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Preliminary Pharmacological Prospection Of Bioactive Compounds Obtained By Organic Synthesis Against Multidrug-Resistant Gram-Negative Rods.

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ABSTRACT

Resistance of pathogenic Gram-negative bacteria to antibiotics is an emerging problem and poses serious challenges for efficient pharmacological treatment. The resistance mechanisms used by these bacteria can lead to therapeutic failure. Given this, there is a search for novel compounds to control the growth of these microorganisms. This work evaluated four substances obtained by organic synthesis (encoded RETRO-2, RETRO-4, MTHP and licarin A) against seven multidrug-resistant Gram-negative rods. The Minimum Inhibitory Concentration (MIC) was determined. All compounds showed activity against at least two strains; the best results were obtained with RETRO-2 and RETRO-4, whose MICs ranged between 10^{-4} and 10^{-9} M. Enterobacteriaceae were more sensitive than Non-fermenting Gram-Negative Bacilli. Through the sorbitol test, the probable mechanism of action of RETRO-2 and RETRO-4 was by damage to the cell wall of these bacteria.

Keywords: Bioactive synthetic compounds, alkaloid derivatives, quinazolidines.

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INTRODUCTION

Microbial resistance to antibiotics commonly used in therapy is a critical public health problem worldwide [1]. Recently WHO listed 12 bacteria as priority pathogens [2]. This encourages the search for new natural bioactive compounds [3]. synthetic bioactive compounds [4], as well as the synthesis of chemical molecules derived from natural compounds [5].

Quinazoline derivatives are alkaloids widely explored because of their diverse pharmacological properties which includes anti-inflammatory, anticonvulsant and antihypertensive [6-8]. There is an emerging interest in the synthesis of quinazoline derivatives in order to obtain compounds with antimicrobial properties through substitutions of halogen atoms or amine group at different positions on the quinazoline ring [9]. According to the literature, the synthesis of quinazoline derivatives may occur from anthranilic acid [10, 11].

Isoquinoline compounds are the most comprehensive plant alkaloids, with approximately 400 molecules. Isoquinoline, tetrahydroisoquinoline, and 1-substituted tetrahydroisoquinoline rings are structures commonly found in these biologically active compounds [12]. They also demonstrate diverse pharmacological properties, as follows: antimalarial and antitumor [13]. analgesic and anti-inflammatory [14] and antimicrobial [15].

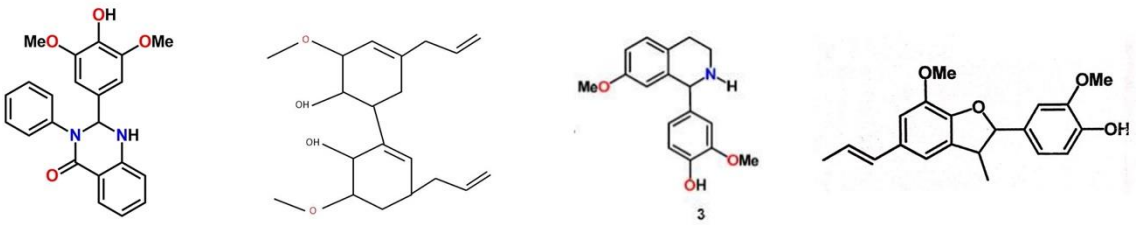
On the other hand, neolignans comprise a class of secondary metabolites with great structural diversity as well as pharmacological activities. They are formed by the coupling of two phenylpropanoid units [16]. They are oxidative dimers of allylphenols and propenylphenols, sequentially or cross-linked, and lack the oxygenated gamma carbon (C-9) found in lignans [17]. The activity described for the class includes anti-schistosomiasis [18], antileishmaniasis [19], antitumor [20] and antibacterial [21]. among others. This work aimed to explore the antimicrobial potential of four substances against seven strains of multidrug-resistant Gram-negative rods.

MATERIALS AND METHODS

Molecules

Four compounds obtained by organic synthesis, with a structure inspired by natural products (Figure 1), encoded by RETRO-2 (2-(4-hydroxy-3,5-dimethoxyphenyl)-3-phenyl-2,3-dihydroquinazolin-4(1H)-one); RETRO-4 (5,5'-dialyl-3,3-dimethoxy-[1,1'-biphenyl]-2,2'-diol); MTHP (1-(3-methoxy-4-hydroxyphenyl)-7-methoxy-1,2,3,4-tetrahydroisoquinoline) and licarin A (dihydrobenzofuran neolignan). The stock solutions of the four compounds were prepared with dimethylsulfoxide (DMSO), obtaining solutions with a concentration of 10^{-1} M, later stored at -20°C.

Figure 1 – bioactive synthetic compounds inspired by natural products: RETRO-2 (A), RETRO-4 (B); MTHP (C) and licarin A (D)



A B C D

Microorganisms

Seven strains of Gram-negative rods were used. They were isolated from sink drains of beauty salons and cosmetic packaging and have been characterized as resistant to different classes of antibiotics and preservative agents [22] *Pseudomonas aeruginosa* RX01, *P. aeruginosa* RX08, *Burkholderia cepacia*

RX02, *Aeromonas hydrophila* RX04, *Escherichia coli* AV12, *Citrobacter freundii* AV13 and *Klebsiella aerogenes* AV14. The strains were maintained in nutrient agar at 4°C.

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC determination was carried out by microdilution technique. The wells were filled with 100 µL of double-concentrated Müeller-Hinton broth and 100 µL of a sample from the respective synthetic compound, diluted in sterile distilled water up from the stock solution, in order to obtain a concentration ranging between 10^{-3} and 10^{-11} M. The suspension from each of the bacterial strains was prepared in 0.85% NaCl solution with turbidity standardized with tube nº 1 of the MacFarland scale and 10 µL of the solution was transferred to each of the wells.

The microplates were incubated at 37°C for 48 hours. Afterwards, aliquots of 25 µL from each well were transferred to 225 µL of Müeller-Hinton broth to verify viability. The MIC was determined by verifying growth inhibition (no turbidity) in each well and comparing with growth in the control [23]. The assay was performed in triplicate. For the control, the viability of the bacteria in broths with and without DMSO was tested.

Identification of the mechanism of action on the cell wall

The most sensitive bacteria in the MIC test were assessed by the sorbitol assay [24]. 225 µL of Müeller-Hinton broth containing the compounds RETRO-2 and RETRO-4 were distributed in microdilution plates, at concentrations MIC÷2, MIC and MICx2 and 25 µL of the suspension of both enterobacteria *E. coli* AV12 and *K. aerogenes* AV14. Both systems, with and without the addition of 0.8 M sorbitol, were tested. After incubation at 37ºC for 48h, aliquots of 25 µL from the wells were transferred to 225 µL of Müeller-Hinton broth to verify viability by turbidity. The assay was performed in triplicate. For the control, the viability of the bacteria in the broth with and without DMSO was tested.

RESULTS

Four strains demonstrated sensitivity to the molecules tested and three were resistant at all concentrations tested (Table 1). Enterobacteriaceae were more sensitive than Non-fermenting Gram-Negative Bacilli. The most sensitive strain was *E. coli* AV12, followed by *K. aerogenes* AV14 and *P. aeruginosa* RX01. The compounds RETRO-2 and RETRO-4 showed the highest potency, recording the lowest MIC values (Table 1). In addition, RETRO-4 was considered the molecule with the greatest spectrum of action, characterizing it as a potential candidate among the tested compounds. On the other hand, MTHP and licarian A were effective against only two strains, with MIC ranging from 10^{-4} to 10^{-5} M, however both were also active against *E. coli* AV12.

The two most sensitive enterobacteria (*E. coli* AV12 and *K. aerogenes* AV14) were evaluated regarding the mechanism of action of the two most active compounds, RETRO-2 and RETRO-4. Later it was confirmed that activity occurred through cell wall damage. In addition, their viability in broth with and without DMSO did not change, compared to the control (data not shown).

Table 1: MIC of the bioactive synthetic compounds on multidrug-resistant Gram-negative rods. The dash (---) indicates that the strains were resistant at all tested concentrations

Strains	Compounds (M)			
	RETRO-2	RETRO-4	MTHP	licarin A
P. aeruginosa RX01	10^{-4}	10^{-5}	10^{-4}	---
P. aeruginosa RX08	---	---	---	---
B. cepacia RX02	---	10^{-5}	---	10^{-4}
A. hydrophila RX04	---	---	---	---
E. coli AV12	10^{-9}	10^{-7}	10^{-5}	10^{-4}
C. freundii AV13	---	---	---	---
K. aerogenes AV14	10^{-7}	10^{-6}	---	---

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DISCUSSION

Microorganism resistance to physical and chemical agents has been known since the beginning of the antibiotic era. With the emergence and clinical use of sulfonamides (1933) and the industrial scale production of penicillin (1941), resistance to antimicrobials has been seen to be wither a natural characteristic of microbes or acquired by individual strains within a sensitive population [25].

The development of effective drugs in the treatment of bacterial infections has drastically transformed medical treatment and caused bacteria to develop defenses to the drugs [26]. This fact highlights the need to stimulate further research and strategies to enhance the efficacy of currently available antibiotics as well as promote the discovery of new classes of antibacterial agents [27-29].

Plants are an important source of biologically active products, serving as models for the synthesis of various drugs [30]. Higher plants evolved from defense selection against microbes. This has led to the belief that synthetic products inspired by botanical bioactive sources may promote less microbial tolerance and resistance [31].

Neolignans are known as a class of secondary metabolites with structural diversity and pharmacological activities [32]. In the present study, licarin A represented this class. Little activity and high MIC values, however, were demonstrated for the tested strains. The data coincided with findings from a previous study in which Gram-negative rods showed limited efficacy [33]. In these cases, differences in permeability barriers are attributed to the lower sensitivity of Gram-negatives compared to Gram-positives. In Gram-negative bacteria, the outer membrane is a very effective barrier against amphipathic compounds [34].

This behavior has also been observed for derivatives of isoquinoline alkaloids, such as MTHP [35, 36]. The activity of this class against Gram-positive bacteria is well described in the literature. Many Gram-negative strains, however, are resistant to isoquinoline derivatives, such as o-methylmoschatolin, isomoschatolin, nornuciferin, isocoreximin, and liriodenine [37-39]. Thus, it is suggested that studies with MTHP should be conducted in Gram-positive pathogens.

On the other hand, quinazoline alkaloids have been considered privileged structures in medicinal chemistry. These structures represent molecules that can bind at multiple sites, showing high affinity. Because of this, they may favor the faster discovery of potentially useful therapeutic compounds [40]. In terms of antibiotic therapy, quinazoline derivatives have demonstrated activity against both Gram-positive bacteria and fungi through interaction with cell walls and DNA structures. Alfuzosin hydrochloride, prazosin hydrochloride, doxazosin mesylate and terazosin hydrochloride are some quinazoline derivatives that have been approved from commercial proposes [41].

Based on the information gathered above, this study analyzed the synthetic quinazoline derivatives, RETRO-2 and RETRO-4 and found them to have antimicrobial activity, differing only in the MIC values. In view of the results obtained, the possible mechanism of action on the permeability of the cell wall was tested, because the chemical skeleton of the synthesized molecules might be able to suggest possible targets in the cell [42]. Thus, the test in the presence of sorbitol, indicated cell wall damage with the compounds RETRO-2 and RETRO-4 proving to be the most active. More studies should be conducted with these compounds, however, in terms of their safety, efficacy and toxicity, and to access the elucidation of their active mechanisms. In addition, to have the information knowing that all strains used in this study are multidrug-resistant, it is suggested that investigations into the association of these compounds with standard antibiotics should also be considered.

CONCLUSION

All alkaloid derivatives evaluated in this study demonstrated activity against at least two species of Gram-negative rods. The compounds RETRO-2 and RETRO-4 showed the best results, considering the MIC values and the number of sensitive strains, especially Enterobacteriaceae and *Pseudomonas aeruginosa*, whose proposed mechanism of action was cell wall disruption.

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