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Synthesis, molecular docking and design of Tetrahydroquinolines as acetylcholinesterase inhibitors

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ABSTRACT

*A series of tetrahydroquinolines (Compounds **1-8**) were synthesized using Povarov reactions, subsequently characterized using spectroscopic methods and evaluated as Acetylcholinesterase/Butirylcholinesterase inhibitors. Bioinformatics tools analyzed active site interactions, leading to the design of a more active new compound. The most potent compound showed moderate in vitro activity (IC_{50} of 215µM) against AChE, while bioinformatics tools such as molecular docking and de novo design allowed establishment of binding sites and design of a new molecule with better activity, decreasing IC_{50} from 618 µM to 215µM. This study describes the application of bioinformatics tools in drug design.*

Keywords: Quinolines, Alzheimer's disease, cholinesterase inhibitors, tetrahydroquinolines, ligand-protein interactions.

INTRODUCTION

Quinolines are a heterocycle with marked biological activity in different therapeutic targets, and are present in different natural sources. Compounds containing quinoline structures are widely used as antiasthmatics, antimalarials, anti-virals, anti-inflammatories, antibacterials, antifungals, and anticarcinogens [1]. Synthetic quinoline derivatives have been reported as base structures in the synthesis new compounds with diverse biological activity including fungicides, virucides, biocides, alkaloids, rubber chemicals and flavoring agents [2]. These and other structural and functional features mean that research efforts have focused on obtaining new, structurally diverse structures to enhance the above activities or act on new therapeutic targets.

Tetrahydroquinolines (THQs) are a group of quinoline derivatives of interest in the area of organic synthesis because of their proven pharmacological properties [3], including anti-HIV[4], anti-cancer[5] and anti-malarial[6]activity, inhibition of the cholesteryl ester transfer protein [7] and anti-diabetic [8] properties, among others.

Alzheimer's disease (AD) is a neurodegenerative disorder affecting a large part of the world's adult population, associated with different causes such as tau protein hyperphosphorylation [9], amyloid-β peptide accumulation[10], mutations in 17q21 chromosome genes [11] and acetylcholine neurotransmitter decreases [12] among others.

Applied therapy for AD treatment is mainly based on palliative treatments that attempt to restore acetylcholine levels by inhibiting the acetylcholinesterase enzyme (AChE) [12] and its action as a partial NMDA receptor antagonist[13]. The AChE enzyme is a serine hydrolase located either in the peripheral (muscles) and/or central nervous (cholinergic) system. The use of AChE blockers to treat Alzheimer's disease is based on their restoration of the levels of this neurotransmitter, minimizing the effects of the disease. Acetylcholinesterase inhibitors (AChEI) also have many other pharmacological applications; for example, they are described as anesthetic test substances that are useful for treating neuromuscular blockade, myasthenia gravis and glaucoma [14].

Many quinoline derivatives have shown anti-cholinesterase activity and may be potential agents for AD treatment [15-17]. Gatta et al reported the synthesis of THQ derivatives as a potential AChEI in 1992[18], Fink et al. described the synthesis and biological evaluation of 5-amino-5,6,7,8-tetrahydroquinolinones as AChEI in 1995[19], and Maalej et al.[20]reported the synthesis, *in vitro* evaluation , and molecular docking of a series of racemic 7-aryl-9,10,11,12-tetrahydro-7H-benzo[7,8]chromeno[2,3-b]quinolin-8-amines as potential compounds for AD treatment in 2012.

Bioinformatics tools have become highly important in drug design in recent years, as they give us an approximation as to the substance's possible behavior in the active site, providing guidelines for work, and optimizing time and resources.

These methods fall into the following natural categories depending on the information available on therapeutic targets and potential drug compounds: Structure-based drug design, ligand-based drug design, *de novo* design and homology modeling [21].

In this study, we have synthesized THQ derivatives for evaluation as potential inhibitors of cholinesterase able to be used as pharmacophores in the design of new drugs effective for treating AD. Bioinformatic tools were used to establish interactions, predict ADME properties [22] and design new compounds with increased activity.

EXPERIMENTAL SECTION

Chemistry

All chemical reactives for synthesis were obtained in Sigma-Aldrich, solvents were reagent grade and usually dried and distilled before use according to standard procedures. Enzymes and substrates were purchased from Aldrich Co.

Reaction was monitored by thin-layer chromatography (TLC) and visualized using UV light; TLC took place on pre-coated silica gel 60 F254 plates (Merck). All solvents were analytical grade. Nuclear Magnetic Resonance (NMR) spectra were recorded in diluted $CDCl_3$ solutions. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 400 instrument. Chemical shifts are reported as parts per million (ppm) and Multiplicities are designated as singlet (s), doublet (d), triplet (t), quadruplet (q) or multiplet (m). Melting points were recorded on a Buchi apparatus and are uncorrected. High-resolution mass spectrometry ESI-MS and ESI-MS/MS analyses were conducted in a high-resolution hybrid quadrupole (Q) and orthogonal time-of-flight (TOF) mass spectrometer (Waters/Micromass Q-TOF micro, Manchester, UK) with a constant nebulizer temperature of 100 °C. Experiments were carried out in positive ion mode, and cone and extractor potentials were set at 10 and 3.0 V, respectively, with a scan range of m/z 105-600. MS/MS experiments were carried out by mass selection of a specific ion in Q1, and then sent for collision-induced dissociation (CID) with helium in the collision chamber. Product ion MS was analyzed using the high-resolution orthogonal TOF analyzer. Samples were dissolved in acetonitrile and directly infused into the ESI source via a syringe pump at flow rates of 5 μL min^{-1}, through the instrument's injection valve. Values expressed are average mass and correspond to the $[M+H]^+$ ion.

Synthetic methodologies

General procedure for synthesizing functionalized tetrahydroquinolines **1-8:** THQs were formed using a Povarov reaction where aldehyde (3.4 mmol) was reacted with an amine (1mmol) in dry CH_3CN (20mL) to generate the corresponding imine, to which $BiCl_3$ (20% mol) was added as Lewis acid. The resulting solution was stirred at room temperature, and a solution of N-vinylpyrrolidin-2-one (1mmol) in CH_3CN was added, with the resulting solution stirred constantly at room temperature in an inert atmosphere (with N_2) for 24 h (scheme1). The progress of the reaction was monitored by TLC compared with precursors.

Once the reactants were exhausted, a saturated aqueous Na_2CO_3 solution was added, and then the resulting mixture was extracted using EtOAc. The organic phase was dried (Na_2SO_4), filtered, and concentrated in reduced pressure. Extracts were purified using column chromatography (SiO_2, hexane-EtOAc) to produce the desired product

(compounds **1**-**8**). All compounds were characterized using spectroscopics and spectrometric methods (IR, NMR and MS). The spectroscopic date of compounds **1**-**7** has been provided above [23].

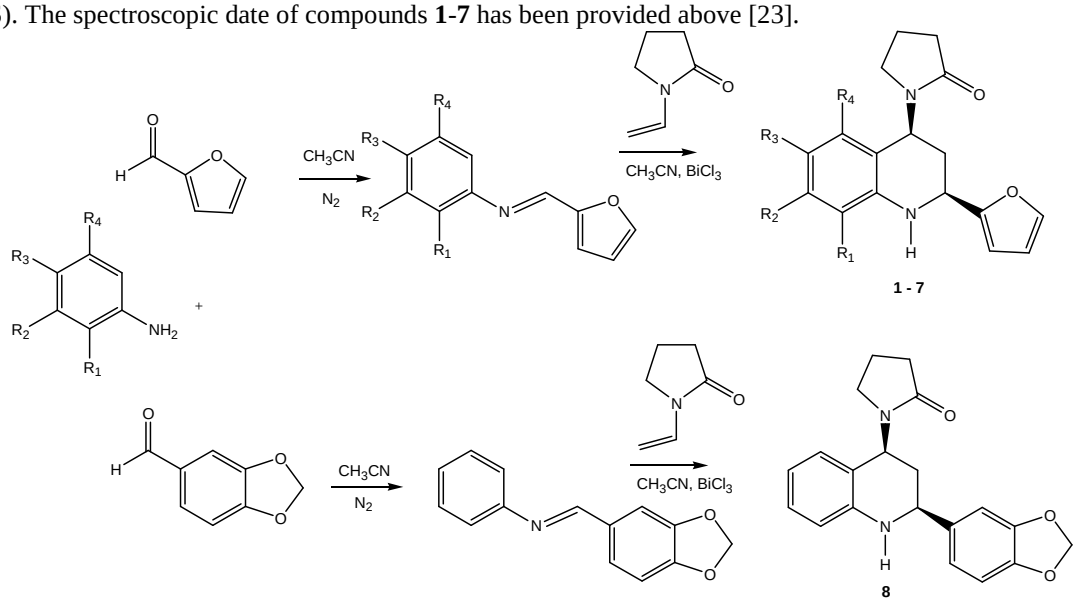

Figure 1: The Synthetic route of tetrahydroquinolines 1-8 derivatives

Biological assays

The biological evaluation of compounds such as cholinesterase inhibitors took place using the methodology described by Ellmann [24] adapted to 96-well plates. Details on the protocol for evaluating cholinesterase inhibitors have been described previously by the authors [25].

All compounds were assayed in the dilution interval of 500 - 15µg/mL, and galantamine was used as reference compound. Assays were carried out in triplicate in independent experiments, including respective targets for each sample and activity and solvent controls on each plate. IC_{50} values were calculated using regression analysis. Values are expressed in µg/mL and µM ±SD. (Table 2)

Molecular docking

Docking with Glide [26] was used to suggest binding modes of some reported inhibitors. The Glide program is part of the Maestro 9.0 software (New York, NY, USA) [27]. Glide docking uses a series of hierarchical filters to find the best possible ligand binding locations in a previously built receptor grid space. The filters include a systematic search approach, sampling the positional, conformational, and orientational space of the ligand before evaluating energy interactions between the ligand and the protein [26].

Protein coordinates were extracted from the X-ray crystal structure of the *Tc*AChE–tacrine [accession code in the Protein Data Bank (PDB): 1ACJ]. The structures of the **4** compounds were sketched using Maestro software, specifically the extra-precision (XP) Glide module. A 30 Å × 30 Å × 30 Å grid box was first located in the center of the *Tc*AChE receptor, and the default docking parameters were used. The docking hierarchy begins with systematic conformational expansion of the ligand followed by placement in the receptor site. The ligand is then minimized in the receptor field using the OPLS-AA force field [28] with a distance-dependent dielectric of 2.0. Subsequently, the lowest energy poses are subjected to a Monte Carlo procedure sampling the nearby torsional minima. The best pose for a given ligand is determined using the Emodel score, while different compounds are ranked using Glide Score. Docking poses for each ligand are analyzed by examining their relative total energy score. The more energetically favorable conformation is selected as the best pose.

***De novo* design**

Briefly, *de novo* design uses structural information and biological activity of known pharmacologically active compounds as input structures in order to generate new structures that share and/or improve the biological activity of the original compounds.

Molecular dynamics simulations

Protein-ligand complexes created by docking were subjected to molecular dynamic (MD) simulations to verify the stability of the interactions established in each complex and to further identify potential interactions accessible during MD.

MD calculations took place using the OPLS-AA force field in explicit solvent under the SPC water model in the Desmond v3.0 package [27] of the Maestro suite. Systems were neutralized by adding Na^+ counter ions to balance the net charge of the systems. An excluded region for counter ions was set at 5 Å from the AChE binding site. Simulation time was set at 10 ns and an isothermal-isobaric ensemble was used, specifying temperature, pressure and number of constant atoms. Temperature was set at 300 K and pressure at 1.01325 bar (1 atm), using the Nosé-Hoover method with a relaxation time of 1 ps applying the MTK algorithm. The SHAKE algorithm [29] was applied to every hydrogen atom and the cut-off for van der Waals forces was set at 9 Å and long-range electrostatic forces were modelled using the particle mesh Ewald (PME) method. Data were collected every 4ps during the MD runs.

ADME properties

ADME data was estimated using the Molinspiration program [30].

Statistical analysis

All assays were performed in triplicate in independent assays, and analyzed and expressed as mean±standard error of mean (SEM)using Statistical Product and Service Solutions,17thversion (SPSS, Inc., Chicago, Il, Usa)). IC_{50} values for THQ derivates against AChE were calculated using regression analysis. Statistical analyses were performed by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Chemistry

All THQ derivatives were synthesized using a Povarov reaction between amines and aldehydes obtaining the corresponding imine, coupled with electron- rich alkene, generally obtaining a good yield and high purity. N-vinylpyrrolidin-2-one was used as an electron-rich alkene in this reaction because it was available, stable and cheap. The amines used were selected for their ability to donate electrons and facilitate imine formation. Reactions were performed under mild conditions (room temperature, 24 h) in the presence of Lewis acid ($BiCl_3$ 20 mol %) in acetonitrile. The substituents employed for synthesizing**1**- **8** are shown in table 1. All THQs were purified using SiO_2 column chromatography and obtained as solids and exclusively as *cis*-diastereoisomers. The *cis* configuration of the substituents was confirmed by measuring the relevant H-H coupling constants in their ^{1}H NMR spectra.

THQs **1**-**8** were characterized using ^{1}H-NMR, ^{13}C-NMR and Mass Spectra. ^{1}H-NMR spectra for compounds **1**-**8** showed characteristic signals distributed mainly over three distinct areas, demonstrating the presence of aromatic protons, protons near heteroatoms and aliphatic protons, resonating in different zones. Mass spectra for compounds **1**-**7** showed similar fragmentation patterns, and the constant presence of the molecular ion $[M+H]^+$in addition to characteristic loss of a fragment of 85 units corresponding to the ring from N-Vinylpyrrolidin-2-one. THQ **8** showed the typical signals of methylenedioxy; a singlet at 5.2 corresponding to $–OCH_2O$- protons and a signal at 100 ppm were detected in ^{1}H NMR and ^{13}C spectrum, respectively.

Table 1: Substituent employed for THQs 1-8

Product	R^1	R^2	R^3	R^4
1	H	H	CH_3	H
2	H	CH_3	H	CH_3
3	CH_3	H	CH_3	H
4	H	H	OCH_3	H
5	H	H	Cl	H
6	H	H	I	H
7	H	H	F	H
8	H	H	H	H

Compounds **1**-**7** were synthesized, characterized and evaluated previously as antifungals against some phytopatogenic fungi, where **4** was the most active compound against *Cladosporium cladosporoides*, with moderate activity [23]. Compound **8** was previously characterized and evaluated as free-radical scavenging and showed the highest TEAC values [31]. Compound **8** and its respective quinoline were also evaluated as antifungal against dermatophyte fungi with both showing poor activity [32, 33].

In order to improve the activity of **4**, in *silico* optimization took place using the part of the structure derived from **4** as a pharmacophore, requiring only modification of the aldehyde, replacing the 2-furaldehyde for piperonal and obtaining compound **8**, which was characterized using NMR spectroscopy.

1-(2-Benzo[1,3]dioxol-5-yl-1,2.3.4-tetrahydro-quinolin-4-yl)-pyrrolidin-2-one (**8**): light brown color mp: 180-182^{o}C; Yield 92%. IR (KBr) *v*3330, 3043, 3018, 2950, 2910 cm^{-1}.^{1}H NMR($CDCl_3$): δ=7.02 (1H, t, *J*= 7.58Hz), 6.91 (1H, d, *J*=1.47 Hz), 6.83 – 6.86 (2H, m); 6.76 (1H, d, *J*=7.83 Hz), 6.88 (1H, ddd, *J*=7.80, 7.22, 0.62 Hz), 6.55 (1H, dd, *J*=8.07, 0.72 Hz), 5.92(2H, s), 5.67 (1H, t, *J*=8.86 Hz), 4.47(1H, t, *J*=7.34 Hz), 3.99 (1H, brs), 3.19 (2H, m), 2.46(2H, m), 2.02(4H,m). ^{13}C NMR ($CDCl_3$) δ=175.68, 147.82, 147.06, 145.78, 136.94, 128.14, 126.63, 119.69, 118.04, 114.85, 108.22, 106.69, 100.99, 56.04, 48.30, 42.18, 35.23, 31.28, 18.13. m/z (%): 327.6 (21.6%) $[M]^{+1}$; 326.16 (100%). Anal. Cald. for $C_{20}H_{20}N_2O_3$: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.53; H, 5.87; N, 8.37. These data concur with the previous report [31].

Inhibition of cholinesterase enzymes

Acetyl and butyrylcholinesterase are enzymes that act on acetylcholine by terminating its actions in the synaptic cleft by cleaving the neurotransmitter to choline and acetate. Both are present in the brain and detected in neurofibrillary tangles and neuritic plaques. This suggests that AChE predominates in the healthy brain and it is believed that BChE plays a minor role in regulating brain acetylcholine levels. However, BChE activity progressively increases in patients with AD, while AChE activity remains unchanged or declines [34]. Cholinesterase inhibitors are standard therapy for patients with AD and are the only class of drugs approved by the Food and Drug Administration (FDA) for this condition, and show a palliative effect for declining cognitive, behavioral, and overall functions, characteristic of AD Compounds **1**-**8** were evaluated against AChE and BuChE. All compounds were inactive against BuChE with IC_{50} values over 500µg/mL. The compounds with the highest activity against AChE were **3** with IC_{50} of 805 µM and **4**, with IC_{50} of 618µM (table 2).

Table 2: IC_{50} values of THQs most actives against AChE

Compound	IC_{50} (µg/mL)	IC_{50} (µM)
3	250± 2.8	805± 3.2
4	**193±3.5**	**618±4.7**
8	**72.30±5.3**	**215±4.2**
Galantamine	1.1	0.54

The concentration of THQs required for inhibiting 50% of enzyme activity (IC_{50}) was calculated using regression analysis. The results tabulated in table 2 are expressed in µg/mL and µM as ± SEM means, and compared using ANOVA analysis. A *P*- value of less than 0.05 was considered significant. The **3** and **4** compounds showed the highest activity with IC_{50} values of 805 and 618, respectively. Although the activity of **3** and **4** is deficient in comparison with the reference, they have proven to be interesting phamacophores useful for in the design of new biologically active compounds against enzymes related with neurodegenerative diseases. Their biological activity as cholinesterase inhibitors was assayed in comparison with galantamine as a reference compound, which was chosen as a naturally occurring substance [35] currently used as a drug for Alzheimer's and also as one of the compounds with the highest number of interactions and lowest free energy bindings with active site amino acid residues [36, 37].

Compound **4** was the most active of the series of the evaluated THQs, with an IC_{50} of 618 µM against AChE. This compound was subjected to molecular docking in order to establish its location and chemical interactions with the active site of the enzyme. Based on biological activity and molecular docking results, compound **4** ─subject to *de novo* design ─was used as a pharmacophore to design new chemically synthesizable structures with greater affinity for the active site of AChE.

Acetylcholinesterase docking studies

In order to understand ligand–protein interactions at the molecular level, detailed automated docking and molecular dynamic simulation studies were performed for the most potent AChE inhibitors reported in this paper (**4** and **8)**. Figure 2.

As seen in Figure 2, compounds **4** and **8** have several interactions along the active-site gorge of AChE, with the main differences being the orientation of the compound in the active site. For compound **4**, the major bindings were π-π stacking with the piperidine ring and Trp 84 (3.8 Å); and two hydrogen bonding between Gly 123 (3.7 Å) and the oxygen of methoxy substituent; and between Phe 300 and oxygen moiety in the furan ring (3.2 Å), withanS-S conformation being used to minimize the steric hindrance caused by surrounding amino acids. When compound **8** is docked onto the AChE receptor, there were indications of hydrogen bonds, hydrophobic and mild polar interactions and π-π stacking. His 440 at the catalytic triad showed hydrogen bonding with carbonyl oxygen of pyrrolidone ring (3.1 Å), Asp 72 showed hydrogen bonding with methylenedioxo ring oxygen (3.2 Å) and the Phe 330 residue showed interaction π-π with an aromatic ring (3.7 Å) of ligand **8.** Similarly, we observed that the oxygen present in methylenedioxo ring forms an additional hydrogen bond, making an important contribution to the binding potency

of compound **8**. Both compounds, when evaluated in the complex using tACHE molecular dynamics, were stable over time (10 ns) and the described interactions remained. The residues present in the interactions between ligand **8** and the enzymes are reported as essential amino acids for binding inhibitors to the active site of the enzyme [38, 39],validating the pose found in the active site.

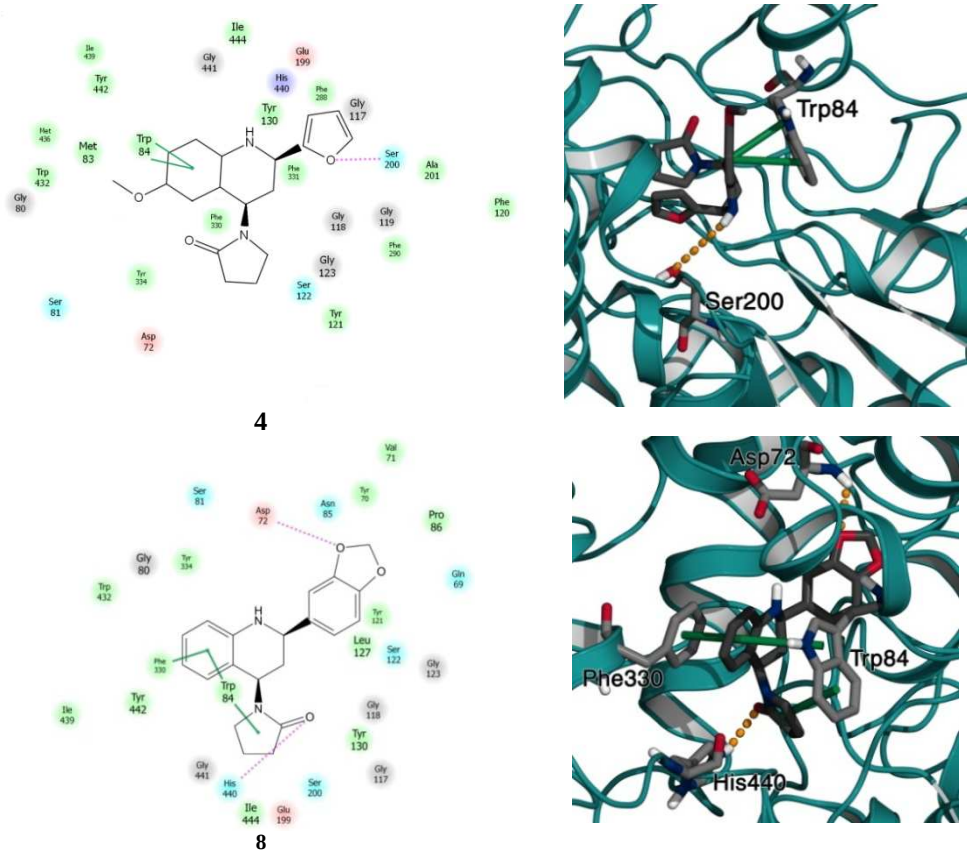

Figure 2: Molecular docking for compounds 4 and 8 within the AChE binding site highlighting the protein residues that form the main interactions with the inhibitors

One important characteristic that any biologically active compound should have for being a candidate drug is the ability to cross biological membranes. Also, and in accordance with the above; a molecule must possess the appropriate ADME (Absorption, Distribution, Metabolism, and Excretion) properties. We utilized Molinspiration software to establish some pharmacological parameters, for example, lipophilicity, expressed as the octanol/water partition coefficient (represented as logP), TPSA as a descriptor characterizing drug absorption, including intestinal absorption, bioavailability and permeability and other parameters related with Lipinski`s rule [22]. Therefore, theoretical prediction of the ADME properties of all compounds took place and was described under different parameters (Table 3) [40].

Table 3: Structural properties of THQs more active. TPSA, topological polar surface area; n-OH, number of hydrogen bond acceptors; n-OHNH, number of hydrogen bond donors. The data were determined with Molinspiration calculation software

Compound	LogP	Molecular weight (g/mol)	TPSA(Å^2)	n-OH acceptors	n-OHNH donors	Volume(Å^3)
3	2.29	310.39	45.48	4	1	293.49
4	2.62	286.38	41.57	4	1	272.77
8	3.06	336.39	50.80	5	1	302.73

When analyzing the theoretical results, no violations of Lipinski's rule (Molecular weight=270-410; Log P= 2.66-4.92, nON=2-6 and nOHNH=0-4) are observed for the THQs described. Additionally, the topological polar surface area, described as a predictive indicator of membrane penetration, was found to be positive for these drug candidates.

The results suggest that rational modifications of the substituent in scaffold THQs would be a suitable basis for developing novel AChE inhibitors. Further studies on this series of derivatives are in progress and will be reported in due course.

Variation in the biological activity of the evaluated compounds may be directly related to an increase in the number of interactions between compound **8** and the active site, and a decrease in the bond length of the compound and amino acidic residues of the active site.

CONCLUSION

In this paper, we have described one-step synthesis of THQs, a biologically important heterocyclic scaffold. Synthesis is based on Lewis acid catalyzed three component Povarov reactions, providing a fast, cheap, and safe way to create THQs with biological activity. We have described the synthesis of a series of THQs with different substitution patterns, their *in vitro* biological evaluation as cholinesterase inhibitors and the application of bioinformatics tools to ensure a rational design and better understanding of the mechanism binding the molecule with its receptor. Compounds follow Lipinski's rule. The results show the pharmacological potential of THQs, and usefulness of bioinformatics tools for designing drugs due to reduced time spent on synthesis and better use of resources. The most active compounds were obtained through *denovo* design and had an IC_{50} value of 215 µM. However, all synthesized compounds showed lower inhibitory activity than the reference drug, galantamine. These studies suggest that THQ derivatives obtained using piperonal as an aldehyde may be a promising structural template for developing novel AChE inhibitors to manage amnesic disorders including AD, and show the effectiveness of bioinformatic tools in rational drug design, which may reduce the time spent on synthesis and ensure a better use of available resources.

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