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# *In vitro* screening of 1-aryl-6-hydroxy-1,2,3,4-tetrahydroisoquinolines: structure related activity against pathogenic bacteria

Moses Njutain Ngemenya[1*], Joelle Ngo Hanna[2], Julios Armand Komtchou[1], Simon Mbua Ngale Efange[2]

[1]*Biotechnology Unit, Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, P.O. Box 63 Buea, South West Region, Cameroon*

[2]*Department of Chemistry, Faculty of Science, University of Buea, P.O. Box 63 Buea, South West Region, Cameroon*

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ABSTRACT

**Objective:** To evaluate the antibacterial activity of ten synthetic tetrahydroisoquinolines against eight bacterial strains.
**Methods:** The ten tetrahydroisoquinolines synthesized via base-catalyzed Pictet-Spengler cyclization were screened against a total of eight bacterial strains comprising control and pathogenic strains by the disc diffusion and micro-dilution methods. The most active compound was then assessed for cytotoxicity on human lymphocytes.
**Results:** Six of the tetrahydroisoquinolines showed broad spectrum bacteriostatic activity. The zones of inhibition produced ranged from 7 to 23 mm for 200 μg per disc. The presence of a lipophilic substituent at the para position of the pendant phenyl group conferred the highest antibacterial activity. Compound 2 [1-(3,4-chlorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline] was the most active and produced zones ranging from 9 to 20 mm against all eight bacterial strains. Compound 2 also showed the lowest minimum inhibitory concentration of 100 μg/mL against *Escherichia coli* ATCC11775 and the lowest minimum bactericidal concentration of 800 μg/mL against pathogenic *Salmonella typhimurium*. Overall, compound 2 was the most active with bacteriostatic and bactericidal activity against three and four bacterial strains respectively. A 50% cytotoxic concentration of 98.2 μg/mL was recorded for compound 2 indicating a low risk of toxicity.
**Conclusions:** The 1-aryl-1,2,3,4-tetrahydroisoquinolines display structure-related antibacterial activity and further chemical exploration of the tetrahydroisoquinoline scaffold may yield more potent non-toxic derivatives for development into new antibacterials.

## 1. Introduction

Bacterial infections feature among the leading causes of death worldwide and contribute significantly to morbidity, disability and mortality[1]. Antibiotic chemotherapy is presently facing two major challenges. Firstly, there is evidence of resistance to almost all classes of antibiotics[2] and secondly, there is a great paucity of new antibacterials approved for clinical use with only three new classes of antibiotics approved for human use in the last fifty years[3,4]. A number of surveys of antibiotic chemotherapy have revealed increasing resistance in several pathogenic bacteria, hence the discovery of new efficacious antibiotics is urgent[5-7]. Several strategies are presently being exploited in the discovery and development of new antibacterials which involve chemical synthesis of novel compounds for antibacterial screening. One such approach is the modification or redesign of new antibacterials from existing antibiotics[8] and another is the synthesis of new antibiotics based on genomics, combinatorial chemistry, high-throughput screening and rational drug design[4]. This study focused on the biological screening of a small library of ten recently described tetrahydroisoquinolines (THIQs)[9].

THIQs, also called 1,2,3,4–THIQs are a class of partially aromatic alkaloids. THIQs abound in nature and have attracted significant attention due to their diverse biological activities. The THIQ skeleton is a structure frequently used in drugs interacting with a number of

*Corresponding author: Moses Njutain Ngemenya, Biotechnology Unit and Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, P.O. Box 63 Buea, South West Region, Cameroon.
Tel: (237) 677 87 53 27
Fax: (237) 233 32 22 72
E-mail: mnngemenya@yahoo.com
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biological systems[10]. The THIQ substructure (Figure 1) is found in many drugs and alkaloids that exhibit antitumor, cardiovascular and wide ranging antimicrobial activity including antibacterial activity[11-14]. These compounds include the naphthylisoquinolines, benzylisoquinolines and bisbenzylisoquinolines. The broad spectrum of biological activities of THIQs prompted us to investigate 1-aryl-1,2,3,4-THIQs for antibacterial activity.

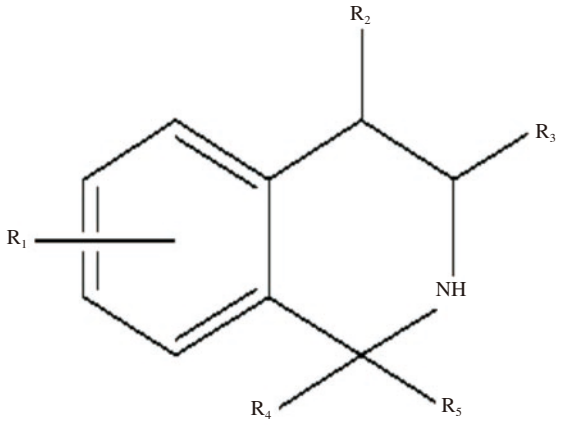


**Figure 1.** Substructure of THIQs.

## 2. Materials and methods

### 2.1. Synthesis of THIQs

The synthesis of the target compounds shown in Figure 2 has been described in detail[9]. The corresponding hydrochlorides were subsequently tested *in vitro* for antibacterial activity.

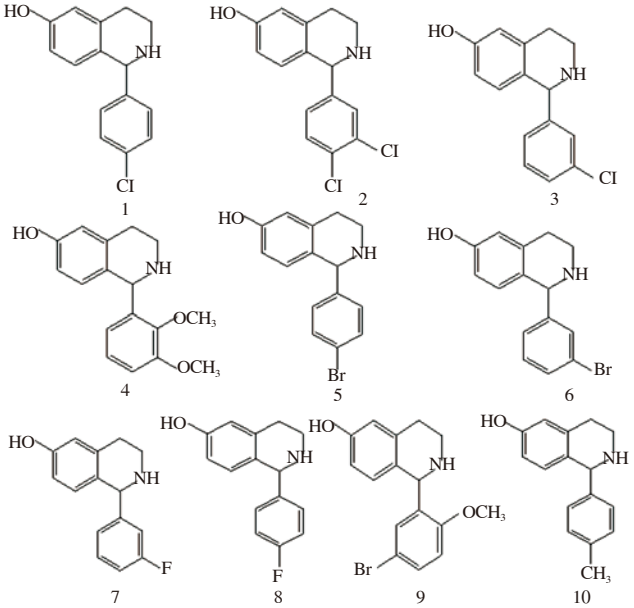


**Figure 2.** Structures of ten THIQs synthesized by base-catalyzed Pictet-Spengler cyclization.

### 2.2. Sources and culture of test bacteria

Five pathogenic strains of bacteria were isolated from clinical specimens in two health facilities: the South West Regional Hospital Annex and the Solidarity Clinic, Molyko, both found in the town of Buea, in the South West Region of Cameroon. The strains were isolated by culturing in appropriate selective media and identified using cultural features and API 20E biochemical test kit (Biomérieux, France). The strains include *Escherichia coli* (*E. coli*), *Klebsiella oxytoca* (*K. oxytoca*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhimurium* (*S. typhimurium*), *Sarratia odorifera* (*S. odorifera*), and *Staphylococcus aureus* (*S. aureus*). Three control strains *E. coli* (ATCC 11775), *P. aeruginosa* (Boston 41501) and *S. aureus* (ATCC 33862) were obtained from American Type Culture Collection (Manassas, USA). The organisms were cultured on Muller Hinton agar (Liofilchem, Italy) in culture plates prepared according to the manufacturer's instructions, and the cultures stored at 4 ℃ and sub-cultured every 48 h during the study period.

### 2.3. Antibacterial screen by disc diffusion

This was performed as described by Mbah *et al*.[15] with some modifications mentioned below. Briefly, sterile discs (5-6 mm diameter) were prepared from Whatman filter paper. A solution of each THIQ was prepared to give 50 μg/10 μL in methanol. Discs containing test substance in the range 50 to 200 μg per disc, gentamicin (1 μg per disc) as positive control and negative control (using methanol) were prepared and the solvent dried-off. The Kirby-Bauer (spread plate) method[16] was used to assess the antibacterial activity of the THIQs. The discs were gently fixed on a uniform spread of bacterial suspension (approximately $1.5 \times 10^8$ CFUs/mL in 0.85% saline equivalent to 0.5 McFarland and incubated for 18 to 24 h at 37 ℃ and the zones of inhibition measured in millimetres. This experiment was conducted twice for each THIQ.

### 2.4. Antibacterial screen by micro-dilution

A 4 mg/mL stock solution of each compound was prepared in dimethyl sulfoxide. The minimum inhibitory concentration (MIC) was determined in duplicate wells in a microtitre plate as described by Mbah *et al*.[15], whereby the stock solution of the pure compound was serially diluted in peptone water sugar medium in tests wells to give final concentration of 50 to 1 000 μg/mL and bacterial suspension added at a final density of approximately $6 \times 10^5$ CFUs/mL in 0.85% sterile saline. Positive (50 μg/mL gentamicin) and negative (bacterial cells without drug) control wells were included and the plate incubated at 37 ℃ for 24 h (Heraeus, Germany). The lowest concentration well without bacterial growth (no colour change of bromothymol blue indicator from blue to yellow) was recorded as the MIC. The minimum bactericidal concentration (MBC) was determined by re-incubation of a 1:10 dilution of the contents of the MIC wells with no bacterial growth. The content of each well was mixed and 100 μL transferred into a sterile Eppendorf (1.5 mL) tube, 900 μL of the peptone medium added and mixed and the tube capped tightly and incubated same as above. The lowest concentration without bacterial growth as above was recorded as the MBC. This experiment was conducted twice.

### 2.5. Cytotoxicity test

The cytotoxicity was performed for compound 2, *i.e.* 1-(3, 4-chlorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline, the most active THIQ against the eight bacterial strains in the disc and micro-dilution experiments on human lymphocytes. The test was conducted following reported methods with modifications[17-19]. Ten millilitres of venous blood was collected from a human volunteer donor by a phlebotomist in a local blood bank into a heparinised tube; and then diluted 1:1 in Roswell Park Memorial Institute (RPMI) 1640 medium (SIGMA, containing 2.5 μg/mL gentamicin 1% Albumax II, 25 mmol/L HEPES buffer, 25 mmol/L $NaHCO_3$) and mixed by gentle swirling[17]. The peripheral blood mononuclear cells were then separated by density gradient centrifugation on Ficoll at 3 900 r/min for 10 min. The layer of peripheral blood mononuclear cells was carefully aspirated into a sterile tube[18]. RPMI medium was added and the cells washed twice by centrifugation at 1 800 r/min for 10 min. The cell pellet was resuspended in 1 mL RPMI medium, thoroughly mixed, then 10 μL diluted with 10 μL of 0.4% trypan blue and counted by light microscopy using a haemacytometer at × 400 magnification[18]. Finally, it was diluted in culture medium to one million cells/mL. A 4 mg/mL stock solution of compound 2 was diluted serially in RPMI medium in duplicate wells in a 96–well microtitre plate giving 100 μL of test solution per well over a range of 1.9 to 2 000 μg/mL. Positive control wells containing 100 μL 0.5 mol/L sodium azide and negative control wells of medium only were included. The cell suspension was mixed and 100 μL (100 000 cells) added into all required wells and the plate loosely covered with aluminium foil to reduce evaporation from the medium and incubated at 37 ℃ in 5 % $CO_2$ for six days in a HERA cell 150 incubator (Thermo electron, Germany). Then the content of each well was mixed and 20 μL diluted 1:1 in trypan blue, and the dead (blue-stained) and living (unstained) cells counted[19].

### 2.6. Data analysis

Most of the zones of inhibition produced by the test substances comprised two portions; an inner clear portion with no visible CFUs and an outer unclear portion with scanty visible CFUs. These two portions were measured for each zone and the total zone equal to the sum was recorded. The average reading for the two experiments were calculated and rounded up to the nearest whole number. The zones of inhibition of each compound was interpreted by making a relative comparison of the total zone with the zones of inhibition of standard antibiotics published by the Clinical and Laboratory Standards Institute[20]. Based on this criterion the antibacterial effect (diameter of zone of inhibition) of each THIQ was then categorised as sensitive, of intermediate sensitivity or insensitive with respect to the bacterial species. Furthermore compounds which were active on more than two bacterial species were classified as having broad spectrum activity. For the cytotoxicity test, the concentration which is cytotoxic to 50% of the cells ($CC_{50}$) was interpolated from a plot of concentration versus percentage cell death; a $CC_{50}$ value > 30 μg/mL was taken as the cut point for lack of cytotoxicity[21].

## 3. Results

### 3.1. Antibacterial activity of the THIQs

Of the ten THIQs tested, the highest total diameter of zone of inhibition observed was 23 mm produced by compound 5, 1-(4-Bromophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline, against *S. aureus* and *S. odorifera*. The highest zone diameter produced by the positive control, gentamicin, was a clear zone of 23 mm against *S. typhimurium* and *S. odorifera* (Table 1). The total zones of inhibition produced by the THIQs ranged from 7 to 23 mm with the highest clear portion of 11 mm produced against *S. aureus* by compounds 1 and 5. Nine of the ten synthetic compounds produced a zone of inhibition on at least one organism. Compound 2, 1-(3,4-chlorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline, was the only compound which produced a zone of inhibition against all eight bacterial strains with the total zone ranging from 9 to 20 mm; and was also the only compound that was active against *P. aeruginosa* (Boston 41501). Four THIQs (1, 2, 5 and 10) produced a zone against more than two species. Compound 3, 1-(3-Chorophenyl)–6-hydroxy-1, 2, 3, 4-tetrahydroisoquinoline, had no effect on any of the organisms as neither a clear nor unclear zone was produced.

**Table 1**

Antibacterial activity of ten THIQs by disc diffusion method.

| Bacterial strains | Diameter of zone of inhibition (mm) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Gentamicin |
| EC * | 8 (13) | 10 (18) | _ | _ | 10 (16) | _ | _ | _ | _ | 8 (15) | 14 |
| EC | 8 (19) | 10 (14) | _ | _ | 9 (19) | _ | _ | _ | _ | 0 (16) | 11 |
| SA * | 11 (16) | 0 (9) | _ | _ | 10 (22) | 0 (8) | _ | _ | _ | 0 (11) | 21 |
| SA | 11 (19) | 0 (11) | _ | _ | 11 (23) | 0 (7) | _ | _ | _ | _ | 22 |
| ST | 8 (17) | 10 (14) | _ | 0 (7) | 9 (18) | _ | 0 (8) | 0 (9) | 0 (7) | 9 (15) | 23 |
| KO | 9 (21) | 10 (20) | _ | _ | 9 (19) | _ | _ | 0 (10) | _ | 10 (14) | 20 |
| PA* | _ | 0 (6) | _ | _ | _ | _ | _ | _ | _ | _ | 20 |
| SO | 7 (14) | 10 (18) | _ | _ | 8 (23) | _ | _ | _ | _ | 7 (12) | 23 |

*: Control strains; EC: *E. coli*; SA: *S. aureus*; ST: *S. typhimurium*; KO: *K. oxytoca*; PA: *P. aeruginosa*; SO: *S. odorifera*; EC*: *E. coli* (ATCC 11775); SA*: *S. aureus* (ATCC 33862); PA*: *P. aeruginosa* (Boston 41501); -: No zone of Inhibition observed; ND: Not done; Zones are average of two experiments at 200 μg of test substance per disc, values in parentheses are unclear zones with scanty colony forming units.

Nine of the ten compounds showed a MIC within the range tested (50 to 1000 μg/mL) with the MIC values ranging from 100 to 1000 μg/mL (Table 2). The lowest MIC value was 100 μg/mL produced by compound 2 on the control (ATCC 11775) and pathogenic strains of *E. coli* respectively. Only compound 4, 1-(2, 3-dimethoxyphenyl)-6-hydroxy-1, 2,3,4-tetrahydroyisoquinoline, did not produce a MIC in the range tested. Of the nine compounds tested for MBC, three showed MBC values ranging from 800 to 1 000 μg/mL (Table 3). Again the highest number of MBC values was recorded for compound 2 *i.e.* against four bacterial strains.

Overall, compound 2 demonstrated the most wide ranging activity; it was active against all bacterial strains in the disc test and also showed the lowest MIC values (100 to 400 μg/mL) and the lowest MBC (800 μg/mL) values.

**Table 2**

MICs of ten THIQs (μg/mL).

| Bacterial species | MIC of compounds | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| *E. coli* (11775)* | 400 | 100 | 600 | > 1 000 | 400 | 400 | 1 000 | > 1 000 | 200 | 600 |
| *E. coli* | 400 | 200 | 600 | > 1 000 | 400 | 400 | 1 000 | 800 | 400 | 600 |
| *S. aureus* (33862)* | 1 000 | 400 | 1 000 | > 1 000 | 400 | 600 | > 1 000 | 1 000 | 800 | 1 000 |
| *S. aureus* | 1 000 | 400 | 1 000 | > 1 000 | 600 | 800 | > 1 000 | 1 000 | 800 | 1 000 |
| *S. typhimurium* | 600 | 400 | 800 | > 1 000 | 600 | 800 | > 1 000 | > 1 000 | 800 | 1 000 |
| *K. oxytoca* | 600 | 400 | 600 | > 1 000 | 600 | 600 | > 1 000 | > 1 000 | 800 | 800 |
| *S. odorifera* | 600 | 400 | 600 | > 1 000 | 400 | 600 | > 1 000 | 1 000 | 800 | 800 |

*: Control strains from ATCC, USA; Names and structures of compounds are shown in Figure 2.

**Table 3**

MBCs of ten THIQs (μg/mL).

| Bacterial species | MBC of compounds | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| *E. coli* (11775)* | > 1 000 | 1 000 | > 1 000 | - | > 1 000 | > 1 000 | > 1 000 | - | > 1 000 | > 1 000 |
| *E. coli* | > 1 000 | > 1 000 | > 1 000 | - | > 1 000 | > 1 000 | > 1 000 | > 1 000 | > 1 000 | > 1 000 |
| *S. aureus* (33862)* | > 1 000 | > 1 000 | > 1 000 | - | > 1 000 | > 1 000 | - | > 1 000 | > 1 000 | > 1 000 |
| *S. aureus* | > 1 000 | > 1 000 | > 1 000 | - | > 1 000 | > 1 000 | - | > 1 000 | > 1 000 | > 1 000 |
| *S. typhimurium* | > 1 000 | 800 | > 1 000 | - | > 1 000 | > 1 000 | - | - | > 1 000 | > 1 000 |
| *K. oxytoca* | > 1 000 | 1 000 | > 1 000 | - | > 1 000 | > 1 000 | - | - | > 1 000 | > 1 000 |
| *S. odorifera* | 1 000 | 1 000 | > 1 000 | - | > 1 000 | 800 | - | > 1 000 | > 1 000 | > 1 000 |

*: Control strains from ATCC, USA; -: Not done; Names and structures of compounds are shown in Figure 2.

### *3.2. Cytotoxicity of compound 2*

The concentrations which were cytotoxic to 50% and 100% of the cells *i.e.* $CC_{50}$ and $CC_{100}$ were determined to be 98.2 μg/mL and 500 μg/mL respectively. These values are higher than the cut- off point for absence of cytotoxicity ($CC_{50}$ > 30 μg/mL), however they are also lower than the concentrations at which antibacterial activity was observed which range from 100 to 1 000 μg/mL.

## 4. Discussion

The findings of this work have revealed that the 1-aryl-1,2,3,4- THIQs are potential broad spectrum antibacterials. Based on the diameter of the zone of inhibition, four compounds (1, 2, 5 and 10) produced clear zones ranging from 7 to 11 mm with no colony visible to the naked eye and a further zone with scanty colonies ranging up to 23 mm similar to the standard antibiotic gentamicin used as positive control in this study. Also, nine of the ten compounds were active on at least one bacterial species. These findings demonstrate the potential efficacy of the THIQs against pathogenic bacterial strains. On the basis of the disc diffusion test four of the ten THIQs demonstrated antibacterial activity with three of them demonstrating broad spectrum activity.

Based on the micro-dilution assay, compound 2 was the most active with the lowest MIC ranging from 100 to 400 μg/mL against all the eight bacterial strains tested, followed by compound 5 (400 to 600 μg/mL). The activity of compounds 2 and 5 in the MIC assay confirms their activity in the disc test. Though compound 2 was the most bactericidal on four bacterial strains the bactericidal activity was weak with an MBC of 800 to 1 000 μg/mL. Overall, two compounds, 1 and 5, showed potential for broad spectrum activity seen from the inhibition of four Gram-negative and one Gram-positive bacteria in the disc and micro-dilution (MIC) assays.

All ten compounds tested contain the same basic scaffold. Therefore, any differences observed in their biological profiles can be attributed to the nature of substituents and the substitution patterns on the scaffold. In reviewing the data, the following four compounds emerge as having the highest potential for antibacterial activity: 1, 2, 5, and 10. A common feature of these analogues is the presence of a lipophilic substituent at the para position of the pendant phenyl group. The activity of these analogues sharply contrasts with that of compound 8 which contains a smaller substituent at this same position (compare 1, 2, 5 *vs* 8), suggesting that the presence of bulky lipophilic substituents at the para position favours antibacterial activity. In contrast, substitution at the meta position of the pendant phenyl group appears to be disfavoured, as the meta-substituted compounds display reduced antibacterial activity (compare 1 *vs* 3 and 5 *vs* 6).

Discs of standard antibiotics contain small amounts of drug, usually 10 to 30 μg with a few containing 50 to 300 μg hence the amount (200 μg) which showed activity in this study falls within the range for antibiotics in clinical use. However, in comparison to standard antibiotics, the bacterial strains showed intermediate sensitivity to the most active THIQs (1, 2 and 5) close to some standard antibiotics singly and in combination such as ampicillin (14-16 mm) and doxycycline (11-13 mm) on Enterobacteriaceae; cefoperazone (16 -20 mm) and trimethoprim (11-15 mm) on *S. aureus*[20]. A study of 1-aryl-6,7-dimethoxy-1,2,3,4-THIQs reported lower MICs of 3.5 to 20 μg/mL, against *E. coli*, *S. aureus* and *P. aeruginosa*[22], suggesting that substitution of different chemical groups in the 1,2,3,4-tetrahydroisoquinoline parent structure significantly affects the antibacterial activity of the parent structure. In this respect, the ring nitrogen atom was particularly sensitive.

The cytotoxicity test on compound 2 showed that the $CC_{50}$ of 98.2 μg/mL and $CC_{100}$ (500 μg/mL) were much higher than the cut-off value ($CC_{50}$ > 30 μg/mL) for lack of cytotoxicity indicating that the

compounds may not possess a high risk of toxicity. High antiplasmodial activity was recorded for compound 2 [ 1-(3,4-chlorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline] with $IC_{50}$ = 0.395 μg/mL against *Plasmodium falciparum* K1 with high very selectivity for the parasite (selectivity index > 70) relative to rat skeletal myoblast cells (L6)[9]. Therefore, subsequent chemical modification of this series should seek to increase potency while reducing toxicity.

The ten THIQs studied have demonstrated moderate but structure-related antibacterial activity. Further exploration of the parent THIQ scaffold may lead to the discovery of more potent and less toxic derivatives for further development into new antibacterials.

## Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgements

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