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RESEARCH ARTICLE

DESIGN AND SYNTHESIS OF *N*-[3-(3-OXOPROP-1-YN-1-YL) PHENYL] BENZENE SULFONAMIDE: A HIGHLY ACTIVE ANTIMICROBIAL COMPOUND

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ABSTRACT

The synthesis of the highly active compound *N*-[3-(3-oxoprop-1-yn-1-yl)phenyl] benzene sulfonamide was performed and its activity against Gram (+), Gram (-), mold and yeast was determined.

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INTRODUCTION

Before the year 1900 only a few therapies were known for the treatment of the following diseases: mercury for syphilis, cinchona bark for malaria and ipecacuanha for dysentery. Paul Ehrlich (1854-1915), a German medicine doctor, was a pioneer looking for dyes that could kill microorganisms, especially Trypanosomes.[1]He was intrigued about the ability of some dyes to stain anatomical tissues selectively (https:// www.sciencehistory.org/historical-profile/paul-erlich). He believed that the staining of the cells by dyes was the result of a chemical reaction. In 1903 he found a dye, that he called Trypan Red I,1, (Figure 1) that healed the mice infected from some types of Trypanosomes. Finally, in 1910, after 15 years of research, he discovered an arsenic compound called Salvarsan, 2, (Figure 1) effective against Spirochaeta, the virus that causes syphilis. Unfortunately, it showed serious side effects including convulsions and dead (Tan, 2010). He was awarded the Nobel Prize in medicine in 1908. Between 1909 and 1935 a great deal of attention was focus on the discovery of synthetic compounds that possessed antibacterial activity. From thousands of chemicals tested, very few were found to have a promising effect, until a compound called Prontosil, 3, (Scheme 1) was discovered, in 1932, by the pharmaceutical division of IG Farbenindustrie, an industrial conglomerate of German companies, including Bayer Company. It was synthesized by chemists Fritz Mietzsch and Josef Klarer, and tested by physician Gerhard Domagk (Tréfouël, 1935). Prontosil, 3, was found to be very effective against bacterial infections in mice. Domagk investigated the antibacterial properties of Prontosil, 3, which was very successful treating several diseases in humans, provoked by staphylococcus and streptococcus. In 1935, Domagk used prontosil, 3, to treat his own daughter, who had contracted a severe streptococcal infection. Domagk treated her with an oral dose of this compound, saving her life within a short time. In 1939, he was awarded the Nobel prize in Medicine, because of his discovery of the first synthetic drug effective against bacterial infections. Since then,a new era in modern chemotherapy begun. Prontosil, 3, was the first synthetic antibacterial drug, with life-saving capability, systematically used for the treatment of bacterial infections in the body. It belongs to a family of compounds called sulfa drugs or sulfonamides. The basic, structural framework contained in these drugs is a sulfonamide group (Scheme 1). Later, in 1936, it was discovered at the Pasteur Institute that Prontosil, 3, is metabolized in the human body to produce a compound named sulfanilamide, 4, (*p*-aminobenzenesulfonamide) (Scheme 1), a colorless substance, which is the active agent against streptococci. Then, prontosil, 3, was reclassified as a pro-drug (Tréfouël, 1935).

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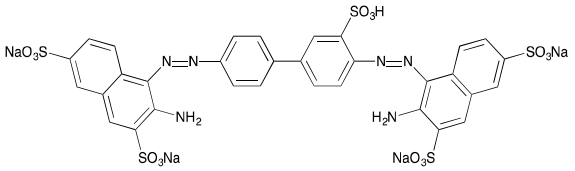
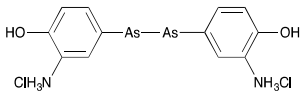
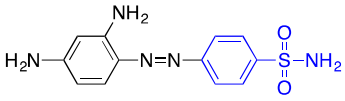


1, Trypan Red I

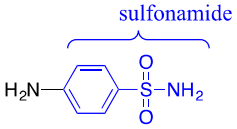


2, Salvarsan

Figure 1. First synthetic compounds used to treat infections and virus



3, Prontosil



4, Sulfanilamide

Scheme 1. Metabolization of prontosil in the body

Prontalbin became the first oral version of sulfanilamide manufactured by Bayer. However, sulfanilamide itself, 4, is very toxic for general use, and thousands of chemical variations were made on the structure of sulfanilamide, in search of better chemotherapeutic effects. Among the most successful sulfa drugs discovered are: sulfadiazine, 5, used in the treatment of toxoplasmosis and in the prevention of rheumatic fever recurrence (WHO Model Formulary 2008), sulfathiazole, 6, used as an oral and topical antimicrobial (Rouf, 2015), sulfacetamide, 7, used for the treatment of acne and seborrheic dermatitits, it also has anti-inflamatory properties as a treatment of blepharitis or conjunctivitis, and sulfamethoxazole, 8, (Figure 2) used to treat urinary tract infections, bronchitis, prostatitis and also is effective against Gram (+) and Gram (-) bacteria. In the mid 1040's and 1950's most of the sulfa drugs were replaced by penicillin and other antibacterial compounds that proved to be more effective against more types of bacteria. Some sulfa drugs such as sulfamethoxazole, 8, in combination with trimethoprim (co-trimoxazole), are still used extensively to inhibit the growth of bacteria that produce opportunistic infections in patients with AIDS, and bacterial infections such as pneumonia, bronchitis and infections of the urinary tract, ears and intestines (https://medlineplus.gov/druginfo/meds/a684026.html).

We would like to report herein the synthesis *N-[3-(3-oxoprop-1-yn-1-yl)phenyl] benzenesulfonamide*, 9, a new highly active sulfanilamide derivative, and the determination of its activity against mold, yeast, Gram (+) and Gram (-) bacteria.

RESULTS AND DISCUSSION

Synthesis: We recently reported (Cabezas, 2019) the synthesis of *N*-[3-(prop-1-yn-1-yl) phenyl] benzenesulfonamide, 10, and the determination of its antibacterial activity against *Staphylococcus aureus* (G+) and *Escherichia coli* (G-) bacteria. Acetylenic benzensulfonamide 10 was prepared by reaction of 3-iodoaniline, 12, with benzenesulfonyl chloride, 11, in the presence of pyridine to obtain, after purification by column chromatography, iodosulfonamide, 13, in 75% yield. This aromatic iodide 13, was treated with propyne, under Sonogashira´s reaction conditions (Sonogashira, 1975) to obtain 10, in 70%. The overall yield of this synthesis was 53% (Scheme 2). Since both compounds, iodide 13 and acetylene 10, are sulfonamide derivatives, we decided to test the antibacterial activity of both of them, and compare the substitution effect, of an iodide in 13, and an acetylene in 10, on their biological activity.

Iodide 13 inhibited *S. aureus* and *E. coli* growth at a concentration of 256 µg/mL and 125 µg/mL respectively. When the antibacterial activity of sulfonamide 10 was tested against *Staphylococcus aureus* and *Escherichia coli*, and the minimum inhibitory concentrations (MIC) were determined values of 12.5 µg/mL and 25.0 µg/mL were obtained respectively (Cabezas, 2019). Remarkably, when the iodide substituent in 13, was replaced by an acetylene group (propyne), in compound 10, the activity against *Staphylococcus aureus* increased by 20.5 times, and the activity against *Escherichia coli* was 5 times as much as compound 13. Our next goal was to make chemical modifications to the structure of sulfonamide 10, to increase, even more, its biological activity.

Microorganisms all have different wall compositions, nevertheless, the mechanical strength afforded by this layer of the cell wall is critical for their ability to survive to different environmental conditions. The chemical reaction or attachment of different substances to the proteins of a cell wall may interfere its biosynthesis. Successful treatment of this cell wall with some biosynthesis inhibitors can result in changes to cell shape and size, inducing cellular stress responses, and culminate in cell lysis. In the same way, attachment to proteins of the cell wall may create "holes" that promote cell lysis because of osmotic shock (Kohanski, 2010). We envisioned that, since the presence of the triple bond attached to the aromatic ring in 10, was responsible to increase the activity of this sulfonamide when compare to 13, then making this acetylene unit chemically more reactive would have a direct impact on its biological activity. Thus, adding a carbonyl group, such an aldehyde, adjacent to the triple bond would make this chemical framework more chemically active. Thus, we proposed structure 9, as our synthetic target. Perhaps, a nucleophilic part of an essential protein or enzyme of a microorganism (an amino NH_2 or sulfur SH group), could react on this part of the sulfonamide 9, and get bounded to it and deactivated, as described in Scheme 3. Sulfonamide 9 was prepared from a Sonogashira reaction of iodobenzenesulfonamide 13, with *tert*-butyldimethyl silyl

propargyl ether, to obtain product 14 in 65% yield. Ether 14, was deprotected by treatment with Bu_4NF to produce propargyl alcohol 15, in 85%.

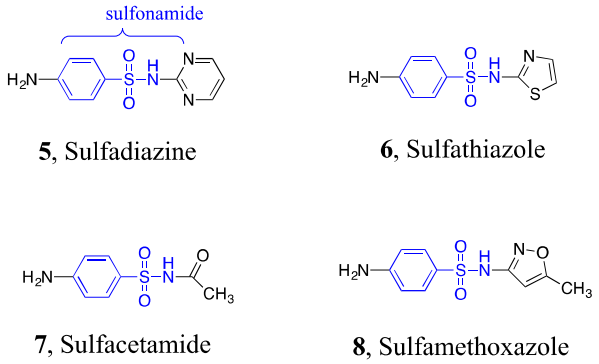
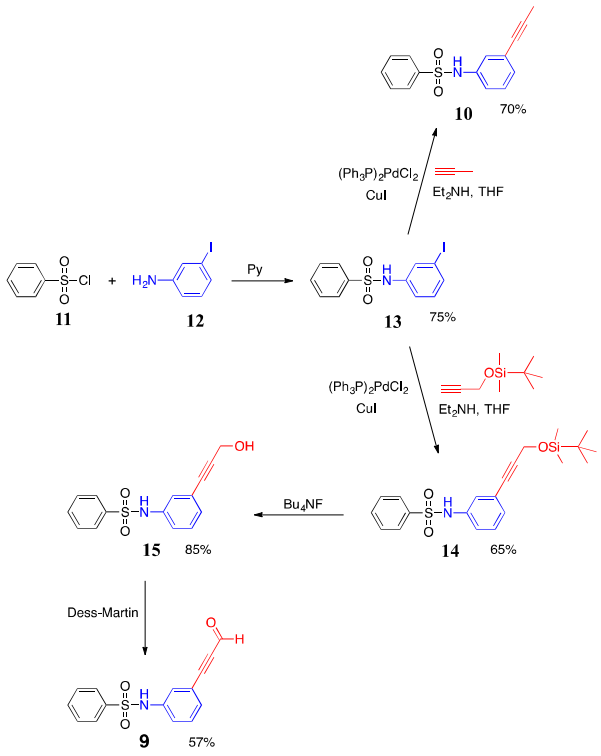
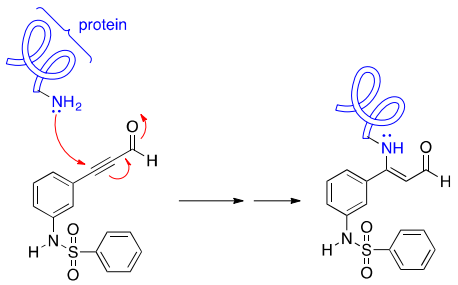


Figure 2. Examples of some sulfa drugs



Scheme 2. Synthesis of *N-[3-(3-oxoprop-1-yn-1-yl)phenyl] benzenesulfonamide*, 9



Scheme 3. Possible mechanism of action of sulfonamide **9**.

Finally, oxidation of propargyl alcohol 15, with Dess-Martin reagent gave the desired title compound 9 in 57%. The overall yield of this synthesis, from 3-iodoaniline, 12, was 24%.

Antibacterial activity: The antibacterial activity of compounds 9,10 and 15, against *Staphylococcus aureus*, (a Gram-positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* (space) and *Salmonella spp*. (Gram-negative bacteria), *Candida albicans*(yeast) and *Aspergillus niger* (mold) was tested and the minimum inhibitory concentrations (MIC) in µg/mL were determined (Table 1).

Table 1. Determination of minimum inhibitory concentration (MIC) of compounds 10,15 and 9 against different microorganisms MIC (µg/mL)

Microorganism	10	15	9
Staphylococcus aureus (G+)	12.5	12.5	12.5
Escherichia coli (G-)	25.0	25.0	4.0
Pseudomonas aeruginosa (G-)	16.0	16.0	4.0
Salmonella spp. (G-)	64.0	32.0	4.0
Candida albicans (yeast)	16.0	8.0	4.0
Aspergillus niger (mold)	128.0	4.0	4.0

As can be seen in Table 1, the presence of an aldehyde group in sulfonamide 9, increased its biological activity considerably, when compared with sulfonamides 10 and 15. For example, the title compound, 9, is 6.2 times more active than sulfonamide 10 against *E. coli*, and 16 times more active against *Salmonella spp*. The title compound 9, is also more active than sulfonamide 10, against yeast *Candida albicans* (4 times more active). Remarkably, sulfonamide, 9, was 32 times more active than 10, when tested against mold *Aspergillus niger*.

Microoganisms have different rolls in nature. Some are classified as beneficial, others as pathogenic and a third group is associated to spoilage. Control of microorganisms associated to illness and spoilage is primordial. Bacterial infections are a major source of morbidity and mortality not just in hospitals but also among community (Sidjui, 2016). At the same time, spoilage microorganisms can cause millionaire loses to different production sectors. Even though there are modern and effective therapies to treat bacterial infections, the gradual increasing resistance of bacterial species has led to the clinical use of some sulfas, and one the most extensively used is the mixture trimethoprim-sulfamethoxazole, 8. *Staphylococcus aureus* is one of the most important pathogens producing most of the hospital and community infection diseases. *Staphylococcus aureus* can become resistant to methicillin, a *β*-lactam antibiotic (Stapleton, 2002). Methicillin-resistant *S. aureus* are often resistant to all other penicillin, carbapenems and beta-lactam inhibitor combinations. Moreover, it has been shown that methicillin-resistant isolates are becoming resistant to some other widely used antibiotics such as quinolones, amino glycosides, tetracyclines, macrolides, clindamicin, chloramphenicol and also trimethoprim –sulfamethoxazole (Genç, 2008).

Antibiotic resistance in E. coli is of particular concern because it is the most common Gram-negative pathogen in humans, also, it is the most common cause of urinary tract infections and a cause of diarrhea. Same time, resistant E. coli strains have the ability to transfer antibiotic resistance determinants not only to other strains of E.coli**,** but also to other bacteria within the gastrointestinal tract and to acquire resistance from other organisms.[14]For example, it has been reported that several strains of commensal *Escherichia coli* from pigs, treated with trimethoprim-sulfamethoxazole, have developed resistance. The isolates from this groups of pigs have shown

resistance to sulfamethoxazole, 9, with MIC >1028 µg/mL. In recent years the increase in antimicrobial resistance, and its persisting as important hospital and community pathogens have become a major concern for to the medical community. The World Health Organization (WHO) has emphasized in the need for the development of new antibacterial compounds (Kaplan, 2004). The sulfone 9, showed very high biological activity against Gram (-), Gram (+) bacteria, mold and yeast. The introduction of a carbonyl group, conjugated with the triple bond, in benzenesulfonamide, 9, resulted in a very active compound. The preliminary results obtained are very promising, and further studies have to be done to determine its activity against some other strains.

EXPERIMENTAL SECTION

Synthesis. General Information: All glassware and syringes were dried in an oven overnight at 140º C and flushed with nitrogen immediately prior to use. Transfers of reagents were performed with syringes equipped with stainless-steel needles. All reactions were carried out under a positive pressure of nitrogen. Nitrogen was passed through a Drierite gas-drying unit. Diethyl ether and tetrahydrofuran were refluxed and freshly distilled from sodium and potassium /benzophenone ketyl respectively, under nitrogen atmosphere. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 400 MHz Bruker spectrometer. Infrared spectra were recorded on a Perkin Elmer FT-IR Spectrum 1000.

Synthesis of sulfone 13: In a round bottom flask, equipped with a magnetic stirring bar, was dissolved 3-iodoaniline (0.876 g, 4 mmol) in dichloromethane (15 mL), and pyridine was added (0.64 mL, 8 mmol). To this solution neat benzensulfonylchloride (0.54 mL, (4.2 mmol) was added dropwise and the mixture stirred at room temperature overnight. The reaction was quenched by addition over water, and extracted with ethyl ether. The organic extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography using a mixture of solvents ether:hexane 40:60, and 1.08 g of product was obtained (75 % isolated yield).

Synthesis of sulfone 10: Propyne gas was bubbled into a THF-Et_2NH mixture of sulfone 13, (0.291 g, 0.8 mmol), $Pd(PPh_3)_2Cl_2$ (0.1 mmol) and CuI (0.05 mmol) according to Sonogashira's procedure (Sonogashira, 1975). The crude reaction was treated with saturated ammonium chloride solution, water and extracted with ether. The organic extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residue obtained was purified by column chromatography using a mixture of hexane: ethyl acetate 75 : 25, to obtain 0.154 g of product (70%). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ 2.01 (s, 3H), 7.01 (m, 1 H), 7.12 (m, 3 H), 7.44 (m, 2 H), 7.53 (dd, 1 H, J: 7.5, 7.5 Hz), 7.79 (m, 2 H). ^{13}C-NMR δ 4.3, 78.9, 86.9, 120.6, 124.3, 125.3, 127.2, 128.5, 129.1, 129.2, 133.1, 136.4, 138.8.

Synthesis of sulfone 14: Same procedure as for the synthesis of 10 was followed, but using tert-butyldimethylsilyl propargyl ether instead of propyne. ^{1}H-NMR ($CDCl_3$, 400 MHz) □ 0.14 (s, 6H), 0.93 (s, 9H), 4.50 (s, 2H), 7.00 (broad s, 1H), 7.06 (m, 1H), 7.12 (m, 1H), 7.16 (m, 2H), 7.44 (m, 2H), 7.54 (m, 1H), 7.79 (m,2H). ^{13}

C-N M
Rδ–5.1, 18.3, 25.9, 52.2, 83.8, 88.8, 121.4, 124.1, 124.2, 127.2, 128. 6, 129.1,129.3, 133.2, 136.5, 138.8

Synthesis of alcohol 15: To a cold (0º C) THF solution of **14** (0.973 g, 2.4 mmol) was added a 1.0 M THF solution of Bu_4NF and stirred overnight. After addition of water the reaction mixture was extracted with ether. The extracts were dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The product was purified by column chromatography using ethyl acetate:hexane (35:65). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.05 (s, 1H), 4.03 (broad s, 1H), 4.46 (s, 2H), 7.07 (m, 1H), 7.15 (m, 3H), 7.42 (m, 2H), 7.52 (m, 1H), 7.78 (m, 2H). ^{13}C-NMR ($CDCl_3$,100 MHz) δ 51. 5, 84.7, 88.1, 121.5, 123.7, 124.3, 127.2, 128.6, 129.1, 129.3, 133.2, 136.7, 138.8.

Synthesis of sulfone 9: A THF solution of a sample of alcohol 15 (0.548 g, 1.9 mmol) and Dess-Martin reagent (1.60 g, 3.77 mmol) was stirred, at room temperature, overnight. The reaction mixture was treated with $Na_2S_2O_3$ in a $NaHCO_3$ buffer. The crude reaction was extracted with ether, dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The product was purified by column chromatography using AcOEt : hexane (3:7). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.24 (m, 2H), 7.27 (m, 1H), 7.33 (m, 2H), 7.47 (m, 2H), 7.57 (m, 1H), 7.81 (m, 2H), 9.39 (s, 1H). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 88.5, 93.7, 120.6, 124.0, 125.2, 127.2, 129.3, 129.9, 130.0, 1 33.4, 137.1, 138.7, 176.7. IR (KBr) 3199, 2186, 1654, 1630, 1155, 1024, 936, 689, 589, 556 cm^{-1}.

Bactereological tests: For the diffusion methods well variant, the solvent used was dimethylsulfoxide (DMSO)

Test bacteria: Antibacterial activity was assessed against *Staphylococcus aureus* (ATCC 25923), *Salmonell*a spp.(ATCC 13076), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). Also *Candida albicans* and *Aspergillus niger* were evaluated.

Suspension preparation: Each microorganism was inoculated into trypticase soy broth (TSB) + yeast (Oxoid®) and cultured at 37°C until the desired concentration was reached. The suspension of bacteria to be cultured was equivalent to 0,5 McFarland standard, (1,5 x10^8 CFU/ml).

Bacteriostatic assays: 96 well tissue culture microtiter plates (Nalge, Nunc International, Rochester, NY) were used for each experiment. The assay mixture consisted of 50 µl TSB (trypticase soy broth) + yeast (Oxoid®) for all strains evaluated. Fifty (50) microliters of each bacteria suspension were used as well as 50 µl of decimal dilutions of the chemical agents tested. One well was used as positive growth control each bacteria, one well was used as negative control (no bacteria added). Growth was determined after 24 h incubation at 35°C using the Biotek Synergy HT multi detection reader (Vermont, US).

Conclusion

In this study the title sulfone, 9, was synthesized and its antibacterial against *Staphylococcus aureus*, (Gram-positive bacteria), *Escheririchia coli*, *Pseudomonas aeruginosa* and *Salmonella spp*. (Gram-negative bacteria), *Candida albicans* (yeast) and *Aspergillus niger* (mold) was tested and the minimum inhibitory concentrations (MIC) in µg/mL were determined (Table 1). In all the microorganisms tested, except in *S. aureus*, sulfone 9, gave MIC values of 4.0 µg/mL. Thus, the title compound resulted to be very active against all of

these microorganisms. The introduction of carbonyl group, adjacent to the acetylene group in benzene sulfonamide 9, was essential to increase the activity of this sulfone, when compare with compounds 10 and 15. (Table 1). The MIC values obtained for this compound, 9, makes of it a suitable candidate for further investigations as a promising antibacterial agent.

Acknowledgement

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Conflicts of interest

The authors declare that there is no conflict of interest.

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He invented the precursor technique to Gram staining bacteria. The methods he developed for staining tissue made it possible to distinguish between different types of blood cells, which led to the capability to diagnose numerous blood diseases.

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