ORIGINAL ARTICLE

European Journal of Advanced Chemistry Research
www.ej-chem.org

Evaluation of the Antibacterial Activity of 5-(thiophen-2-yl)-1H-tetrazole and Its Oxime Derivative against ATCC Reference Strains and Strains Isolated from the Hospital Environment of a Provincial Public Hospital in the City of Fez

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ABSTRACT

Bacterial resistance to antibiotics and disinfectants has become a real concern. The hospital presents a favorable environment for the colonization and development of bacteria resistant to antibiotics and disinfectants. The search for new antimicrobial compounds is essential to combat this phenomenon. Tetrazole derivatives may represent a solution due to their interesting antibacterial activity. In this work, two tetrazole derivatives; thiophene-2-carbaldehyde (T2C) and 5-(thiophen-2-yl)-1H-tetrazole (5TPh-1HT), were evaluated for their antibacterial activities against a set of reference strains and strains isolated from the hospital environment. The antibacterial effect was studied by the disc diffusion method and by determination of MIC and MBC. The 5-(thiophen-2-yl)-1H-tetrazole (5TPh-1HT) has a broader spectrum of activity than its oxime derivative (T2C). The latter has bactericidal activity only on gram-negative Escherichia coli, Pseudomonas aeruginosa with MICs ranging from 0.62 mg/ml to 2.5 mg/ml, while 5TPh-1HT has a bactericidal effect on all strains with MICs ranging from 0.62 mg/ml to 1.25 mg/ml. Both products have a significant inhibitory activity on the strains tested in particular E. coli H, S. aureus H, P. aeruginosa and Streptococcus spp A. It was found that these activities vary depending on the microbial strain tested and the product applied.

Keywords: Hospital environment, Resistant bacteria, Antibacterial activity, Tetrazole, MIC, MBC.

Published Online: March 26, 2021

ISSN: 2684-4478

DOI :10.24018/ejchem.2021.2.2.54

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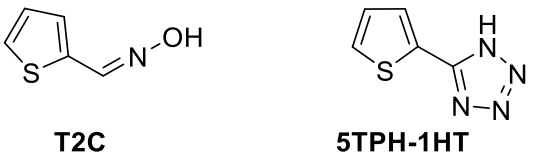
I. INTRODUCTION

The hospital is a place of care, but also a place where one can contract infectious diseases [1]. Contamination contracted in the hospital is at the origin of infections, known as nosocomial infections. The latter is a serious public health problem with considerable consequences both for individuals and for the economy [2]. Bacteria can be transmitted through direct contact or through the hospital environment (air, water, food, materials). Most of the bacteria responsible for nosocomial infections are multi-resistant bacteria [3]. The acquisition of bacterial resistance to antimicrobial agents, particularly antibiotics, is due to the overuse of TBAs in human medical practice, whether in hospital or community settings. The use of disinfectants with sub-inhibitory concentrations also causes bacterial resistance to a range of disinfectants [4].

Faced with this world-wide problem, which is growing considerably and constitutes a major threat to public health, it is necessary to find new antimicrobial molecules that can be the basis of new antibiotics or disinfectants. Indeed, the chemical synthesis of new antibacterial molecules is essential to combat the phenomena of bacterial resistance. In recent years, many teams of researchers have been interested in the synthesis and study of new heterocyclic compounds. Tetrazoles constitute an important class of these compounds which have received a lot of interest through their various biological activities [5]-[16] in particular antibacterial activity [17]-[21]. Tetrazoles and their derivatives also occupy an important place in the drug market [22]. Examples of drugs based on tetrazole or its derivatives include Pentetrazol, Candesartan cilexetil and Ceftezole.

Thus, in the continuation of our previous work on the evaluation of antibacterial activity of tetrazolic compounds and their precursors, synthesized in our laboratory [23]-[24], we present in this paper the results of the tests of antibacterial activity of two synthetic chemical molecules derived from tetrazole, namely thiophene-2-carbaldehyde oxime (T2C) and the 5-(thiophen-2-yl)-1H-tetrazole **(5TPH-1HT)** [17]-[18] on different types of bacterial strains: ATCC reference strains and strains isolated from the environment of the Hassan II University Hospital Center in Fez.



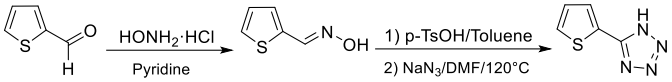
T2C **5TPH-1HT**

Fig. 1. structures of the two synthetic molecules tested.

II. RESULTS AND DISCUSSION

A. Chemistry

The two organic compounds tested are the 5-(thiophen-2-yl)-1H-tetrazole, substituted in position 5 by an electron-attracting group, and its derivative thiophen-2-carbaldehyde oxime. These two compounds are resynthesized in the laboratory according to the same protocol used by Alami [25]. The following reaction diagram summarizes the steps in this synthesis.



Scheme 1. Synthesis strategy of tetrazole derivative and its precursor oxime.

B. Biology

The evaluation of the antibacterial activity of the two synthesised compounds was carried out on different types of bacterial strains.

- Strains of ATCC References:
  - ✓ Esherichia coli ATCC 25922 (E. coli R),
  - ✓ Staphylococcus aureus ATCC 29213 (S. aureus R),
  - ✓ Bacillus subtilis ATCC 3366 (B. subtilis R), Pseudomenas aeruginosa ATCC 27853 (P. aeruginosa R).

Strains isolated from the hospital environment (intensive care unit) of the provincial public hospital of the city of Fez (CHU of Fez). The strains were identified by our research team [20]:

- ✓ Esherichia coli (E. coli H)
- ✓ Staphylococcus aureus (S. aureus H).

- Isolated strains of Foods for Hassan II Hospital patients, purified and preserved at -80 °C in the Laboratory of the Faculty of Medicine and Pharmacy of Fez:
  - ✓ Esherichia coli (E. coli A),
  - ✓ Staphylococcus aureus (S. aureus A),
  - ✓ Pseudomenas aeruginosa (P. aeruginosa A),
  - ✓ Streptococcus spp (Streptococcus A).

1. Evaluation of the sensitivity of bacteria to tetrazole derivatives by disk diffusion

The sensitivity of the four ATCC reference strains, as well as the six strains isolated from the hospital environment, is evaluated by the disc diffusion method.

1.1. Identification of antibacterial activity against ATCC strains

Table 1 shows the results of the diameters of the growth inhibition aureoles of ATCC strains by the tetrazole derivative and its oxime precursors (expressed in mm).

TABLE I: DIAMETERS OF INHIBITION HALOS (IN MM) OF THE T2C AND 5TPH-1HT PRODUCTS AGAINST ATCC STRAINS

ATCC strains	T2C	5TPh-1HT	Ampicilline*
E. coli R	9±1.4	4.5±0.7	23,5±1
S. aureus R	2±0	7,5±0.3	21±0.7
B. subtilis R	2±0	6,5±0.3	42±1.4
P. aeruginosa R	12±2.8	12,5±1.1	23,5±1

*Positive control: Ampicillin (100 µg/ml).

The oxime product has an inhibitory activity on Gram-negative bacteria; E. coli R and P. aeruginosa R, with a halo diameter of inhibition of 9 and 12 mm respectively. It has a low inhibitory activity on Gram positive S. aureus R and B. subtilis R (2 mm) (Table I). These results corroborate with the work of Dhayanithi et al. [31] on tetrazole derivatives containing the piperazine group. The latter show inhibitory activity on E.coli (8-10 mm) and P.aeruginosa (7-12 mm).

As for the tetrazolic derivative, it is highly active on P. aeruginosa R (12.5 mm), moderately active on S. aureus R (7.5 mm) and B. subtilis R (6.5 mm), and weakly active on E. coli R (4.5 mm) (Table I). Similar results have been obtained in numerous studies, notably those carried out by Mohite et al. [26] with 5-phenyl-tetrazoles and Kategaonkar et al. [27]

with tetrazolo [1,5-a] quinolone derivatives. These compounds caused inhibition of E. coli, S.aureus and B.subtilis strains with inhibition diameters of 5, 7 and 6-9 mm respectively. Benzyl-substituted tetrazole derivatives have inhibitory activity on P. aeruginosa with a 13 mm halo of inhibition [28]. The effects of our products on P. aeruginosa are promising compared to those obtained by Yildirir et al. [29] with phenylselanyl-1-(toluene-4-sulfonyl)-1H-tetrazole to which P. aeruginosa was resistant.

1.2. Identification of antibacterial activity against strains of hospital origin

Table II shows the results of the diameters of the inhibition aureoles of the two compounds on strains of hospital origin.

TABLE II: DIAMETERS OF INHIBITION HALOS (IN MM) OF THE T2C AND 5TPH-1HT PRODUCTS AGAINST STRAINS OF HOSPITAL ORIGIN

Strains of hospital origin	T2C	5TPh-1HT	Ampicilline*
E. coli A	4.5±0.71	5±0	6±1.4
E. coli H	14,5±0.71	7±1.41	14.5±0.71
S. aureus H	4.5±0.71	8,5±0.71	25.5±1
S. aureus A	6,5±0.71	7±0	26±0.71
P. aeruginosa A	14±1.41	4.5±0.71	5±0
Streptococcus A	7±0	5,5±0.71	11±0

*Positive control: Ampicillin (100 µg/ml).

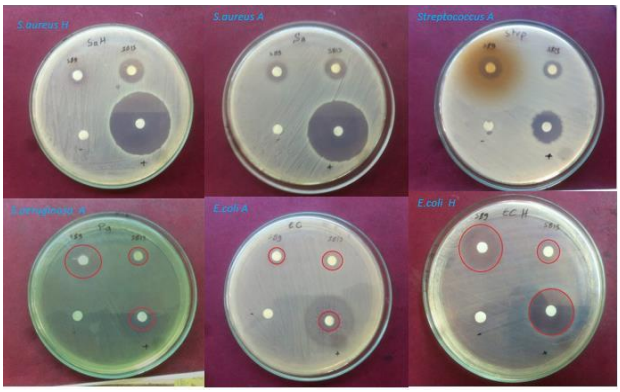


Fig. 2. Expression of activity of both Oxime and Tetrazole on bacterial strains tested. The oxime sensitivity (T2C) test of the different strains of hospital origin showed high inhibitory activity on E. coli H and P. aeruginosa A (14.5 and 14 mm respectively) and moderate activity on S. aureus A and Streptococcus spp A (7 and 6.5 mm respectively). However, the bacterial strains E. coli A and S. aureus H were resistant (4.5 mm).

The 5-(thiophen-2-yl)-1H-tetrazole (5TPh-1HT) has a high inhibitory activity on S. aureus H (8.5 mm), and a moderate inhibitory activity on E. coli H and S. aureus A (7 mm). However, this inhibitory activity is low on E. coli A, P. aeruginosa A and Streptococcus A with 5, 4.5 and 5.5 mm inhibition diameters, respectively (Table II).

To our knowledge, there is not much work already done on the antimicrobial activity of tetrazole derivatives on strains of hospital origin except a study by Morjan et al. [30] on 5-oxo and 5-thio-tetrazoles 1,4-disubstituted derivatives. The results of this study show that some 5-oxo-tetrazole derivatives have activities on E. coli, Streptococcus spp and S. aureus with halos of inhibition of 5 mm, 5-6 mm and 6 mm respectively. These 5-oxo-tetrazole derivatives are not active on P. aeruginosa, while other 5-thio-tetrazole derivatives are highly active on P. aeruginosa, S. aureus, E. coli, and Streptococcus spp.

All reference strains are highly sensitive to ampicillin with halos of inhibition between 23.5 mm and 42 mm. On the other hand, E. coli A and P. aeruginosa A have been shown to be more resistant to ampicillin with inhibition diameters ≤6 mm.

Also, the role of tetrazole ring substituents on antibacterial activity, which can modify the chemical properties of these tetrazoles (hydrophobicity...), by influencing their mode of action.

2. Determination of Minimum Inhibitory Concentrations (MICs)

Tables III-VI summarize the results of the determination of minimum inhibitory concentrations of tetrazole derivative (5TPh-1HT) and its oxime precursor (T2C) against the reference strains ATCC (E. coli R, S. aureus R, B. subtilis R and P. aeruginosa R) and strains isolated from hospital environments (E. coli A, E. coli H, S. aureus A, S. aureus H, P. aeruginosa A and Streptococcus spp A). The MIC corresponds to the lowest concentration that has shown no visible bacterial growth.

2.1. Minimum Inhibitory Concentrations against ATCC reference strains

Tables III and IV show that both compounds have antibacterial activity on ATCC strains and confirm the qualitative test results. Indeed, the oxime derivative inhibits the growth of P. aeruginosa R strain at a MIC of 0.62 mg/ml, E. coli R strain at a MIC of 1.25 mg/ml and S. aureus R and B. subtilis R strains at a MIC of 2.5 mg/ml (Table III).

TABLE III: MINIMUM INHIBITORY CONCENTRATIONS (MG/ML) OF T2C ON ATCC STRAINS

Concentration (mg/ml)	2.5	1.25	0.625	0.31	0.15	0.07	Témoin
E. coli R	-	-	+	+	+	+	+
S. aureus R	-	+	+	+	+	+	+
B. subtilis R	-	+	+	+	+	+	+
P. aeruginosa R	-	-	-	+	+	+	+

5TPh-1HT inhibits the growth of all ATCC strains with a 1.25mg/ml MIC, except P. aeruginosa A, which is inhibited at a 0.62mg/ml MIC (Table IV).

TABLE IV: MINIMUM INHIBITORY CONCENTRATIONS (MG/ML) OF 5TPH-1HT ON ATCC STRAINS

Concentration (mg/ml)	2.5	1.25	0.625	0.31	0.15	0.07	Témoin
E. coli R	-	-	+	+	+	+	+
S. aureus R	-	-	+	+	+	+	+
B. subtilis R	-	-	+	+	+	+	+
P. aeruginosa R	-	-	-	+	+	+	+

In the literature, the results of the CMIs of tetrazole derivatives vary widely, ranging from µg to mg. Indeed, the studies of Dhayanithi et al. [31] from tetrazole derivatives show that E. coli and S. aureus strains were inhibited with a 6.25 mg/ml MIC and P. aeruginosa was inhibited with a 50 mg/ml MIC. In a second study with other tetrazole derivatives, the same authors found that these products inhibited S. aureus, E. coli, and P. aeruginosa with an IMC of 12.5 mg/ml [28]. Other studies have shown that the MIC values of tetrazole derivatives on E. coli, B. subtilis, S. aureus, and P. aeruginosa range from 10 µg/ml to 250 µg/ml [27], [32]-[33].

2.2. Minimum Inhibitory Concentrations against strains of hospital origin

The results of Table V show that thiophene 2-carbaldehyde (T2C) has an inhibitory activity against the strains tested. The strains E. coli H and P. aeruginosa A were found to be more sensitive (CMI = 1.25 mg/ml) than E. coli A, S. aureus A, S. aureus H, and Streptococcus spp A (CMI = 2.5 mg/ml) (Table V).

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TABLE V: MINIMUM INHIBITORY CONCENTRATIONS (MG/ML) OF T2C ON STRAINS OF HOSPITAL ORIGIN

Concentration (mg/ml)	2.5	1.25	0.625	0.31	0.15	0.07	Témoin
E. coli A	-	+	+	+	+	+	+
E. coli H	-	-	+	+	+	+	+
S. aureus A	-	+	+	+	+	+	+
S. aureus H	-	+	+	+	+	+	+
P. aeruginosa A	-	-	+	+	+	+	+
Streptococcus spp A	-	+	+	+	+	+	+

MIC values obtained with 5TPh-1HT indicate that this product has a greater inhibitory effect than that obtained with T2C. In fact, all strains were inhibited at a concentration of 1.25 mg/ml, except E. coli strain which was found to be less sensitive to the tetrazolic derivative with a MIC of around 2.5 mg/ml (Table VI).

TABLE VI: MINIMUM INHIBITORY CONCENTRATIONS (MG/ML) OF 5TPH-1HT ON STRAINS OF HOSPITAL ORIGIN

Concentration (mg/ml)	2.5	1.25	0.625	0.31	0.15	0.07	Témoin
E. coli A	-	+	+	+	+	+	+
E. coli H	-	-	+	+	+	+	+
S. aureus A	-	-	+	+	+	+	+
S. aureus H	-	-	+	+	+	+	+
P. aeruginosa A	-	-	+	+	+	+	+
Streptococcus spp A	-	-	+	+	+	+	+

It should be noted that the work of Morjan et al. [30] have shown that 5-oxo and 5-thio-tetrazoles 1,4-disubstitute derivatives inhibit the growth of isolated strains in the hospital environment; S.aureus and E.coli with a MIC of 0.2 mg/ml and 0.6 mg/ml respectively.

2.3. Comparison of the effects of tetrazole derivatives on the different strains tested

Based on the data in Tables VII and Fig. 2, both T2C and 5TPh-1HT products have the same MIC on Gram-negative strains (E. coli and P. aeruginosa). Tetrazole 5TPh-1HT is more active on Gram-positive strains (S. aureus, B. subtilis, and Streptococcus spp) than the oxime derivative.

TABLE VII: COMPARISON OF MICS OF T2C AND 5TPH-1HT ON THE STRAINS TESTED

Souches bactériennes	CMI du SB 9 (mg/ml)	CMI du SB 13 (mg/ml)
E. coli R	1.25	1.25
P. aeruginosa R	0.62	0.62
S. aureus R	2.5	1.25
B. subtilis R	2.5	1.25
E. coli A	2.5	2.5
E. coli H	1.25	1.25
P. aeruginosa A	1.25	1.25
S. aureus A	2.5	1.25
S. aureus H	2.5	1.25
Streptococcus spp A	2.5	1.25

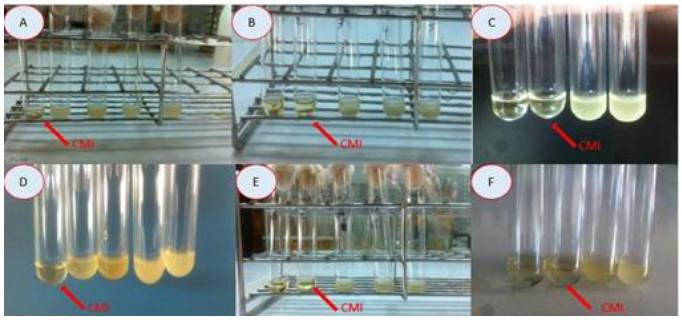

Fig. 2. Expression of MICs in liquid medium (Macrodilution): A: MIC of T2C on E. coli A, B: MIC of 5TPh-1HT on E. coli H, C: MIC of 5TPh-1HT on P. aeruginosa A, D: MIC of T2C on S. aureus A, E: MIC of 5TPh-1HT on S. aureus H, F: MIC of 5TPh-1HT on Streptocuccus spp A.

2.4. Determination of minimum bactericidal concentrations (MBCs)

The minimum bactericidal concentration of the two T2C and 5TPh-1HT products, which corresponds to the lowest concentration of these products with a maximum number of 5 colonies on a box, was determined. Tables VIII-IX represent the results of CMBs obtained on ATCC and hospital-derived strains.

2.4.1. Minimum Bactericidal Concentrations (MBCs) against ATCC reference strains

The CMB of the oxime product is 2.5 mg/ml for both E. coli R, P. aeruginosa R. It is greater than 2.5 mg/ml for S. aureus R and B. subtilis R (Table VIII).

TABLE VIII: MINIMUM BACTERICIDAL CONCENTRATIONS (MG/ML) OF T2C ON ATCC STRAINS

Concentration (mg/ml)	2,5	1,25	0,625
E.coli R	-	+	+
S.aureus R	+	+	+
B.subtilis R	+	+	+
P.aeruginosa R	-	+	+

Table IX shows that the Minimum Bactericidal Concentration of 5TPh-1HT is 2.5 mg/ml for all ATTC strains.

TABLE IX: MINIMUM BACTERICIDAL CONCENTRATIONS (MG/ML) OF 5TPH-1HT ON ATCC STRAINS

Concentration (mg/ml)	2,5	1,25	0,625
E.coli R	-	+	+
S.aureus R	-	+	+
B.subtilis R	-	+	+
P.aeruginosa R	-	+	+

Thus, the oxime derivative has a bactericidal effect on E. coli R and P. aeruginosa R, while the tetrazole derivative has a bactericidal effect on all ATCC strains tested (CMB/CMI ≤ 4). It should be noted that the work of Rao et al [34] has shown that the MBCs of some tetrazole derivatives vary between 0.5 and 1 mg/ml against the strains S. aureus and B. subtilis. Whereas the BMC against E. coli is of the order of 6.25 mg/ml.

2.4.2. Minimum Bactericidal Concentrations (MBCs) against hospital strains

The MBC of T2C is 2.5 mg/ml on the P.aeruginosa A and Streptococcus spp A strain. While the other strains are resistant to the T2C product, the MBC remains above 2.5 mg/ml (Table X).

TABLE X: MINIMUM BACTERICIDAL CONCENTRATIONS (MG/ML) OF T2C ON STRAINS OF HOSPITAL ORIGIN

Concentration (mg/ml)	2,5	1,25	0,625	0,31	0.15
E. coli A	+	+	+	+	+
E. coli H	+	+	+	+	+
S. aureus A	+	+	+	+	+
S. aureus H	+	+	+	+	+
P. aeruginosa A	-	+	+	+	+
Streptococcus spp A	-	+	+	+	+

As for the product 5TPh-1HT, it presents an MBC of 2.5 mg/ml on both strains of E. coli, as well as on P. aeruginosa A. The BMC of the tetrazole derivative on S. aureus A, S. aureus H and Streptococcus spp A is 1.25 mg/ml (Table XI).

TABLE XI: MINIMUM BACTERICIDAL CONCENTRATIONS (MG/ML) OF 5TPH-1HT ON STRAINS OF HOSPITAL ORIGIN

Concentration (mg/ml)	2,5	1,25	0,625	0,31	0.15
E. coli A	-	+	+	+	+
E. coli H	-	+	+	+	+
S. aureus A	-	-	+	+	+
S. aureus H	-	-	+	+	+
P. aeruginosa A	-	+	+	+	+
Streptococcus spp A	-	-	+	+	+

In summary, the thiophene 2- carbaldehyde (T2C) compound has a bactericidal effect on P. aeruginosa A and Streptococcus ssp A, whereas 5-(thiophen-2-yl)-1H-tetrazole (5TPh-1HT) has a bactericidal effect on all hospital-derived strains tested (CMB/CMI) ≤ 4).

III. MATERIALS AND METHODS

This study was conducted in the Biotechnology Laboratory of the Faculty of Sciences, Sidi Mohamed Ben Abdellah University-Fez. Our study focused on the evaluation of the antibacterial activity of thiophene-2-carbaldehyde (T2C) and 5-(thiophen-2-yl)-1H-tetrazole (5TPh-1HT) compounds against four ATCC reference strains (E. coli R, S.aureus R, B.subtilis R, P.aeruginosa R) and six strains isolated from the hospital environment (E.coli A, E.coli H, S.aureus H, S.aureus A, P.aeruginosa A and Streptococcus spp A). The sensitivity of these strains is evaluated by the disc diffusion method. The evaluation of the antibacterial activity of a synthetic chemical molecule is carried out in two tests:

Qualitative method: It is a test that allows the demonstration of antibacterial activity.

Quantitative method: It is a test that allows to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) that can inhibit the growth of the bacteria tested.

A. Diffusion Method in Solid Media: Antibiogram

The antibiotic susceptibility test or diffusion method is one of the oldest approaches to determining the sensitivity of bacteria to antibiotics, and remains one of the most commonly used methods in medical analysis laboratories. It is recommended by the European Committee for Antibacterial Susceptibility Testing (EUCAST) [35] and the National Committee for Clinical Laboratory Standards (CLSI) [36].

1. Principle

This method consists of placing a disk soaked with the antibacterial agent in a petri dish previously sown by a bacterial species. The diffusion of the antibacterial agent into the agar creates a halo of growth inhibition of the bacteria around the disk. The susceptibility of bacterial strains to the products tested could be classified into three profiles according to the halo diameter of inhibition [37]:

- Highly active: inhibition zone > 12 mm.
- Moderately active: 6-11 mm inhibition zone.
- Low active: <5 mm inhibition zone.

1.1. Experimental Protocol

The antibacterial activity by diffusion on disks of the two tested compounds (T2C, 5TPh-1HT), was performed according to the Protocol recommended by EUCAST and CLSI, with some modifications [35]-[36].

1.1.1. Preparation of solutions of tetrazole derivatives

A quantity of 15 mg of T2C or 5TPh-1HT was solubilized in 60 µl of DMSO to obtain a final concentration of 250 mg/ml.

1.1.2. Inoculum preparation

From a 24-hour pure bacterial culture on LB medium (Appendix 1), 3-4 colonies were collected with a handle and transferred to 5 ml of LB medium to prepare a bacterial suspension. The latter has been homogenized. The turbidity of the bacterial suspension was adjusted to a DO=0.1 at 600 nm, equivalent to that of the McFarland 0.5 standard, which corresponds to an inoculum with a bacterial load of 108 CFU/ml [36] (Appendix 2).

1.1.3. Inoculation of the boxes

The inoculation was carried out by the swabbing method on boxes containing the LB medium. A sterile cotton swab was dipped into the bacterial suspension. Excess liquid was removed by turning the swab on the walls of the tube.

1.1.4. Disc deposit

Whatman paper discs, N°1, 6 mm in diameter, previously sterilised (prepared and autoclaved for 20 minutes at 121 °C) were deposited on the surface of agar sown with the bacterial strains to be tested. 10 µl solutions of SB 9 or SB 13 were deposited on the discs. 10 µl of Ampicillin (100 µg/ml) were deposited on discs used as positive controls. The negative control was a disc containing 10 µl of DMSO. The plates were incubated at 37 °C for 24 hours. Each test was carried out in two replicates. After 24 hours of incubation, the product with antibacterial activity formed an inhibition halo around the disc. Inhibition diameters were measured in (mm). Disc diameter is excluded.

2. Determination of MICs and MBCs

The action of the antibacterial agent on a bacterial strain can be characterised by its minimum inhibitory concentration (MIC) and its minimum bactericidal concentration (MBC).

2.1. Determination of the Minimum Inhibitory Concentration in a liquid medium

The MIC is defined according to the Antibiogram Committee of the French Microbiology Society (CA-SFM) as the lowest concentration that results in the inhibition of visible bacterial growth [35].

2.1.1. Principle

The determination of the MIC was carried out by preparing a series of dilutions from ½ of the antimicrobial agent to be tested on liquid medium (microdilution or macrodilution). The MIC is the lowest concentration of the antibacterial agent present in the tube, well or canister that shows no visible bacterial growth [36].

2.1.2. Experimental protocol

2.1.2.1. Preparation of stock solutions of chemicals

An amount of 15 mg of T2C or 5TPh-1HT product was solubilised in 0.5ml of LB medium to which 0.2 ml of DMSO was added. 2.3 ml of LB medium was added to give a final concentration of 5 mg/ml. During this work, the MIC was determined by the liquid dilution method by macro and micro dilution.

2.1.2.2. Method of dilution in liquid medium: Macrodilution

The macrodilution method consists of preparing dilution series in test tubes with a final volume of 1 ml [36]. The MIC determination of the products tested by macrodilution is performed according to the protocol recommended by CLSI [36] and Rao et al. [34], with some modifications.

- Preparation of the inoculum

From a 24-hour pure bacterial culture on LB medium of the strains tested, a pre-culture of 3-4 hours was carried out by taking 3 to 4 colonies using a loop and then transferring them into 5 ml of LB medium in order to prepare a bacterial suspension. The turbidity of the latter was adjusted to an OD=0.1 at 600 nm, equivalent to that of the McFarland 0.5 standard and which corresponds to an inoculum with a bacterial load of 108 CFU/ml. Next, 5ml of the inoculum of the strains tested was prepared by adding 4.950 ml of LB medium and 50 µl of the bacterial suspension to dilute the bacterial load by 1/100, to have a final concentration of 106 CFU/ml.

- Preparation of the dilution series of T2C and 5TPh-1HT products

250 µl of the LB medium were distributed in sterile test tubes. Then 500 µl of T2C and 5TPh-1HT stock solutions were added to the first tube. After stirring, a cascade dilution series was performed by adding 250 µl of the solution from the first tube to the second and so on until the last tube where 250 µl was removed to have the same volume in all tubes. The concentrations obtained ranged from 2.5 mg/ml to 0.07 mg/ml for the two products tested.

250 µl of bacterial inoculum was added to the tubes containing the dilution series. The final volume in the tubes is 0.5 ml, and the bacterial load is 5×10^5 CFU/ml. The final concentrations of the products tested range from 2.5 mg/ml to 0.07 mg/ml for T2C and 5TPh-1HT.

A negative control tube has been made, containing the bacterial inoculum at 5×10^5 CFU/ml. All tubes were incubated at 37 °C for 24 hours.

The MIC is the tube containing the lowest concentration of the test product that showed no visible bacterial growth.

2.1.2.3. Method of dilution in liquid medium: Microdilution

The microdilution method consists of preparing dilution series in a 96-well polypropylene microplate. The MIC determination by microdilution was performed according to the protocol recommended by CLSI, Hellal et al. [38].

- Preparation of the inoculum

The preparation of the inoculum was carried out as described above for the macrodilution except instead of preparing 5 ml of the inoculum we prepared 2 ml.

- Preparation of the dilution series of T2C and 5TPh-1HT products

100 µl of LB medium were distributed in all wells except those in the first line. Then 200 µl of the stock solutions of the tested products were added to the first line. The dilution series was carried out by taking 100 µl from the first well in the first column and adding it to the second well belonging to the same column, and so on until the penultimate well. The same steps were repeated for the other columns. The wells were inoculated with 100 µl of the bacterial suspension at a concentration of 10^6 CFU/ml. The wells in the last row of the microplate contain only the inoculum (control). The microplate was incubated at 37 °C for 24 hours. The MIC corresponds to the well, containing the lowest concentration of the test product, which showed no visible bacterial growth.

2.1.3. Determination of the Minimum Bactericidal Concentration (MBC) in solid media

The MBC is the lowest concentration of the tested product capable of killing 99.9% of the bacteria and allowing only 0.01% to grow [39].

- Protocol

From the tubes and wells used for the MIC determination, 5 µl of each tube or well was deposited on LB medium, then streaked and incubated at 37 °C for 24 hours. The BMC was considered to be the lowest concentration of test material that showed no growth [38].

The calculation of the BMC/MIC ratio is used to assess whether an antibacterial agent has a bactericidal (BMC/CMI≤ 4) or bacteriostatic (BMC/MIC >4) effect [40], [41].

3. Data analysis and processing

The calculation of averages and standard deviations was developed using the Excel program.

4. Annexes

4.1. Appendix 1

Composition of the environment Luria-Bertani (LB)

- Liquid LB medium

Peptone	10 g
Yeast extract	5 g
NaCl	10 g
Distilled water	1000 ml

- Solid LB Medium

Peptone	10 g
Yeast extract	5 g
NaCl	10 g
Agar	15
Distilled water	1000 ml

4.2. Appendix 2

Protocol for the preparation of the McFarland 0.5 standard

The McFarland 0.5 standard is prepared by adding a 0.048 mol/l $BaCl_2$ solution and 0.18 mol/l H_2SO_4.

Preparation of a 0.048 mol/l $BaCl_2$ solution (1.175% w/v $BaCl_2.\ _{2H2O}$): 10 ml $BaCl_2$ and 0.1175 g $BaCl_2\ .2H_2O$ are solubilised in 10 ml distilled water.

Preparation of a solution of 100 ml of H_2SO_4 0.18 mol/l (1% v/v) in 100 ml of distilled water.

Preparation of McFarland 0.5

Add 0.5 ml of the $BaCl_2$ solution to 99.5 ml of a 0.18 mol/l H_2SO_4 solution and shake vigorously.

The optical density of the resulting solution should be between 0.08 and 0.13 at 625 nm.

IV. CONCLUSION

Thiophene 2-carbaldehyde (T2C) and 5-(thiophen-2-yl)-1H-tetrazole (5TPh-1HT) were evaluated for their antibacterial activities against a set of reference strains and strains isolated from the hospital environment. Minimum inhibitory concentrations were determined by two methods: the method of macrodilution and microdilution in liquid medium.

The oxime T2C product was more active on E. coli R (9 mm), P. aeruginosa R (12 mm) and E. coli H (14.5 mm). This product exhibited strong inhibitory activity on P.aeruginosa A with a 14 mm halo of inhibition that is greater than that obtained by ampicillin (5 mm). This product inhibits all strains with an MIC ranging from 1.25 mg/ml to 2.5 mg/ml with the exception of P. aeruginosa R (MIC= 0.62 mg/ml). The tetrazole 5TPh-1HT derivative is more active on P. aeruginosa R (12.5 mm) and S. aureus H (8.5 mm). CMI values show that 5-(thiophen-2-yl)-1H-tetrazole is more active than 2-carbaldehyde thiophene. The tetrazoolic derivative inhibits all strains with an MIC of 1.25 mg/ml, with the exception of P. aeruginosa R (MIC=0.62 mg/ml) and E. coli A (MIC=2.5 mg/ml).

CMB results from the products tested showed that T2C has a bactericidal effect against E. coli R, both P. aeruginosa and Streptococcus spp A. While 5TPh-1HT has a bactericidal effect on all strains tested. It can be concluded that both products have an interesting activity on hospital-derived strains, in particular E. coli H, S. aureus H, Streptococcus spp A and P. aeruginosa A.

This work constitutes an approach in the design of tetrazole derivatives compounds that can be used as an alternative to antibiotics and disinfectants. it would therefore be interesting to study the effect of the combination.

ACKNOWLEDGMENT

This work was supported by Sidi Mohammed Ben Abdellah University (USMBA) and National Center for Scientific and Technical Research (CNRST).

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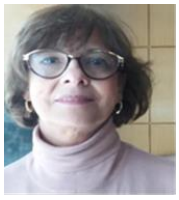

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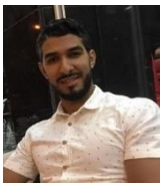

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