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Research Article

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Tetrahydroquinolines and Isoxazoles: Nitrogen heterocycles as potential antibacterial agents

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ABSTRACT

Nitrogen heterocycles are a part of a special group of organic substances found in many biologically active natural and synthetic products with properties pharmacologically relevant. The synthesis and spectroscopic characterization of tetrahydroquinolines (THQs) and isoxazole are shown. These compounds were evaluated for their in vitro antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Acinetobacter baumannii. With the purpose of evaluate the structural features in the antibacterial activity, in the present work, some substituents such as: methyl, methoxy and hydrogen in different positions on the THQ ring are reported.

Keywords: Isoxazoles; tetrahydroquinolines; antibacterial activity.

INTRODUCTION

The nature has been responsible for bring us countless chemical molecules nitrogenous structurally and biologically relevant. Among these heterocycles the isoxazoles and THQs[1] have shown significant biological activity on different therapeutic targets. They show several applications in diverse areas such as pharmaceuticals [2, 3], agro chemistry, and industry [4]. They are also found in natural sources showing insecticidal, plant growth regulation, pigment functions [5], and with antibacterial properties [6].

Within the field of activities of the heterocycles is found the antibacterial activity, which has had a growing interest because the inadequate use of antibiotic has increased the resistance of bacterial to the commercial antibiotics, even the appearance of bacterial strain with no treatment knows[7]. Similarly, the emergence of antibiotic resistance in some bacterial populations is yet a relevant field of study in some areas of science included the organic chemistry. Hence there create a need to generate new and potent antibacterial compounds to help with this problem.

Because of their importance as substructures in a broad range of natural and synthetic products, significant efforts continue to be directed into the development of new structure quinoline and isoxazole with biological perspectives.

Due to the mentioned previously, the THQs and isoxazoles compounds have been considered attractive molecules as good starting material for the search of novel antimicrobial agents; therefore we include them in this research.

EXPERIMENTAL SECTION

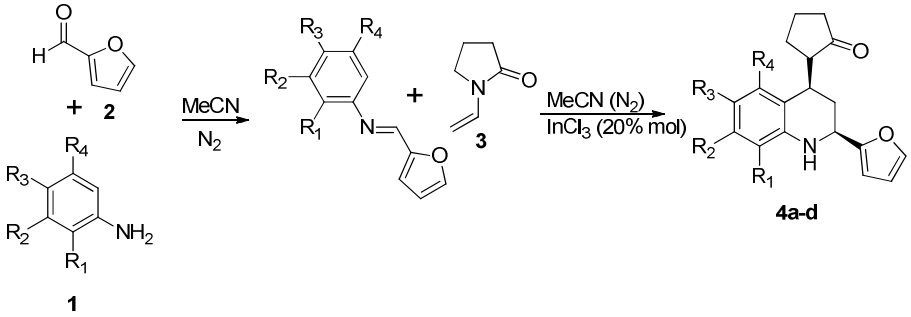
Chemistry

All solvents used were of analytical grade. Melting points were determined by open capillary methods on a Bucchi apparatus and are uncorrected. Infrared (IR) spectra, KBrpellets 500-4000 cm^{-1} were recorded on a Thermo Nicolet

NEXUS 670 FT-IR spectrophotometer with a 0.125 cm^{-1} spectral resolution. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer (400 MHz), using $CDCl_3$ as solvent. TMS was used as an internal standard. Chemical shifts (δ) and *J* values are reported in ppm and Hz, respectively relative to the solvent peak ($CHCl_3$ in $CDCl_3$ at 7.27 ppm for protons and 77.00 ppm from carbon). Signals are designated as follow: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; br.s, broad singlet. ESI-MS(/MS) data were collected using a high resolution mass spectrometer (Q-TOF, Micromass UK) with constant nebulizer temperature of 100 ºC. The ESI source and the mass spectrometer 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 ca 5 µL/min via a microsyringe pump. ESI-MS/MS experiments were carried out by selection of a specific ion in Q1 and by performing its collision-induced dissociation (CID) with argon in the collision chamber. The values expressed are average mass and correspond to the $[M+H]^+$ ion. The collision energy ranged from 10-25 eV, depending on the stability of the precursor ion undergoing collision-induced dissociation.

Synthetic Methodologies

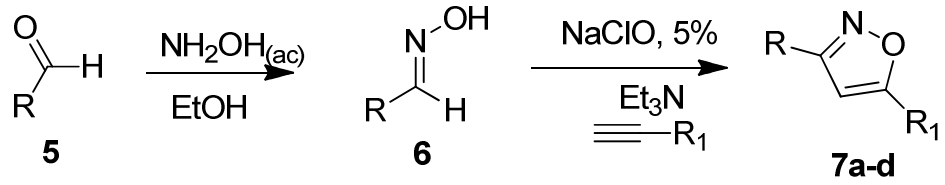
We report here the synthesis of 4 THQs**4a-d** and 4 isoxazoles**7a-d**. The THQs were prepared by imino Diels-Alder cycloaddition [8], between different substituted anilines **1** selected by their ability to donate electrons and facilitate the formation of the imines, aromatic aldehyde **2** (2-furaldehyde), and N-vinylpyrrolidin-2- one **3** as alkene, it last was used as electron-rich alkene because it was an available, stable and cheap reagent, also we used acetonitrile as dissolvent in the presence of 20 mol% of Indium trichloride (III) as catalyst. These coupling reactions were performed under mild conditions (room temperature, 24 h). The synthetic approach adopted to obtain the target compounds is depicted in Scheme 1.



Scheme 1. Synthesis of THQs, through the Diels-Alder cycloaddition promoted by $InCl_3$ and MeCN, rt

Reaction progress was monitored by means of TLC using Merck Silica gel 60. All reagent were purchase from commercial suppliers and used without further purification. Final purification of all products for analysis was carried out by recrystallisation. Acetonitrile was distilled from calcium hydride and dried over 4 A molecular sieves.

Isoxazoles derivatives were synthesized by 1,3 dipolar cycloaddition[9] using two steps: First we prepared the oxime derivatives through Hydroxylamine (NH_2OH 50%) and one Aldehyde **5** in ethyl alcohol at room temperature with subsequent extraction with dichlorometane. Second we proceeded to the preparation of 3,5-disubstituted isoxazoles**7a-d** using the Oxime**6** in dichloromethane a 0°C which was added dropwise to a mixture of alkyne, triethylamine and 5% aqueous sodium hypochlorite, the reaction mixture was stirred 60 min in iced water and extracted with dichloromethane. The solid obtained was suspended in hot ethyl alcohol and crystallized obtained the isoxazole (Scheme 2).



Scheme 2. Synthesis of 3,5-disubstituted isoxazoles by 1,3 dipolar cycloaddition

Biological activity

Microorganisms: Collection of Clinical isolated:

Bacterial cultures used in the present study were clinical isolates obtained from samples(Samples used: Blood, Pus, Catheter, Sputum, Urine; etc)of infectious patients of Talca hospital, Chile. The cultures comprise of 3 Gram negative bacterial isolates namely *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, *a*nd one Gram positive bacterial isolates, namely *Staphylococcus aureus.*

Maintenance of bacterial cultures
All the bacterial isolates were cultured and maintained in LB (Luria Bertain) medium (1% tryptone, 1% sodium chloride, 0.5% yeast extract) during all the experiments of the study until mentioned. The bacterial cultures were refreshed fortnightly.

Antibiotic used:
The antibiotics used in this investigation were: Penicillin G and Streptomycin. The control strains were run simultaneously with the test organisms.

Preservation of Isolates:
Glycerol stocks were prepared and stored at -80^{o}C for long term preservation. Pure cultures strains were incubated at 37^{o}C for 48 h in isolation broth. Then 0.5 mL of each of the cultures was transferred into cryotubes and 0.5 ml broth containing 40% glycerol was added. The samples were mixed gently and stored at -80^{o}C.

Antibacterial Activity assay: Antibacterial activity was assessed by spectrophotometric measurements, determining the MIC for each compound against the four bacterial, *E. coli*, *P. aeruginosa*, *S. aureus* and *A. baumannii*, using the doubling dilution method in 96-well microtiter plates reported by Gutierrez *et al*, 2005[10] . Bacterial suspensions were obtained from overnight cultures, incubated at 25 °C, in Luria Broth Base nutrient broth (Gibco BRL, Scotland). The starting cultures were diluted to approximately 10^5 colony-forming units (CFU)/well in fresh medium. The organic extracts were dissolved up to 5 mg/mL in MeOH, as stock solution. Stock solutions of the extracts were diluted to give serial 2-fold dilutions, added to each well, giving concentrations ranging from 1.25 to 0.078 mg/mL. The final concentration of MeOH in the assays did not exceed 2%. Plates were kept at 25 °C overnight (12 h). After incubation, 20 µL of 0.5 mg/mL aqueous 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Co., St. Louis, MO) was added in each well and re-incubated for 30 min to detect living bacteria. The change of color indicated that the bacteria were biologically active. The MIC was taken to the well, where no change of colour of MTT was observed. The MIC values were done in triplicate. Penicillin G (Sigma-Aldrich, St. Louis, MO) and streptomycin (Laboratorio Chile, Santiago, Chile) were used as standard antibacterial.

RESULTS AND DISCUSSION

The isoxazoles and THQs were obtained with yield between 78-90% (table 1-2). All THQs were purified by SiO_2 column chromatography and were obtained as solids with *cis*-diastereoisomers; the configuration of the substituents was determined by ^{1}H NMR spectra and assigned on the basis of coupling constants.

Tetrahydroquinolines were characterized by ^{1}H-NMR, ^{13}C-NMR spectra and Mass Spectra. ^{1}H-NMR spectra of THQs **4a-d** were similar, these showed three groups of signals: aromatics protons, protons near heteroatoms and aliphatic protons, with different displacements. Also, these THQs were compared with previous reports[11-13] for analogous THQs.

The structures of the C-2 substituted THQs were confirmed on the basis of analytical and spectral data and were supported by inverse-detected 2D NMR experiments. The ^{1}H NMR spectrum of this compound presented the 4-H proton signal at 5.70 ppm, observed as a double doublet with the coupling constants 4.0 Hz and 11.0 Hz. This fact suggested *axial-axial* and *axial-equatorial* interactions between 4-H and 3-H protons. On the other hand, the 2-H proton signal was observed at 4.60 ppm with the coupling constants 1.0 Hz and 11.0 Hz that indicated at vicinal *axial-axial* and *axial-equatorial* interactions. The high value of the coupling constant (11.0 Hz) of the 4-H and 2-H protons confirmed the *axial* proton configurations; therefore, substituents of the C-2 and C-4 positions of THQ ring have the equatorial disposition, respectively. The spectroscopic dates of compounds **4a-d** are provided below [14].

The mass spectra showed similar fragmentation patterns between compounds, showing characteristic loss of a fragment of 85 units corresponding to the ring from N-Vinylpyrrolidin-2-one.

N-vinylpyrrolidin-2-one was used as electron-rich alkene. Amines used were selected from their ability to donate electrons and facilitate the formation of the imines.The chosen catalyst Indium trichloride ($InCl_3$) has emerged as a mild and water-tolerant Lewis acid imparting high regio- and chemoselectivity in various organic transformations. The catalyst can be conveniently used inaqueous and non-aqueous media and can also be recovered from the aqueous layer on work-up and recycled for use in subsequent reactions. The catalyst $InCl_3$ has been reported as highly efficient in activating nitrogen-containing compounds such as imines and hydrazones, especially in acetonitrile as solvent [15]. In general the overall yields ranged from 85-90%.

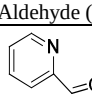
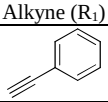
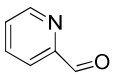
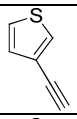
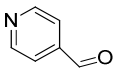
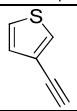
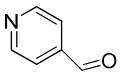
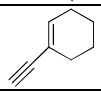
Table 1. Substituent's of THQs synthesized.

Product	R^1	R^2	R^3	R^4	Yield (%)
4a	H	H	CH_3	H	85
4b	H	CH_3	H	CH_3	87
4c	H	H	OCH_3	H	87
4d	CH_3	H	CH_3	H	90

All isoxazoles were purified by recrystallization process with good yield and few byproducts. Isoxazoles**7a-d** was characterized by ^{1}H-NMR, ^{13}C-NMR and Mass Spectra. ^{1}H-NMR spectra of all isoxazoles synthesized were very similar, and characterized by signals for aromatics protons, protons near heteroatoms and aliphatic protons with different shift, but in all ^{1}H-NMR spectra showed the presence of characteristic isoxazole proton as a singlet with a shift near seven. Their IR spectra exhibited a band corresponding to –C–O–N (1230 cm–1), which confirmed the presence of the isoxazole ring. The appearance of a peak due to C=N and disappearance of the peak due to C=O in the ^{13}C-NMR spectra further supported the formation of isoxazole.

We have developed a method for formation of isoxazolic compounds with good bond-forming efficiency and high yields. The use of hypochlorite showed good yields, crystal structures, shortening reaction times and decrease in reagents cost. Easy work-up, atom economy, convenience, and the formation of no by-products is other attractive feature of this method. We anticipate that the building blocks rendered, readily accessible by the chemistry, will prove useful for the selective synthesis of more complex targets.

Table 2.Start products for synthesis of Isoxazoles

Product	Aldehyde (R)	Alkyne (R_1)	Yield (%)
7a			87
7b			78
7c			83
7d			78

Biological results

The data from Antibacterial activity of synthetic compounds are expressed as MICs, but all compounds had MIC upper 500 μg/mL and weren't comparable with standard antibacterial. Low activity exhibited by these compounds may be due to its mechanism of action against microorganism may be using another therapeutic target, so it is necessary to evaluate these compounds on the same microorganisms but through another trial to provide different information.

Chemical data

Compound **4a**: 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 **4b**: 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 **4c**: 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 **4d**: 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^+).

2-(5-Phenyl-isoxazol-3-yl)-pyridine**7a**: $C_{14}H_{10}N_2O$; Yield 87%. Mp80-82ºC.^{1}H NMR δppm ($CDCl_3$-400 MHz): 8.73 (1H, ddd, *J* = 8.0; 4.8; 1.2 Hz); 8.15(1H, d, *J* = 8.0); 7.87 (2H, dd, *J* = 8.0; 1.6 Hz), 7.84 (1H, dt, *J* = 7.6, 1.6), 7.49 (3H, m), 7.38 (1H, ddd, *J* = 7.5, 5.2, 1.2 Hz), 7.20 (1H, s). ^{13}C 170.64; 163.756; 149.71; 148.55; 136.91; 130.24; 129.02; 127.42; 125.84; 124.52; 121.66; 98.31. MS *m/z* (EI): 222.98 (M^++1). IR (KBr) cm-1: 3413; 1649; 1456; 1230.

2-(5-Thiophen-3-yl-isoxazol-3-yl)-pyridine**7b**:$C_{12}H_8N_2OS$; Yield 78%. Mp119ºC. 1H NMR δppm ($CDCl_3$-400 MHz): 8.72 (1H, ddd, *J* = 8.0, 4.8, 1.2 Hz); 8.13 (1H, d, *J* = 8.0),7.83 (2H, m), 7.47 (1H, t, *J* = 6.0 Hz), 7.45 (1H, dd, *J* = 5.0, 3.2 Hz), 7.37 (1H, ddd, *J* =7.5, 4.8, 1.2 Hz),7.06 (1H, s). MS *m/z* (EI): 228.93 (M^++1). IR (KBr) cm-1 3442; 2102, 1632, 1227.

4-(5-Thiophen-3-yl-isoxazol-3-yl)-pyridine**7c**:$C_{12}H_8N_2OS$; Yield 83%. Mp162-163ºC.^{1}H NMR δppm ($CDCl_3$-400 MHz): 8.76 (2H, d, *J* = 6.0 Hz), 7.88 (1H, s), 7.74 (2H, d, *J* = 6.0 Hz), 7.48 (2H, m), 6.75(1H, s). MS *m/z* (EI): 228.93 (M^++1). IR (KBr) cm-1: 3438; 2075; 1637; 1232; 950.

4-(5-Cyclohex-1-enyl-isoxazol-3-yl)-pyridine **7d**: $C_{14}H_{14}N_2O$; 14 Yield 78%. Oil.^{1}H NMR δppm ($CDCl_3$-400 MHz): 8.41 (2H, d, *J* = 8.0 Hz), 7.92 (2H, d, *J* = 8.0 Hz), 6.31 (1H, brs), 6.25 (1H, s), 2.02 (4H, m), 1.39 (4H, m).MS *m/z* (EI): 227.054 (M++1). IR (KBr) cm-1: 3420; 2130; 1649; 1220; 832.

Spectroscopic data of compounds**7a-7d**; has been previously reported [16]; and evaluated as acetylcholinesterase inhibitors.

CONCLUSION

The study reports the facile synthesis of THQs and isoxazoles using a Povarov reaction and 1,3 dipolar cycloaddition, respectively. The methodology reported is clean, simple and efficient giving good yields of the reported compounds. Our results suggest that this kind of structures with these substituents don't have good activity against these bacteria but this don't mean that the same structures have good activity against other kind of microorganisms. Further investigation with appropriate structural modification of the above compounds may result in therapeutically useful products.

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