Nitrogen heterocycles as potential antibacterial agents

[1]Yorley Duarte, [1]Bárbara Arévalo, [1]Gonzalo Martínez, [1]Francisca Matus, [1]Tomas Poblete, [1]Margarita Gutiérrez, [1]Jessica Amigo, [1]Luis Astudillo

Laboratorio Síntesis Orgánica, Instituto de Química de RecursosNaturales, Universidad de Talca, Casilla 747, Talca 3460000, Chile; E-Mails: yduarte@utalca.cl; mgutierrez@utalca.cl.

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 of a new series of tetrahydroquinolines (THQs) and isoxazole is shown. These compounds were screened for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginos*a, *Staphylococcus aureus* and *Acinetobacter baumannii* by spectrophotometric measurements.

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 tetrahydroquinolines (THQs)[1] have shown significant biological activity on different therapeutic targets. They show several applications in diverse areas such as pharmaceuticals,[2] agrochemistry, and industry.[3] They are also found in natural sources showing insecticidal, plant growth regulation, pigment functions,[4] and with antibacterial propierties.[5]

Within the field of activity of the heterocycles is found the antibacterial activity, which has had a growing interest because the in appropriated use of antibiotic has increased the resistance of bacterial to the commercial antibiotics, even the appearance of bacterial strain with no treatment knows.[6] 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.

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.

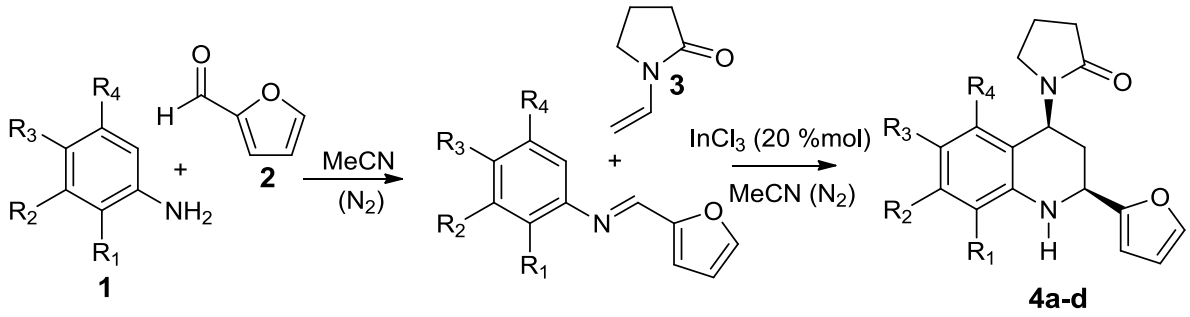
Due to mentioned previously, the THQs e isoxazoles compounds have been considered attractive as good starting material for the search of novel antimicrobial agents, therefore we include them in this research.

MATERIALS AND METHODS

Chemistry

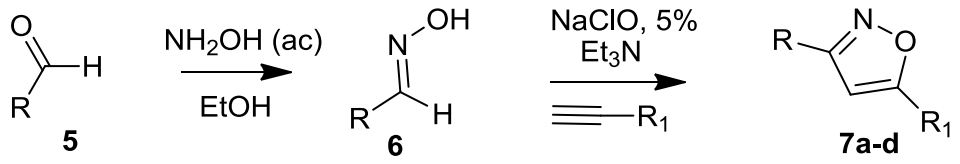
We report here the synthesis of a new series of 4 tetrahydroquinolines **4a-d** and 4 isoxazoles **7a-d**. The THQs were prepared by imino Diels-Alder cycloaddition[7] between

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 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 novel series of bis-THQs, through the Diels-Alder cycloaddition promoted by $InCl_3$ and MeCN, rt.

Isoxazoles derivatives were synthesized by 1,3 dipolar cycloaddition[8] 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

Antibacterial activity was assessed by spectrophotometric measurements, determining the MIC for each compound against the four bacterial, *Escherichia coli*, *Pseudomonas aeruginos*a, *Staphylococcus aureus* and *Acinetobacter baumannii,* This activity was did through the next methodology.

Bacterial suspensions were obtained from overnight cultures in Luria Broth Base nutrient broth (Gibco BRL, Scotland) cultured at 25 °C and diluted to approximately 10^5 colony-forming units (CFU)/well in fresh medium. The compounds were dissolved to give 1 mg/mL in DMSO as a stock solution. Stock solutions of compounds were diluted to give serial 2-fold dilutions that were added to each medium resulting in concentrations ranging from 250 to 1.92 μg/mL. The final concentration of DMSO in the assays did not exceed 2%. The 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 absorbance was read in a universal microplate reader (Bio-Tek Instruments, Inc., VT) at 405 nm.[9] The results were transformed to the percentage of controls, and the median inhibitory concentration (IC_{50}) values were graphically obtained from the dose-response curves. Comercial antibiotics were used as standard antibacterials.

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 very 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[10-12] for analogous THQs. 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.

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

Table 1. Substituent's of THQs synthesized.

All isoxazoles were purified by recristalization process with good yield and few byproducts. Isoxazoles **7a-d** were 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.

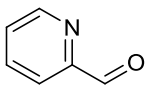
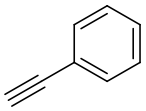
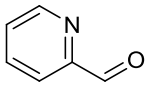
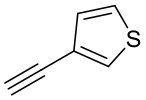
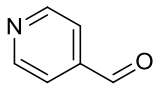
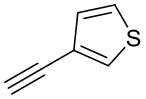
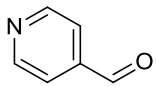
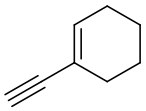
Compound	Aldehyde (R)	Alkyne (R_1)	Yield (%)
7a			87
7b			78
7c			83
7d			78

Table 2. Start products for synthesis of Isoxazoles.

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 antibacterials.

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** : 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)..MS *m/z* (EI): 222.98 (M^++1). IR (KBr) cm^{-1}: 3413; 1649;1456.

2-(5-Thiophen-3-yl-isoxazol-3-yl)pyridine **7b**: Yield 78%. Mp119ºC. ^{1}H 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).

4-(5-Thiophen-3-yl-isoxazol-3-yl)pyridine **7c**: 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).

4-(5-Cyclohex-1-enyl-isoxazol-3-yl)-pyridine **7d**: 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).

CONCLUSIONS

Our results suggest that this kind of structures with these substituents don't have good activity against this bacteria but this don't mean that the same structures have good activity against other kind of microorganisms.

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