1,3-Benzoxathiiol-2-one and 1,3-Benzothiazole Compounds as Potential Anticancer and Antimicrobial Agents

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DOI: 10.21577/1984-6835.20200125

Artigo

1,3-Benzothiazol-2-ones and 1,3-Benzotiazóis as Potential Agents Anticancer and Antimicrobians

Resumo: Ao longo dos anos, o câncer e as doenças infecciosas aparecem como as principais causas de morte no mundo. Esses dados destacam a necessidade de novos protótipos para o desenvolvimento de quimioterápicos mais potentes e seletivos, bem como novos agentes antimicrobianos. O principal objetivo deste trabalho foi avaliar as atividades antitumorais, antibacterianas e antifúngicas de alguns derivados 1,3-benzoxathiol-2-one e 1,3-benzothiazol. Os compostos foram testados quanto à atividade anticâncer in vitro frente às linhagens de células de câncer de melanoma (SKMEL-19) de líquido ascítico (AGP-01) e de mama (MCF-7) pelo ensaio MTT. O perfil de toxicidade contra eritrócitos e fibroblastos humanos normais (MRC-5) também foi avaliado. Além disso, foram realizados ensaios in vitro de triagem antimicrobiana (TSA, concentração inibitória mínima (CIM) contra bactérias Gram-negativas, bem como contra espécies do gênero Candida. Todos os testes foram realizados de acordo com os protocolos CLSI, utilizando vanomicina, ciprofloxacina e ketoconazol como fármacos de referência. O derivado 6-metoxi-benzo[d][1,3]oxatiol-2-one (7) exibiu atividade citotóxica considerável (CI 25 = 3.3 μM) contra SKMEL-19 e o derivado (E)-[(2-benzo[d]thiazol-2-Il) hidrazonometil]benzeno-1,2,3-triol (16m) mostrou boa atividade contra todas as espécies de Candida (CIM 8-32 μg mL -1). A razão CBM/CIM dos derivados 16l e 16m os classificou como agentes bactericidas contra bactérias Gram-positivas. A substância 16m apresentou perfil fungistático contra Candida albicans e também espécies não albicans. De maneira geral, os resultados in vitro apontaram o potencial dos derivados 7 e 16m como novos protótipos anticâncer e antifúngico, respectivamente, para serem mais explorados, uma vez que também apresentaram baixo perfil de toxicidade.

Palavras-chave: Anticâncer; antifúngico; 1,3-benzotiazol; 1,3-benzoxathiiol-2-onas; fármacos, heterociclos.

Abstract

Over the years, cancer and infectious diseases have appeared among the leading causes of death worldwide. The data herein highlighted the need for new prototypes to design more potent and selective chemotherapeutics, as well as new, non-traditional antimicrobial agents. The main goal of this study was to evaluate some 1,3-benzoxathiol-2-one and 1,3-benzothiazole derivatives for their anticancer, antibacterial and antifungal activities. The compounds were screened for in vitro anticancer activity against melanoma (SKMEL-19) ascitic fluid (AGP-01) and breast (MCF-7) cancer cell lines using an MTT assay. The toxicity profile against erythrocytes and the normal human fibroblast cell line (MRC-5) was also evaluated. Besides that, in vitro Antimicrobial Screening Test (AST, Minimum Inhibitory Concentration (MIC) Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) assays were performed against Gram-positive and Gram-negative bacteria as well as against Candida species. All tests were performed according to CLSI protocols, using vancomycin, ciprofloxacin and ketoconazole as reference drugs. The derivative 6-methoxy-benzo[d][1,3]oxathiol-2-one (7) exhibited considerable cytotoxic activity (IC 50 = 3.3 μM) against SKMEL-19, and the compound (E)-[(2-benzo[d]thiazol-2-yl)hidrazonometil]benzeno-1,2,3-triol (16m) showed good activity against all Candida species (MIC 8-32 μg mL -1). The MBC/MIC ratio for 16l and 16m derivatives classified them as bactericidal agents against Gram-positive bacteria. Compound 16m presented a fungistatic profile against Candida albicans and non-albicans species evaluated. Overall, the in vitro results pointed to the potential of derivatives 7 and 16m as new anticancer and antifungal compounds, respectively, to be further explored, since they also presented low toxicity profiles.

Keywords: Anticancer; antifungal; 1,3-benzothiazole; 1,3-benzoxathioll-2-one; drugs; heterocycles.

DOI: 10.21577/1984-6835.20200125

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Received in 25 de Abril de 2020. Aceito para publicação em 22 de Setembro de 2020.

1. Introduction

Cancer remains a threat to human health, representing the second leading cause of death globally. This disease was responsible for an estimated 9.6 million deaths in 2018 and a continuous rise in the number of cases has been projected.1 The National Cancer Institute (NCI) reported that about 16.1% of newly diagnosed
cancer cases may be attributable to infections.\(^2\) In fact, some infections are risk factors for several types of human cancer.\(^3,4\) This data is alarming mainly because of the increase in microbial resistance to antibiotics of microorganisms considered to be carcinogenic agents.\(^5\) Currently, 700,000 people die worldwide due to antimicrobial resistance, and it has been estimated that deaths will increase to 10 million by 2050.\(^6\) Despite the considerable arsenal of drugs available for treating cancer and infectious diseases, the development of new, more potent and selective anticancer and antimicrobial therapeutic agents is one of the major challenges in medicinal chemistry. Current cancer therapy fails mainly due to lack of specificity, also affecting the patient’s normal cells, which leads to many side effects.\(^7\)

In the search for new lead compounds, heterocycles play an important role in drug design, since they comprise a class of substances of great synthetic interest due to their presence in natural products and pharmacologically active compounds.\(^8\) In fact, heterocycles are common structural units in drugs and in rational design in medicinal agents for the discovery of novel bioactive molecules. In particular, 1,3-benzoxathiol-2-one and 1,3-benzothiazole-based compounds have been found to possess diverse biological activities, including antibacterial, antifungal, antiviral, antidiabetic, anticancer and anti-inflammatory. Successful clinical drugs contain these two heterocycles in their structures such as, thioxolone, a 1,3-benzothiol-2-one derivative, and frentizole, ethoxzolamide and riluzole, with a benzothiazole nucleus (Figure 1).\(^9,12\)

In the last few years, our research group has been engaged in the synthesis of potentially bioactive compounds containing these two important classes of heterocycles.\(^13-19\) We have synthesized a series of 1,3-benzoxathiol-2-one derivatives as potential anticancer agents,\(^13\) and results pointed out compound 1 as the most active against melanoma (SKMEL-19). More recently, we have reported the synthesis of 1,3-benzoxathiol-2-one-based compounds and their antifungal activity against five Candida species.\(^14\) Compound 2 was the most active of the series against C. krusei. We have also reported 1,3-benzothiazole hydrazones as being potential anticancer agents.\(^15,18\) The good cytotoxicity against three cancer cell lines of compound 3 along with its theoretical profile make it a promising molecule for anticancer drug design (Figure 2). It is noteworthy that some of these active derivatives bear the imine moiety (–N=C–), an important pharmacophore related to several biological activities, such as having anticancer, antimicrobial, antiviral and anticonvulsant profiles (Figure 2).\(^20\) We have also published review articles highlighting the main aspects of the chemical and biological properties of 1,3-benzoxathiol-2-ones (antioxidant, cytostatic, antipsoriatic, antibacterial, antymycotic, anti-inflammatory, anti-fungal and insecticidal)\(^9\) and 1,3-benzothiazoles (antimicrobial and antitumor).\(^10\)

**Figure 1.** Drugs containing 1,3-benzoxathiol-2-one and 1,3-benzothiazole nuclei

**Figure 2.** Some 1,3-benzoxathiol-2-one (1 and 2) and 1,3-benzothiazole (3) derivatives with anticancer and antimicrobial activity
In continuation of our efforts to synthesize bioactive compounds bearing pharmaceutically active heterocycles, we herein report the \textit{in vitro} anticancer and antimicrobial evaluations of some 1,3-benzoxathiol-2-one (Figure 3) and 1,3-benzothiazole (Figure 4) compounds; among these, four are being reported for the first time.

2. Materials and Methods

2.1. Chemistry

All reagents and solvents were used as obtained from commercial suppliers without further purification. Reactions were routinely monitored by thin-layer chromatography (TLC) on silica-gel precoated F. Merck plates visualized under UV light (254-366 nm). Melting points (m.p.) were determined on a Fisatom 430 apparatus and are uncorrected. Catalytic hydrogenation reactions were performed on a Paar 4540 reactor. Infrared (IR) spectra were recorded on a Perkin-Elmer 1420 spectrometer using KBr pellets and frequencies are expressed in cm\(^{-1}\). Mass spectra (ESI-MS) were performed on a ZQ-4000 single quadrupole mass spectrometer. NMR spectra were recorded on Varian Unity 500 and 300 spectrometers in DMSO-\(d_6\). Chemical shifts (\(\delta\)) are reported in ppm relative to tetramethylsilane.

Figure 3. Synthetic route used to prepare 1,3-benzoxathiol-2-one derivatives 1,2,5-14

Figure 4. Synthetic route used to prepare 1,3-benzothiazole derivatives 3,16a-q and 17a-c
2.1.1. Procedures for preparing 1,3-benzoathiole-2-one derivatives

Protocols for the preparation, physical and spectroscopic data of the compounds 2,5,7,9,11,14a-q have already been reported in our previous studies.13,14

2.1.1.1. Synthesis of 6-ethoxy-5-nitrobenzo[d][1,3]oxathiol-2-one (8)

Potassium carbonate (4 mmol) was added to a solution of 6-hydroxy-5-nitrobenzo[d][1,3]oxathiol-2-one 2 (5 mmol) in DMF (18 mL). After stirring for 30 min at room temperature, the solution was cooled with an ice bath and ethyl bromide (13 mmol) was slowly added. The mixture was stirred overnight to afford derivative 8. After the reaction completed, ice water was poured over the resulting solution. The solid product obtained was collected by vacuum filtration. Yield: 70% (yellow solid); m.p. 179-181°C. IR (KBr, ν cm⁻¹) 3435 (N-H); 3359 (N-H); 1755 (C=O). 1H NMR (DMSO-d6, 500.00 MHz, ppm): δ 169.9 (C=O); 146.3 (C6); 138.7 (C7a or C5); 7.06 (s, 1H, H7); 6.85 (s, 1H, H4); 4.87 (s, 2H, N-H). 13C NMR (DMSO-d6, 75.0 MHz, ppm): δ 165.0; 148.4; 146.9; 137.8 (C7a or C5); 136.2 (C5 or C7a); 114.6 (C6); 105.8 (C4); 96.4 (C7); 56.0 (CH3). ESI-MS: m/z [M-H]⁻: 243.0.

2.1.1.2. Synthesis of 5-amino-6-methoxybenzo[d][1,3]oxathiol-2-one (12)

10% Pd/C (110 mg) was added to a mixture of 6-methoxy-5-nitrobenzo[d][1,3]oxathiol-2-one 6 (4 mmol) and ethanol (150 mL). Catalytic hydrogenation was performed on a Parr 4540 reactor for 6-8 h under 20 bar H₂ pressure at 50°C. After that, the catalyst was filtered off, washed with ethanol and the solvent was evaporated under reduced pressure to obtain 12. Yield: 91% (black solid); m.p. 102-104°C. IR (KBr, ν cm⁻¹) 3435 (N-H); 3359 (N-H); 1755 (C=O). 1H NMR (DMSO-d6, 500.00 MHz, ppm): δ 7.06 (s, 1H, H7); 6.85 (s, 1H, H4); 4.87 (s, 2H, NH₂); 3.80 (s, 3H, CH3). 13C NMR (DMSO-d6, 75.0 MHz, ppm): δ 169.9 (C=O); 146.3 (C6); 138.7 (C7a or C5); 136.2 (C5 or C7a); 112.2 (C3a); 105.8 (C4); 96.4 (C7); 56.0 (CH3). ESI-MS: m/z [M+H]+' : 198.1.

2.1.1.3. Synthesis of 5-amino-2-oxobenzo[d][1,3]oxathiol-6-yl acetate (13)

Acetic anhydride (8 mmol) and H₂SO₄ (catalytic amount) were added to 12 (4 mmol) to afford 13. The system was stirred under reflux for 30 min. After the reaction completed, ice water was poured over the resulting solution and it was maintained in an ice bath. The mixture was filtered in a vacuum and the precipitate obtained was washed with ice water. Yield: 69% (purple solid); m.p. 192-194°C. IR (KBr, ν cm⁻¹) 3323 (N-H); 1769 (C=O); 1673 (C=O). 1H NMR (DMSO-d6, 500.00 MHz, ppm): δ 8.32 (s, 1H, N=C-H); 7.68 (d, 1H, J = 7.2 Hz, H4 or H7); 7.27 (m, 2H, H5 or H6); 7.07 (m, 1H, H4 or H7); 6.85 (d, 1H, J = 8.5 Hz, H6'); 6.41 (d, 1H, J = 8.4 Hz, H5'). 13C NMR (DMSO-d6, 75.0 MHz, ppm): δ 165.0; 148.4; 146.9; 132.6; 126.2; 121.8; 121.3; 120.3; 111.6; 107.8. ESI-MS: m/z [M-H]⁻: 300.27.

2.2. Biological assays

2.2.1. Cytotoxicity against cancer and normal cell lines

Cell viability was determined through reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product after 72 h as described by
Mosmann. Derivatives (0.312–20 µM) were tested for cytotoxic activity against SKMEL-19 (melanoma), AGP-01 (ascitic fluid) and MCF-7 (breast) cancer cell lines and human lung fibroblast cell line (MRC-5). All cell lines were maintained in DMEM (Dulbecco’s Modified Eagle Medium) medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U mL⁻¹ penicillin, and 100 µM streptomycin at 37°C with 5% CO₂. Each derivative was dissolved in DMSO and diluted with water to obtain a concentration of 20 µM. They were incubated with the cells for 72 h. The negative control received the same amount of DMSO (0.005% at the highest concentration). Doxorubicin was used as a positive control. The IC₅₀ values were calculated by nonlinear regression using the program GraphPad (Intuitive Software for Science, San Diego, CA). SI (selectivity index) values were measured using the ratio between IC₅₀ of the compound against MRC-5 (normal cell line) and IC₅₀ of the same compound against a cancer cell line.

2.2.3. Antimicrobial Susceptibility Testing (AST)

Antibacterial tests were carried out using Staphylococcus aureus (ATCC 25923), Staphylococcus epidermidis (ATCC 12228), Staphylococcus simulans (ATCC 27851), Enterococcus faecalis (ATCC 29212), Enterobacter cloacae (ATCC 23355), Serratia marcescens (ATCC 14756) and Escherichia coli (ATCC 25922) strains. The disk diffusion susceptibility test was performed and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The bacterial suspension was spread with a cotton swab on Mueller Hinton plates and the disks containing the test derivatives (5 mg mL⁻¹) were placed on the inoculated agar surface. Plates were incubated for 24 h at 37°C. The activity of each derivative was compared with vancomycin (30 µg/disk) and ciprofloxacin (5 µg/disk), standard drugs for Gram-positive and Gram-negative strains, respectively. For the antifungal assays, Candida albicans (ATCC 24433), Candida krusei (ATCC 34135), Candida parapsilosis (ATCC 90018), Candida glabrata (ATCC 90030) and Candida tropicalis (ATCC 750) strains were used. The disk diffusion assay was performed according to CLSI guidelines. The inoculum was prepared using 24-hour plate cultures of Candida sp. and was suspended in 0.85% sterile saline. The fungal suspension was spread on a surface with Sabouraud dextrose agar supplemented with 2% glucose using a sterile swab. The disks with derivatives (5 mg mL⁻¹) were placed on an agar surface and incubated at 35°C for 24 h. Ketoconazole (50 µg/disk) was used as the positive control. This assay was used as a screening for the selection of compounds to be evaluated in the assay for determining the Minimum Inhibitory Concentration.

2.2.4. Minimum Inhibitory Concentration (MIC)

Antibacterial activity was evaluated through Minimum Inhibitory Concentration (MIC) assay using the serial dilution method in 96-well microplates. Compounds were dissolved in dimethyl sulfoxide (DMSO) and the stock solution was serially diluted in Mueller Hinton growth medium and incubated at 37°C. For Candida strains, the MIC assay was performed according to CLSI guidelines using RPMI 1640 buffered with 0.165 M MOPS (3-[N-morpholino]propane sulfonic acid) as the test medium. The test derivatives were serially diluted in a 96-well microplate and incubated at 35°C for 24 h. The analyses were performed in triplicate. The MIC value is defined as the lowest concentration of the derivative that inhibits the visible growth of the microorganism tested.

2.2.5. Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The Minimum Bactericidal Concentration (MBC) was determined according to the standardized set of conditions described in guideline M26-A from the Clinical and Laboratory Standards Institute and the study by Peterson and Shanholzer. The MBC assay was performed through transferring the culture medium from each well in MIC microplate with no visible growth (10 µL) to agar plates. After the plates dried, a sterile spreading rod was used...
to evenly disperse the inoculum over the entire surface of the plate. These plates were incubated for 24 h at 37°C and the MBC was determined based on the minimum concentration of derivatives capable of inhibiting 99.9% of bacterial growth. The Minimum Fungicidal Concentration (MFC) was determined as described by Cantón et al.\(^\text{29}\) in which the content of each well with no growth, seen from the MIC assay, was subcultured. The inoculum was homogenized with a micropipette and 100 µL was removed from each of these wells and subcultured onto Sabouraud dextrose agar (Difco) plates. Each aliquot was deposited as a spot onto an agar plate and once they dried, streaking was performed to separate any conidia and remove them from the derivative source. The plates were incubated at 35°C for 48 h. The number of colony forming units was counted in the plates where there was microbial growth. The MFC was the lowest derivative concentration that killed ≥ 99.9% of the initial inoculum. Compounds were classified as bactericidal or fungicidal \textit{a priori} if the MBC/MIC or MFC/MIC ratios did not exceed a value of 4. However, if the ratio was greater than 4, they were considered bacteriostatic or fungistatic.\(^\text{30,31}\)

3. Results and Discussion

3.1. Chemistry

1,3-Benzoxathiol-2-one derivatives \(1,2,5-14\) were prepared as shown in Figure 3.\(^\text{13,14}\) Commercially available 6-hydroxy-benzo[d][1,3]oxathiol-2-one \(4\) was submitted to selective nitration at position 5 leading to the intermediate 2. Nitro derivative 2 was converted to derivatives \(5, 6, 8\) and \(10\) through catalytic hydrogenation, methylation, ethylation and acetylation conditions, respectively. Derivatives 7, 9 and 11 were obtained from 4 through methylation, ethylation and acetylation reactions, respectively. Derivative 6 afforded 12 through catalytic hydrogenation and subsequently 13, under acetylation conditions. Schiff bases \(14a-q\) were obtained in good yields from reactions between intermediate 5 and appropriate benzaldehydes or heteroaromatic benzaldehydes in ethanol at room temperature. Derivatives \(3,16a-q\) and \(17a-c\) were synthesized from reactions between the commercially available 2-hydrazinyl-1,3-benzothiazole \(15\) and aromatic aldehydes (Figure 4).\(^\text{15,16,18}\)

Spectral data (IR, \(^1\)H NMR, \(^13\)C NMR and ESI-MS) of new compounds \(8, 12, 13\) and \(16m\) are in full agreement with the proposed structures (See Supplementary Material).

The synthesis and characterization of the 1,3-benzothiazol-2-one derivatives \(5-7, 9-11\) and \(1,14a-q\) (Figure 3) and 1,3-benzothiazole compounds, \(3, 16a-l,n-q\) and \(17a-c\) (Figure 4) have already been reported in our previous studies.\(^\text{13-16,18}\)

3.2. Biological assays

3.2.1. Cytotoxic Activity and Hemocompatibility

The anticancer activity of compounds \(1-3, 5, 14a-q, 16a-l,n-q\) and \(17a-c\) was previously reported in our studies.\(^\text{13,15,18}\)

In vitro cytotoxic activity of derivatives \(6-13\) was assessed against melanoma (SKMEL-19), ascitic fluid (AGP-01), breast (MCF-7) cancer cells and human lung fibroblast cell line (MRC-5) and compared to doxorubicin using an MTT assay.\(^\text{21}\) As shown in Table 1, derivative 7 was active against SKMEL-19 with an IC\(_{50}\) value of 3.3 µM and SI value > 3, indicating good selectively for this cancer cell line. The SI reveals the differential activity of a compound; therefore, the higher the SI value is, the more selective it is. On the other hand, an SI value < 2 suggests general toxicity of the compound.\(^\text{32}\) Compound 8 displayed good cytotoxicity against AGP-01 and MCF-7 with IC\(_{50}\) values of 3.0 µM and 3.2 µM, respectively. These results are in accordance with National Cancer Institute (NCI) protocols, where compounds exhibiting IC\(_{50}\) values < 10 µM or 15 µM are considered active.\(^\text{33}\) However, this compound had a lower selectivity for cancer cells when compared to normal cells.

Among the alkylated or acetylated derivatives \(6-13\), derivative 7, with a methoxy group at position C-6, and derivative 8, containing a nitro group and an ethoxy group at positions C-5 and C-6, respectively, were found to be active. Although derivative 8 also exhibited cytotoxicity against the human lung fibroblast cell line with an IC\(_{50}\) of 1.8 µM, this cytotoxicity is 9 times less than that of doxorubicin, the control drug, known to present severe side effects in cancer treatment.\(^\text{34}\) Further studies with long or cyclic side chains (e.g. propyl, butyl, pentyl, benzyl and cyclopropyl) may enable the exploration of new lead molecules containing a 1,3-benzoxathioli-2-one core with the nitro group at position C-5 as well as no substitution at this position.
The mechanical stability of red blood cells is a good parameter for in vitro screening of hemocompatibility, since the erythrocyte membranes can suffer significant changes in their structural properties depending on the drug used in treatment.22 Interestingly, derivatives 6-13 showed no hemolytic activity (EC50 > 200 μg mL−1) (Table 1). Therefore, we may suggest that the mechanism involved in cytotoxicity against cancer cells is most likely not related to nonspecific membrane damage.

### 3.2.2. Antimicrobial Activity

Compounds 2, 5-7 and 9-11 have already been evaluated in vitro against seven bacterial strains, including Gram-positive (Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus simulans and Enterococcus faecalis) and Gram-negative (Enterobacter cloacae, Serratia marcescens and Escherichia coli) bacteria and five Candida strains (Candida albicans, Candida krusei, Candida parapsilosis, Candida glabrata and Candida tropicalis). Derivatives 2, 7 and 11 displayed poor antibacterial activity, when compared to vancomycin and ciprofloxacin. On the other hand, significant antifungal activity was exhibited by compounds 2 and 10, highlighting derivative 2 with MIC value of 4 μg mL−1 (compared to ketoconazole) against C. krusei.

In this study, in vitro antimicrobial screening of derivatives 1,14a-q, 3, 16a-q and 17a-c was performed using the same seven bacterial strains and five Candida strains previously mentioned. Results, expressed as inhibitory growing zone diameters (halo = mm), pointed to 1,3-benzoxathiol-2-one derivatives 1, 14c, 14d and 14n, as well as 1,3-benzothiazole derivatives 16h, 16j, 16k, 16l and 16m, as having antimicrobial activity against some Gram-positive, Gram-negative or Candida strains (Table 2).

All derivatives that were active in the disk diffusion assay were also active in the MIC assay. However, the microdilution broth assay is a quantitative and more appropriate method for assessing the compounds’ activity.23

The tested derivatives showed a varying degree of inhibition when compared to the standard drugs vancomycin, ciprofloxacin and ketoconazole (Table 3). The minimal inhibitory concentration (MIC) evaluation showed that among the 1,3-benzoxathiol-2-one derivatives, compounds 1 and 14n, with a hydroxyl group at position C-2’, exhibited the highest antibacterial activity. Derivative 14n was found to be the most active against S. epidermidis with MIC value of 32 μg mL−1. All the tested 1,3-benzoxathiol-2-one derivatives were inactive against Candida species. Among the 1,3-benzothiazole derivatives, 16l and 16m were the most active against Gram-positive bacteria and Candida species, respectively. Compound 16l showed a MIC value of 32 μg mL−1 against S. aureus and S. simulans, whereas 16m exhibited MIC values ranging from 8 to 32 μg mL−1 against Candida species. Although the MIC values of the derivatives are higher than those obtained for standard drugs, the search for new active

### Table 1. Cytotoxic activity of 1,3-benzoxathiol-2-one derivatives against cancer (SKMEL-19, AGP-01, MCF-7) and normal (MRC-5) cell lines2

| Compound | MTT IC50(µM)/Selectivity index (SI) | Hemolysis |
|----------|----------------------------------|-----------|
|          | SKMEL-19 | AGP-01 | MCF-7 | MRC-5 | EC50b (µg mL−1) |
| 6        | >10      | >10    | >10    | >10    | >200 |
| 7        | 3.3 (3.0–3.6)         | >10    | >10    | >10    | >200 |
| 8        | >10      | 3.0 (2.6–3.4)         | >10    | >10    | >200 |
| 9        | >10      | >10    | >10    | >10    | >200 |
| 10       | >10      | >10    | >10    | >10    | >200 |
| 11       | >10      | >10    | >10    | >10    | >200 |
| 12       | >10      | >10    | >10    | >10    | >200 |
| 13       | >10      | >10    | >10    | >10    | >200 |
| Dox      | 0.03 (0.031–0.041) | 0.25 (0.19–0.33) | 0.95 (0.73–1.24) | 0.20 (0.16–0.25) | >200 |

Notes: In vitro antimicrobial screening of derivatives 1,14a-q, 3, 16a-q and 17a-c was performed using the same seven bacterial strains and five Candida strains previously mentioned. Results, expressed as inhibitory growing zone diameters (halo = mm), pointed to 1,3-benzoxathiol-2-one derivatives 1, 14c, 14d and 14n, as well as 1,3-benzothiazole derivatives 16h, 16j, 16k, 16l and 16m, as having antimicrobial activity against some Gram-positive, Gram-negative or Candida strains (Table 2). All derivatives that were active in the disk diffusion assay were also active in the MIC assay. However, the microdilution broth assay is a quantitative and more appropriate method for assessing the compounds’ activity.23

The tested derivatives showed a varying degree of inhibition when compared to the standard drugs vancomycin, ciprofloxacin and ketoconazole (Table 3). The minimal inhibitory concentration (MIC) evaluation showed that among the 1,3-benzoxathiol-2-one derivatives, compounds 1 and 14n, with a hydroxyl group at position C-2’, exhibited the highest antibacterial activity. Derivative 14n was found to be the most active against S. epidermidis with MIC value of 32 μg mL−1. All the tested 1,3-benzoxathiol-2-one derivatives were inactive against Candida species. Among the 1,3-benzothiazole derivatives, 16l and 16m were the most active against Gram-positive bacteria and Candida species, respectively. Compound 16l showed a MIC value of 32 μg mL−1 against S. aureus and S. simulans, whereas 16m exhibited MIC values ranging from 8 to 32 μg mL−1 against Candida species. Although the MIC values of the derivatives are higher than those obtained for standard drugs, the search for new active
Table 2. Antimicrobial Susceptibility Testing results for 1,3-benzoxathiol-2-one and 1,3-benzothiazole derivatives against Gram-positive and Gram-negative bacteria, as well as Candida strains using the disk diffusion method$^{a,b}$

| Compound | Gram-positive | Gram-negative | Fungi |
|----------|---------------|---------------|-------|
|          | S.a. | S.e. | S.s. | E.f. | E.c. | S.m. | E.co. | C.a. | C.k. | C.p. | C.g. | C.t. |
| 1        | 25923 | 12228 | 27851 | 29212 | 23355 | 14756 | 25922 | 24433 | 34135 | 90018 | 90030 | 750 |
| 13228    | 10    | 0    | 0    | 0    | 0    | 0    | 0    | -    | -    | -    | -    | -    |
| 14c      | 0     | 0    | 0    | 0    | 0    | 0    | 0    | -    | -    | -    | -    | -    |
| 14d      | 13    | 0    | 0    | 0    | 0    | 0    | 0    | -    | -    | -    | -    | -    |
| 14n      | 8     | 10   | 7    | 10   | 0    | 0    | 0    | -    | -    | -    | -    | -    |
| 16h      | 0     | 0    | 9    | 0    | 0    | 0    | 0    | -    | -    | -    | -    | -    |
| 16j      | 0     | 0    | 0    | 0    | 13   | 0    | 0    | -    | -    | -    | -    | -    |
| 16k      | 0     | 0    | 10   | 0    | 0    | 10   | 0    | -    | -    | -    | -    | -    |
| 16l      | 5     | 5    | 12   | 6    | 0    | 0    | 0    | -    | -    | -    | -    | -    |
| 16m      | 6     | 6    | 5    | 0    | 0    | 0    | 0    | 9    | 10   | 8    | 8    | 10   |
| Van      | 14    | 15   | 18   | 16   | -    | -    | -    | -    | -    | -    | -    | -    |
| Cip      | -     | -    | -    | -    | -    | 128  | -    | -    | -    | -    | -    | -    |
| Keto     | -     | -    | -    | -    | -    | -    | 32   | 29   | 32   | -    | -    | -    |
| DMSO     | -     | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |

$^a$Zones of inhibition in millimeters. $^b$Abbreviations: S.a.: Staphylococcus aureus, S.e.: Staphylococcus epidermidis, S.s.: Staphylococcus simulans, E.f.: Enterococcus faecalis, E.c.: Enterobacter cloacae, S.m.: Serratia marcescens, E.co.: Escherichia coli, C.a.: C. albicans, C.k.: C. krusei, C.p.: C. parapsilosis, C.g.: C. glabrata, C.t.: C. tropicalis, Van: vancomycin, Cip: ciprofloxacin, Keto: ketoconazole, (-) Not tested. Experiments were performed in triplicate

Table 3. Minimum Inhibitory Concentration (MIC), in μg mL$^{-1}$ of 1,3-benzoxathiol-2-one and 1,3-benzothiazole compounds against Gram-positive and Gram-negative bacteria and Candida strains

| Comp. | Gram-positive | Gram-negative | Fungi |
|-------|---------------|---------------|-------|
|       | S.a. | S.e. | S.s. | E.f. | E.c. | S.m. | E.co. | C.a. | C.k. | C.p. | C.g. | C.t. |
| 1     | 25923 | 12228 | 27851 | 29212 | 23355 | 14756 | 25922 | 24433 | 34135 | 90018 | 90030 | 750 |
| 13228 | 128  | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| 14c   | -     | -    | -    | -    | -    | 256  | -    | -    | -    | -    | -    | -    |
| 14d   | 64    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| 14n   | 64    | 32   | -    | 128  | -    | -    | -    | -    | -    | -    | -    | -    |
| 16h   | -     | 256  | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| 16j   | -     | -    | -    | -    | 256  | -    | -    | -    | -    | -    | -    | -    |
| 16k   | -     | -    | -    | -    | -    | 256  | -    | -    | -    | -    | -    | -    |
| 16l   | 32    | 64   | 32   | 64   | -    | -    | -    | -    | -    | -    | -    | -    |
| 16m   | 64    | 256  | 128  | 256  | -    | -    | -    | 32   | 32   | 16   | 8    | 16   |
| Van   | 0.25  | 0.25 | 0.5  | 2    | -    | -    | -    | -    | -    | -    | -    | -    |
| Cip   | -     | -    | 0.03 | 0.125| 0.125| -    | -    | -    | -    | -    | -    | -    |
| Keto  | -     | -    | 0.125| 1    | 0.03 | 2    | 0.125| -    | -    | -    | -    | -    |

$^a$Abbreviations: S.a.: Staphylococcus aureus, S.e.: Staphylococcus epidermidis, S.s.: Staphylococcus simulans, E.f.: Enterococcus faecalis, E.c.: Enterobacter cloacae, S.m.: Serratia marcescens, E.co.: Escherichia coli, C.a.: C. albicans, C.k.: C. krusei, C.p.: C. parapsilosis, C.g.: C. glabrata, C.t.: C. tropicalis, Van: vancomycin, Cip: ciprofloxacin, Keto: ketoconazole, (-) Not tested. Experiments were performed in triplicate
Compounds is extremely important. This fact is justified by the toxicity of available drugs and their antimicrobial resistance.\textsuperscript{36,37}

Taken together, the structural analysis and the biological data showed the importance of the two hydroxyl groups at positions C-3' and C-4', which revealed 16l as the most active derivative against Gram-positive strains. The analysis also pointed out three neighboring hydroxyl groups (derivative 16m) as having some role in the compound’s antifungal profile, especially against \textit{C. glabrata} (MIC = 8 μg mL\textsuperscript{-1}) (Table 3).

Currently, the literature reports \textit{C. glabrata} as being the second most common cause of mucosal and invasive infection with a resistant profile against several clinical azole antifungals (e.g. fluconazole and miconazole).\textsuperscript{38,39} Thus, the results obtained in this study can be used for further research aimed to develop new antifungal agents containing 1,3-benzothiazole moiety.

Compounds 14c, 16j and 16k showed low inhibition profiles against Gram-negative strains (MIC = 256 μg mL\textsuperscript{-1}), which reinforced the problem of finding new derivatives targeting these pathogens. Gram-negative bacteria have a complex cell wall with an extra membrane layer that provides a barrier for drugs that penetrate the cell wall and makes them more resistant to antimicrobials.\textsuperscript{40}

Data on bactericidal/fungicidal or bacteriostatic/fungistatic effects may provide important information on the potential action of derivatives \textit{in vitro}.\textsuperscript{41,42} In Table 4, the results of MBC/MIC ratio values for compounds 16l and 16m against \textit{S. epidermidis} ATCC 12228 and \textit{S. simulans} ATCC 27851 (≤ 2), allowed us to classify them as bactericidal agents despite their modest activity. For the other species of bacteria and fungi, the active derivatives (1, 14c, 14d, 14n, 16j, 16k, 16l and 16m) showed a bacteriostatic or fungistatic profile.

**Table 4.** MBC, MFC and ratios (MBC/MIC or MFC/MIC) for 1,3-benoxathiol-2-one and 1,3-benzothiazole derivatives

| Species         | Compound   | MBC or MFC (μg mL\textsuperscript{-1}) | MBC/MIC ratio | MFC/MIC ratio |
|-----------------|------------|----------------------------------|---------------|---------------|
| **Bacteria**    |            |                                  |               |               |
| S. aureus ATCC 25923 | 1           | 512                              | 8             | *             |
|                 | 14d        | > 512                            | ≥ 16          | *             |
|                 | 14n        | 256                              | 4             | *             |
|                 | 16l        | 256                              | 8             | *             |
|                 | 16m        | 512                              | 8             | *             |
|                 | 14n        | 128                              | 4             | *             |
| S. epidermidis ATCC 12228 | 16l        | 128                              | 2             | *             |
|                 | 16m        | 256                              | 1             | *             |
|                 | 1          | 512                              | 4             | *             |
|                 | 16h        | > 512                            | ≥ 4           | *             |
|                 | 16l        | > 512                            | ≥ 32          | *             |
|                 | 16m        | 128                              | 1             | *             |
|                 | 14n        | 512                              | 4             | *             |
| S. simulans ATCC 27851 | 16l        | > 512                            | ≥ 16          | *             |
|                 | 16m        | > 512                            | ≥ 4           | *             |
| E. faecalis ATCC 29212 | 16j        | > 512                            | ≥ 4           | *             |
| E. cloacae ATCC 23355 | 14c        | > 512                            | ≥ 4           | *             |
| S. marcencens ATCC 14756 | 16k        | > 512                            | ≥ 4           | *             |
| **Yeast**       |            |                                  |               |               |
| C. albicans ATCC 24433 |            | 128                              | *             | 4             |
| C. krusei ATCC 34135 |            | 128                              | *             | 4             |
| C. parapsilosis ATCC 90018 | 16m       | 128                              | *             | 8             |
| C. glabrata ATCC 90030 |            | 32                               | *             | 4             |
| C. tropicalis ATCC 750 |            | 128                              | *             | 8             |

MBC/MIC or MFC/MIC ≤ 2 = bactericidal or fungicidal activity; MBC/MIC or MFC/MIC ≥ 4 = bacteriostatic or fungistatic activity. *Not applicable
4. Conclusion

In summary, 1,3-benzoxathiol-2-one and 1,3-benzothiazole compounds, among which four are herein reported for the first time, have been evaluated for in vitro anticancer and antimicrobial activity. Results point to derivative 7, 6-methoxybenzo[d][1,3]oxathiol-2-one, as the most promising molecule for anticancer drug design, since it exhibited considerable cytotoxicity against melanoma (SKMEL-19) but not against normal cells (MRC-5). Regarding antimicrobial activity, derivative 16m appears to be an interesting antifungal prototype that should be further explored, since it was active against all Candida strains, highlighting its activity against C. glabrata (MIC = 8 µg mL⁻¹).

Acknowledgements

The authors thank UFF, FAPERJ, CNPq, CAPES and Farmanguinhos/FIOCRUZ for their support. Fellowships granted to E.L.C., L.T., L.F.E.M., M.F.C., J.S.N. by CAPES and to P.S.S., L.C.P., M.V.N.S., R.C.M., A.M.S.F., H.C.C. and T.R.A.V. by CNPq.

References

1. World Health Organization (WHO). Disponível em: <http://www.who.int/mediacentre/factsheets/fs297/en/>. Acesso em: 23 março 2020.
2. National Cancer Institute (NCI). Disponível em: <https://epi.grants.cancer.gov/infectious-agents/>. Acesso em: 23 março 2020.
3. Casper, C.; Fitzmaurice, C. Infection-related cancers: prioritising an important and eliminable contributor to the global cancer burden. *Lancet Global Health* 2016, 4, e580. [CrossRef][PubMed]
4. Plummer, M.; de Martel, C.; Vignat, J.; Ferlay, J.; Bray, F.; Franceschi, S. Global burden of cancers attributable to infections in 2012: a synthetic analysis. *Lancet Global Health* 2016, 4, e609. [CrossRef][PubMed]
5. Kagan, R. M.; Dunn, K. J.; Snell, G. P.; Nettles, R. E.; Kaufman, H. W. Trends in HIV-1 Drug Resistance Mutations from a U.S. Reference Laboratory from 2006 to 2017. *AIDS Research and Human Retroviruses* 2019, 35, 1. [CrossRef]
6. Ghosh, C.; Sarkar, P.; Issa, R.; Halder, J. Alternatives to conventional antibiotics in the era of antimicrobial resistance. *Trends in Microbiology* 2019, 27, 323. [CrossRef][PubMed]
7. Burotto, M.; Wilkerson, J.; Stein, W. D.; Bates, S. E.; Fojo, T. Adjuvant and neoadjuvant cancer therapies: A historical review and a rational approach to understand outcomes. *Seminars in Oncology* 2019, 46, 83. [CrossRef][PubMed]
8. a) Katritzky, A. R. Preface. *Tetrahedron* 1996, 52, xiii; [CrossRef] b) Balaban, A. T.; Oniciu, D. C.; Katritzky, A. R. Aromaticity as a cornerstone of heterocyclic chemistry. *Chemical Reviews* 2004, 104, 2777; [CrossRef] c) Patel, A. B.; Raval, R.; *Importance of Heterocycles in Medicinal Chemistry*. LAP Lambert Academic Publishing: New York, 2015; d) Gomtsyan, A. Heterocycles in drugs and drug discovery. *Chemistry of Heterocyclic Compounds* 2012, 48, 7. [CrossRef]
9. Vellasco Júnior, W. T.; Gomes, C. R. B.; Vasconcelos, T. R. A. Chemistry and biological activities of 1,3-benzoxathiols-2-ones. *Mini-Reviews in Organic Chemistry* 2011, 8, 103. [CrossRef]
10. Facchinetti, V.; Reis, R. R.; Gomes, C. R. B.; Vasconcelos, T. R. A. Chemistry and biological activities of 1,3-benzothiazoles. *Mini-Reviews in Organic Chemistry* 2012, 9, 44. [CrossRef]
11. Prajapati, N. P.; Vekariya, R. H.; Borad, M. A.; Patel, H. D. Recent advances in the synthesis of 2-substituted benzothiazoles: a review. *RSC Advances* 2014, 4, 60176. [CrossRef]
12. Kamal, A.; Syed, M. A. H.; Mohammed, S. M. Therapeutic potential of benzothiazoles: a patent review (2010-2014). *Expert Opinion on Therapeutic Patents* 2015, 25, 335. [CrossRef][PubMed]
13. Chazin, E. L.; Sanches, P. S.; Lindgren, E. B.; Vellasco Júnior, W. T.; Pinto, L. C.; Burbano, R. M. R.; Yoneda, J. D.; Leal, K. Z.; Gomes, C. R. B.; Wardell, J. L.; Wardell, S. M. S. V.; Montenegro, R. C.; Vasconcelos, T. R. A. Synthesis and biological evaluation of novel 6-hydroxy-benzo[d][1,3] oxathiol-2-one Schiff bases as potential anticancer agents. *Molecules* 2015, 20, 1968. [CrossRef]
14. Terra, L.; Chazin, E. L.; Sanches, P. S.; Saito, M.; de Souza, M. V. N.; Gomes, C. R. B.; Wardell, J. L.; Wardell, S. M. S. V.; Montenegro, R. C.; Vasconcelos, T. R. A. Evaluation of 1,3-benzoxathiol-2-one derivatives as potential antifungal agents. *Medicinal Chemistry* 2018, 14, 304. [CrossRef][PubMed]
2011, 1036, 19. [CrossRef]

2021, 22, 381. [CrossRef] [PubMed]

2020, 103, 121. [CrossRef] [PubMed]

2020, 78, 4. [CrossRef] [PubMed]

2020, 38, 3778. [CrossRef] [PubMed]

2020, 46, 1448. [CrossRef] [PubMed]

2020, 7, 551. [CrossRef] [PubMed]

2020, 3, 851. [Link]

2020, 11:18. [CrossRef] [PubMed]

1998, 38, 3778. [CrossRef] [PubMed]

1997, 30, 1036. [CrossRef] [PubMed]

1996, 12, 1448. [CrossRef] [PubMed]

1995, 37, 121. [CrossRef] [PubMed]

2019, 67, 8. [CrossRef] [PubMed]

1994, 37, 8. [CrossRef] [PubMed]

1993, 36, 3778. [CrossRef] [PubMed]

1992, 10, 121. [CrossRef] [PubMed]

1991, 30, 1036. [CrossRef] [PubMed]

1990, 29, 1036. [CrossRef] [PubMed]

1989, 28, 1036. [CrossRef] [PubMed]

1988, 27, 1036. [CrossRef] [PubMed]

1987, 26, 1036. [CrossRef] [PubMed]

1986, 25, 1036. [CrossRef] [PubMed]

1985, 24, 1036. [CrossRef] [PubMed]

1984, 23, 1036. [CrossRef] [PubMed]

1983, 22, 1036. [CrossRef] [PubMed]

1982, 21, 1036. [CrossRef] [PubMed]

1981, 20, 1036. [CrossRef] [PubMed]

1980, 19, 1036. [CrossRef] [PubMed]

1979, 18, 1036. [CrossRef] [PubMed]

1978, 17, 1036. [CrossRef] [PubMed]

1977, 16, 1036. [CrossRef] [PubMed]

1976, 15, 1036. [CrossRef] [PubMed]

1975, 14, 1036. [CrossRef] [PubMed]

1974, 13, 1036. [CrossRef] [PubMed]

1973, 12, 1036. [CrossRef] [PubMed]

1972, 11, 1036. [CrossRef] [PubMed]

1971, 10, 1036. [CrossRef] [PubMed]

1970, 9, 1036. [CrossRef] [PubMed]

1969, 8, 1036. [CrossRef] [PubMed]

1968, 7, 1036. [CrossRef] [PubMed]

1967, 6, 1036. [CrossRef] [PubMed]

1966, 5, 1036. [CrossRef] [PubMed]

1965, 4, 1036. [CrossRef] [PubMed]

1964, 3, 1036. [CrossRef] [PubMed]

1963, 2, 1036. [CrossRef] [PubMed]

1962, 1, 1036. [CrossRef] [PubMed]

2013, 86, 12. [CrossRef]

2012, 19. [CrossRef]

2011, 86, 12. [CrossRef]

2010, 12, 121. [CrossRef] [PubMed]

2009, 55, 3778. [CrossRef] [PubMed]

2008, 37, 3778. [CrossRef] [PubMed]

2007, 36, 3778. [CrossRef] [PubMed]

2006, 35, 3778. [CrossRef] [PubMed]

2005, 34, 3778. [CrossRef] [PubMed]

2004, 33, 3778. [CrossRef] [PubMed]

2003, 32, 3778. [CrossRef] [PubMed]

2002, 31, 3778. [CrossRef] [PubMed]

2001, 30, 3778. [CrossRef] [PubMed]

2000, 29, 3778. [CrossRef] [PubMed]

1999, 28, 3778. [CrossRef] [PubMed]

1998, 27, 3778. [CrossRef] [PubMed]

1997, 26, 3778. [CrossRef] [PubMed]

1996, 25, 3778. [CrossRef] [PubMed]

1995, 24, 3778. [CrossRef] [PubMed]

1994, 23, 3778. [CrossRef] [PubMed]

1993, 22, 3778. [CrossRef] [PubMed]

1992, 21, 3778. [CrossRef] [PubMed]

1991, 20, 3778. [CrossRef] [PubMed]

1990, 19, 3778. [CrossRef] [PubMed]

1989, 18, 3778. [CrossRef] [PubMed]

1988, 17, 3778. [CrossRef] [PubMed]

1987, 16, 3778. [CrossRef] [PubMed]

1986, 15, 3778. [CrossRef] [PubMed]

1985, 14, 3778. [CrossRef] [PubMed]

1984, 13, 3778. [CrossRef] [PubMed]

1983, 12, 3778. [CrossRef] [PubMed]

1982, 11, 3778. [CrossRef] [PubMed]

1981, 10, 3778. [CrossRef] [PubMed]

1980, 9, 3778. [CrossRef] [PubMed]

1979, 8, 3778. [CrossRef] [PubMed]

1978, 7, 3778. [CrossRef] [PubMed]

1977, 6, 3778. [CrossRef] [PubMed]

1976, 5, 3778. [CrossRef] [PubMed]

1975, 4, 3778. [CrossRef] [PubMed]

1974, 3, 3778. [CrossRef] [PubMed]

1973, 2, 3778. [CrossRef] [PubMed]

1972, 1, 3778. [CrossRef] [PubMed]
Selective Cytotoxic Activities of Two Novel Synthetic Drugs on Human Breast Carcinoma MCF-7 Cells. *Anticancer Research* 2009, 29, 2993. [Link] [PubMed]

33) National Cancer Institute (NCI). Disponível em: <https://dtp.cancer.gov/discovery_development/nci-60/default.htm>. Acesso em: 10 de janeiro de 2020.

b) NCI/NIH Developmental Therapeutics Program. Disponível em: http://dtp.nci.nih.gov/branches/btb/handlingprep.html. Acesso em: 20 abril 2020.

34) Tacar, O.; Sriamornsak, P.; Dass, C. R. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. *Journal of Pharmacy and Pharmacology* 2013, 65, 157. [CrossRef] [PubMed]

35) De Bona, E. A. M; Pinto, F. G. S.; Fruet, T. K.; Jorge, T. C. M.; de Moura, A. C. Comparação de métodos para avaliação da atividade antimicrobiana e determinação da concentração inibitória mínima (cim) de extratos vegetais aquosos e etanólicos. *Arquivos do Instituto Biológico* 2014, 81, 218. [CrossRef]

36) Guan, D.; Chen, F.; Qiu, Y.; Jiang, B.; Gong, L.; Lan, L.; Huang, W. Sulfonium, an underestimated moiety for structural modification, alters the antibacterial profile of vancomycin against multidrug-resistant bacteria. *Angewandte Chemie International Edition* 2019, 58, 6678. [CrossRef] [PubMed]

37) Krishnasamy, L.; Krishnakumar, S.; Kumaramanickavel, G.; Saikumar, C. Molecular mechanisms of antifungal drug resistance in *Candida* species. *Journal of Clinical and Diagnostic Research* 2018, 12, DE01. [CrossRef]

38) Rodrigues, C. F; Silva, S.; Henriques, M. *Candida glabrata*: a review of its features and resistance. *European Journal of Clinical Microbiology & Infectious Diseases* 2014, 33, 673. [CrossRef] [PubMed]

39) Sanguinetti, M.; Posteraro, B.; Fiori, B.; Ranno, S.; Torelli, R.; Fadda, G. Mechanisms ofazole resistance in clinical isolates of *Candida glabrata* collected during a hospital survey of antifungal resistance. *Antimicrobial Agents and Chemotherapy* 2005, 49, 668. [CrossRef] [PubMed]

40) Delcour, A. H. Outer membrane permeability and antibiotic resistance. *Biochimica et Biophysica Acta (BBA) – Proteins and Proteomics* 2009, 1794, 808. [CrossRef]

41) Pankey, G. A.; Sabath, L. D. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clinical Infectious Disease* 2004, 38, 864. [CrossRef]

42) Ocampo, P. S; Lázár, V.; Papp, B.; Arnoldini, M.; zur Wiesch, P. A.; Busa-Fekete, R.; Fekete, G.; Pál, C.; Ackermann, M.; Bonhoeffer; S. Antagonism between bacteriostatic and bactericidal antibiotics is prevalent. *Antimicrobial Agents and Chemotherapy* 2014, 58, 4573. [CrossRef]