Synthesis and Characterization of Tetrahydroquinolines as acetylcholinesterase inhibitors

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Abstract: A serie of tetrahydroquinolines were synthesized in good yields using the Povarov reaction and evaluated as inhibitors of Acetilcholinesterase *in vitro*. The most active compounds showed an IC_{50} of 193µg/mL, the analysis of result suggest modification in the patters of substitution to increassed activity.

Keywords: Quinolines, Alzheimer's disease, Acetylcholinesterase inhibitors, tetrahydroquinolines.

Introduction

Quinoline is a heterocyclic scaffold of paramount importance to human race. Compounds containing quinoline structure are widely used as antiasthmatic, antimalarials, anti-viral, anti-inflammatory, antibacterials, antifungals, and anticancer (1). Quinoline derivatives have been used in the synthesis of fungicides, virucides, biocides, alkaloids, rubber chemicals and flavoring agents (2). The importance as substructures in a broad range of products, significant efforts continue to be directed into the development of new quinoline-based structures.

Among quinoline derivatives, tetrahydroquinolines (THQs) are an important structural subunit of natural and synthetic products and many THQs derivatives exhibit interesting biological and pharmaceutical activities (3), including anti-HIV (4), anti-cancer (5), anti-malarial (6), cholesteryl ester transfer protein inhibitors (7), anti-diabetic (8), etc.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with cognitive, functional and behavioral impairments. The current therapies for AD, yet symptomatic and palliative, rely mainly on the restoration of acetylcholine levels (9) and on the partial antagonism of NMDA receptor (10). Since cholinergic transmission is involved in a variety of physiological systems, AChE is a pursued pharmacological target for treatment of several pathologies besides AD. AChE inhibitors (AChEIs) have therapeutic application in surgical anesthesia, in the treatment of neuromuscular blockade, in myasthenia gravis and in glaucoma (11).

Many quinoline derivatives have shown anti-cholinesterase activity and can be potential agents for treatment of AD (12-14). Gatta et al in 1992(15) reported the synthesis of THQs derivatives as potential AChEI, Fink et al in 1995(16) reported the synthesis and evaluation of 5-amino-5,6,7,8-tetrahydroquinolinones as AChEI, and in 2012 Maalej et al (17)reported the synthesis, biological assessment, and molecular modeling of racemic 7-aryl-9,10,11,12-tetrahydro-7H-benzo[7,8]chromeno[2,3-b]quinolin-8-amines as potential drugs for the treatment of AD.

We reported a serie of THQs synthetic as potencials AChE inhibitors that could be employed in the design of new drugs for AD treatments.

Material and methods

Materials

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

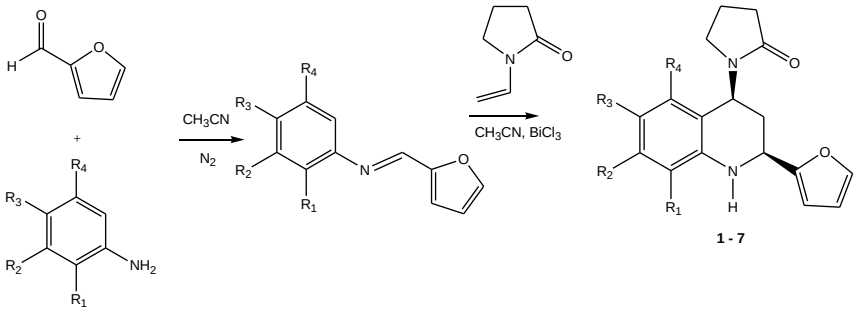
Measurements

All compounds was characterized by ^{1}H NMR with on Bruker AM-400 spectrometer (400 MHz), using $CDCl_3$ as solvent and TMS was used as an internal standard. Melting point (uncorrected) was measured on a Fischer-Johns melting point apparatus. ESI-MS(/MS) data were collected using a high resolution hybrid quadrupole (Q) and orthogonal time-of-flight (TOF) mass spectrometer (Q-Tof, Micromass UK) with constant nebulizer to 100 ºC. The equipment were operated in the positive ion mode, and the cone and extractor potentials were set to 40 and 5 V, respectively, with a scan range of *m/z* 80-1000. Samples were infused into the ESI source at flow rates of 5μL/min via a microsyringe pump.

Synthesis

General Procedure for the Three-Component Reaction of alquene with Aldehydes and Anilines.

The Lewis acid $BiCl_3$(20% mol) was added to imine obtained from a mixture of a solution of aldehyde (3.4 mmol) and amine (1 mmol) in dry CH_3CN (15 mL), this mixture was stirred at room temperature. A solution of alquene (1 mmol) in dry CH_3CN (15 mL) was then added, and the resulting suspension was stirred at room temperature under N_2 atmosphere for 18-24 h (Scheme 1). The reaction progress was monitored by means of TLC using Merck Kiesel-gel 60 (230-240 mesh). A saturated aqueous $NaHCO_3$ solution was added, and the resulting mixture was extracted with EtOAc. The organic phase was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, hexane-EtOAc) to give the desired product (compounds **1-7**). All compounds was purified against chromatographic technical and characterized for spectroscopic methods. The spectroscopic data of compounds **1-7** was describe previously (18).



Scheme 1. Synthesis of tetrahydroquinolines **1**-**7** using Povarov Reaction.

Acetylcholinesterase inhibitors

To assess AChE inhibition, an adapted version of the Ellmann (19) assay in 96 well plates was used (20).The enzyme activity was calculated as a percentage compared to a control using only the buffer and enzyme solution. The compounds were assayed in the dilution interval of 500 - 15 μg/mL, and the alkaloid galanthamine 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. The values were obtained and expressed in μM ±SD.

Results and Discussion

Seven THQs were synthesized by imino Diels-Alder cycloaddition between different substituted anilines, 2-furaldehyde, and N-Vinylpyrrolidin-2-one used as alkene, with acetonitrile as solvent in the presence of 20 mol% of bismuth trichloride (III) as catalyst (Scheme 1). It must be taken into consideration that bismuth compounds have attracted attention due to their low toxicity, low cost, water tolerant catalyst and good stability in several reactions such as the imino Diels-Alder (Povarov) reaction obtaining good yield with almost no by-products. The substituents employed are show in table 1. All THQs were purified by SiO_2 column chromatography and obtained as solids and exclusively as the *cis*-diastereoisomers. The *cis* configuration of the substituents was determined by measurement of the relevant H-H coupling constants in their ^{1}H NMR spectra.

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

Table 1. Substituent employed for THQs **1**-**7**

Chemical data

Compound **1**: 1-[2-(furan-2-yl)-6-methyl-1,2,3,4-tetrahydroquinolin-4-yl]pyrrolidin-2-one, Orange powder, mp 190-193 ºC, yield 85.0%. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.40 (1H, d, *J* = 4.0 Hz); 6.87 (1H, d, *J* = 8.0 Hz,); 6.68 (1H, s); 6.53 (1H, d, *J* = 8.0 Hz); 6.36 (1H, dd, *J* =8.0 and 4.0 Hz); 6.26 (1H,d , *J* =4.0 Hz); 5.68 (1H,dd, *J* = 11.0 and 4.0 Hz); 4.62 (1H,dd, *J* = 11.0 and 1.0 Hz); 4.00 (1H, br.s, NH); 3.29 – 3.15 (2H, m); 2.60 – 2.45 (2H, m); 2.30 – 2.18 (2H, m); 2.22 (3H, s, -CH_3); 2.07-1.99 (2H, m). MS *m/z* (EI): 296.36 (M^+).

Compound **2**: 1-[2-(furan-2-yl)-5,7-dimethyl-1,2,3,4-tetrahydroquinolin-4-yl]pyrrolidin-2-one, Rose powder, mp 163-165, yield 87.0%. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.37 (1H, s); 6.42 (1H, s); 6.33 (2H, s); 6.21 (1H, d, *J* = 4.0 Hz); 5.49 (1H, t, *J* = 8.0 Hz); 4.44 (1H, dd, *J* = 11.0 and 1.0 Hz); 4.14 (1H, br.s, NH); 3.01 – 2.72 (2H, m); 2.44 - 2.37 (2H, m); 2.38 – 2.28 (2H, m); 2.20 (3H, s); 2.04 (3H, s); 1.86 – 1.78 (2H, m). MS *m/z* (EI): 310.39(M^+).

Compound **3**: 1-[2-(furan-2-yl)-6,8-dimethyl-1,2,3,4-tetrahydroquinolin-4-yl]pyrrolidin-2-one, Yellow powder, mp 170-173, yield 90.0%. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.41 (1H, s,); 6.79 (1H, s); 6.58 (1H, s); 6.38 (1H, br.s); 6.29 (1H, d, *J* = 4.0 Hz); 5.70 (1H,dd, *J* = 11.0 and 4.0 Hz); 4.64 (1H,dd, *J* = 11.0 and 1.0 Hz); 3.92 (1H, br.s, NH); 3.40 – 3.13 (2H, m,); 2.60 – 2.45 (2H, m,); 2.30 – 2.18 (2H, m,); 2.20 (3H, s); 2.10 (3H, s); 2.05 – 1.98 (2H, m). MS *m/z* (EI): 310.39 (M^+).

Compound **4**: 1-[2-(furan-2-yl)-6-methoxy-1,2,3,4-tetrahydroquinolin-4-yl]pyrrolidin-2-one, Coffee powder, mp 165-167, yield 87.0%. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.44 (1H, s); 7.20 (1H, d, *J* = 4.0 Hz); 6.74 (1H, d, *J* = 4.0 Hz); 6.64 (1H, br.s); 6.58 (1H,d, *J* = 2.0 Hz); 6.38 (1H, d, *J* = 2.0 Hz); 5.66 (1H,dd, *J* = 11.0 and 4.0 Hz); 4.64 (1H,dd, *J* = 11.0 and 1.0 Hz); 3.97 (1H, br.s, NH); 3.76 (3H, s, -OCH_3); 3.38 – 3.12 (2H, m); 2.75 – 2.37 (2H, m); 2.55 – 2.43 (2H, m); 2.07 – 1.95 (2H, m). MS *m/z* (EI): 312.36 (M^+).

Compound **5**: 1-[6-chloro-2-(furan-2-yl)-1,2,3,4-tetrahydroquinolin-4-yl]pyrrolidin-2-one, Orange powder, mp 175-177, yield 85.5%. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.40 (1H, d, *J* = 2.0 Hz); 7.01 (1H, dd, *J* = 8.0 and 4.0 Hz); 6.83 (1H, d, *J* = 2.0 Hz); 6.52 (2H, d, *J* = 8.0 Hz); 6.37 (1H, dd, *J* = 4.0 and 2.0 Hz); 6.28 (1H, d, *J* = 4.0 Hz,); 5.65 (1H,dd, *J* = 11.0 and 4.0 Hz); 4.67 (1H,dd, *J* = 11.0 and 1.0 Hz); 4.13 (1H, br.s, NH); 3.30 – 3.16 (2H, m); 2.61 – 2.44 (2H, m); 2.26 – 2.18 (2H, m); 2.10 – 2.03 (2H, m). MS *m/z* (EI): 316.78 (M^+).

Compound **6**: 1-[2-(furan-2-yl)-6-iodo-1,2,3,4-tetrahydroquinolin-4-yl]pyrrolidin-2-one, Coffee powder, mp 190-192 yield 90.0%. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.40 (1H, s); 7.31 (1H, dd, *J* = 8.0 and 2.0 Hz); 7.12 (1H, s); 6.38 (1H, m); 6.36 (1H, m); 6.27 (1H, d, *J* = 4.0 Hz); 5.63 (1H,dd, *J* = 11.0 and 4.0 Hz); 4.67 (1H,dd, *J* = 11.0 and 1.0 Hz); 4.15 (1H, br.s, NH); 3.28 – 3.16 (2H, m); 2.63 – 2.44 (2H, m); 2.24 – 2.20 (2H, m); 2.08 – 2.02 (2H, m). MS *m/z* (EI): 408.23 (M^+).

Compound **7**: 1-[6-fluoro-2-(furan-2-yl)-1,2,3,4-tetrahydroquinolin-4-yl]pyrrolidin-2-one, Beige powder, mp160-162, yield 90%. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.40 (1H, s,); 6.79 (1H,m); 6.61 (1H,ddd, *J* = 9.0; 3.0 and 1.0 Hz); 6.55 (1H,dd, *J* = 8.0 and 4.0 Hz); 6.37 (1H,dd, *J* = 3.0 and 2.0 Hz); 6.27 (1H, d, *J* = 3.0 Hz); 5.66 (1H,dd, J = 11.1 and 7.0 Hz); 4.65 (1H, dd, *J* = 11.0 and 1.0 Hz); 4.04 (1H, br.s, NH); 3.31 – 3.15 (2H, m); 2.57 – 2.44 (2H, m); 2.27 – 2.17 (2H, m); 2.09 – 2.02 (2H, m). MS *m/z* (EI): 300.32 (M^+).

THQs **1-7** were characterized by ^{1}H-NMR, ^{13}C-NMR and Mass Spectra. ^{1}H-NMR spectra of THQs **1-7** were very similar, and characterized by the presence of three groups of signals showing presence of aromatics protons, protons near heteroatoms and aliphatic protons, which resonated in different zones. The mass spectra of compounds **1-7,** showed similar fragmentation patterns between compounds, always showing the molecular ion $[M+H]^{+}$and the characteristic loss of a fragment of 85 units corresponding to the ring from N-Vinylpyrrolidin-2-one.

All compounds were evaluated as potential AChE inhibitors; compounds **1**, **2**, and **5-7** were not actives against AChE with values of IC_{50} over 1000 µM. The compound with the highest activity was **3** with IC_{50} of 805± 3.2 µM and **4**, with IC_{50} of 618± 4.7µM, the ppor activity showed for the compounds is not comparable with galanthamine used as reference (IC_{50}=0.54±0.07µM).

Conclusions

Our results suggest that synthetic heterocyclic tetrahydroquinolines obtained using the Povarov reaction, may possess anticholinesterasic effects. The analysis of results suggests new modifications in the patters of substitution for increased activity. These findings encourage us to continue the efforts towards the optimization of the pharmacological profile of these structural types as important scaffolds in the neurodegenerative diseases.

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References

1. Madapa S, Tusi Z, Batra S. *Curr Org Chem* **2008**; 12: 1116-1183.

2. Mistry B, Jauhari S. *Arch. Appl. Sci. Res* **2010**; 2: 322-343

3.Hiessbock R, Wolf C, Richter E, et al. *J. Med. Chem.* **1999**; 42: 1921-1926.

4. Su D-S, Lim J, Tinney E, et al, *Bioorg Med ChemLett.* **2009**;19:5119–5123.

5.Ghorab M, Ragab F, Hamed M. *Eur J Med Chem* **2009**; 44: 4211–4217.

6. Bulbule V, Rivas K, Verlindes C, et al, *J. Med. Chem.* **2008**; 51: 384–387.

7. Rano T, Sieber-Mc Master E, et al, *Bioorg Med Chem Lett* .**2009**; 19: 2456–2460.

8. Kim H, Gim H, Yang M, Ryu J-H, Jeon R. *Heterocycles* **2007**; 71: 2131-2154.

9. Francis P, Palmer A, et al, *J Neurol Neurosurg Psychiatry* **1999**;66:137–147.

10. Danysz W, Parsons C. *Int J Geriatr Psychiatry* **2003**; 18: S23–S32

11. Catto M, Pisani L, Leonetti F, et al, *Bioorg Med Chem* **2013**; 21: 146–152

12.Isomae K, Ishikawa M, Ohta M, et al, *Jpn. J. Pharmacol.* **2002**; 88: 206 – 212.

13.Marco JL, Carreiras MC. *Mini Rev. Chem.* **2003**; 3: 518-524.

14. Szymański P, Laznickova A, et al, *Int. J. Mol. Sci.* **2012**;13: 10067-10090.

15.Gatta F, Del Giudice M, Pomponi M, Marta M. *Heterocycles* **1992**; 34: 991-1004.

16. Fink D, Bores G, Effland R, Huger F, et al, *J. Med. Chem.* **1995**; 38: 3645-3651.

17. Maalej E, Chabchoub F, et al. *Eur J of Med Chem* **2012**; 54: 750-763.

18. Gutiérrez M. Carmona U. Vallejos G. et al, *Z. naturforsch* 67c **2012**; 551-556.

19. Ellman GL, Courtney DK, Andreas V, et al, *Biochem.Pharmacol.***1961**; 7: 88–95.

20. Gutierrez M, Theoduloz C, et al, *J. Agric. Food Chem*. **2005**, 53, 7701-7708.