1	2	1
A-F	207.24	2.27	−2.59	23.47	2	2	1
P-(CO)Me	229.27	1.68	−2.10	40.54	2	2	1
Galantamine	287.35	1.92	−2.93	41.93	1	4	1
Ascorbic acid	176.12	−1.28	0.23	107.22	2	6	4

Finally, Figure 2 shows the correlation of Log P and Log S for ABTS activity, to evaluate if the effectiveness of the scavenging activity can be explained by the physicochemical parameters of each compound. Higher solubility of the molecule showed a moderate correlation with higher ABTS activity, but the R^2 value is very close to 0.6 suggesting that activity could be better explained by other mechanisms.



(A) (B)

Figure 2. Correlation of Log P (**A**) and Log S (**B**) for ABTS activity, done in RStudio with an exponential curve model. Values of R^2: <0.4 very weak; >0.4 weak; >0.6 moderate and >0.8 for strong correlation.

4. Conclusions

In summary, the synthesis of a new series of substituted *N*-propargyl-THQs derivatives has been developed in mild conditions and simple procedure through the Imino Diels-Alder reactions using $InCl_3$ as catalyst. The antioxidant activity is dependent on the

concentration of the compounds, likewise, the compound P-Me presented the most favorable antioxidant activity and AChE inhibition values, whereas A-Me was the most effective inhibiting BChE. Hemolysis assay and physicochemical descriptors calculated theoretically, indicated that the new compounds have a low toxicity risk and could potentially cross the BBB. Structurally the *N*-propargyl THQs are attractive for the production of a second generation of compounds due to the reactivity of the allyl/propargyl fragment. In this study, we obtained compounds with higher antioxidant capacity than the reference compound and selectivity for BChE.

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