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## RESEARCH ARTICLE

# SYNTHESIS OF N-[3-(PROP-1-YN-1-YL) PHENYL] BENZENE SULFONAMIDE AND DETERMINATION OF ITS ANTIBACTERIAL ACTIVITY

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**ABSTRACT**

The synthesis of N-[3-(prop-1-yn-1-yl)phenyl] benzenesulfonamide was performed and its antibacterial activity against Staphylococcus aureus and Escherichia coli was determined. Minimalinhibitory concentrations (MIC) of 12.5 µg/mL and 25.0 µg/mL were obtained respectively.



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## INTRODUCTION

Before the year 1900 only about three chemical therapies were known for the treatment of diseases: mercury for syphilis, cinchona bark for malaria and ipecacuanha for dysentery. Paul Ehrlich (1854-1915), a German medicine doctor, was very passionate about the ability of some dyes to stain anatomical tissues selectively.[1]In 1890 he noted that methylene blue, 1, (Figure 1) deposited only at the living end of the nerves. He believed that the staining of the cells by dyes was the result of a chemical reaction. This encouraged him to look for dyes that could also kill microorganisms, especially Trypanosomes.[2] In 1903 he found a dye, that he called Trypan Red I, 2, (Figure 1) that healed the mice infected from some types of Trypanosomes. Finally, in 1910, after 15 years of research, trying to discover a substance that he calleda "magic bullet", he discovered an arsenic compound called Salvarsan, 3, (Figure 1) effective against Spirochaeta, the bacteria that causes syphilis. Unfortunately, it showed serious side effects including convulsions and death.[3]Ehrlich used the term "chemotherapy" as the treatment with chemicals ("magic bullets") that would be toxic to infectious microorganisms but harmless to humans. He was awarded the Nobel Prize in medicine in 1908. In the 1020's and 1930's common bacterial infections, especially caused by *Staphylococcus* and *Streptococcus*, were widely spread in Europe and the United States; and along with pneumococcal and tubercular infections were responsible of multiple deaths.[4] Between 1909 and 1935 the antibacterial activity of thousands of chemicals was tested however, very few were found to have a promising effect, until a compound called Prontosil, 4, (Scheme 1) was discovered by the pharmaceutical division of IG Farbenindustrie, an industrial conglomerate of German companies, including Bayer Company. It was discovered in 1932, and synthesized by chemists Fritz Mietzsch and Josef Klarer, and tested by physician Gerhard Domagk. They worked, as a team, on a research program synthesizing dyes and testing their activity against bacterial diseases.[4]Prontosil, 4, was found to be very effective against bacterial infections in mice. For the following three years Domagk investigated the antibacterial properties of Prontosil, 4, which was very successful treating several diseases in humans, provoked by *Staphylococcus* and *Streptococcus*. In 1935, Domagk used prontosil, 4, to treat his own daughter, who had contracted a severe streptococcal

[1]Ehrlich developed the initial technique to Gram staining bacteria and tissue. These methods made it possible to distinguish between different types of blood cells, which led to the capability to diagnose numerous blood diseases.
[2]https://www.sciencehistory.org/historical-profile/paul-ehrlich
[3]S. Y. Tan and S. Grimes (2010). Medicine in Stamps. Paul Ehrlich (1984-1915): man with the magic bullet. Singapure Med J, vol. 51 pp 842-843.
[4]https://www.sciencehistory.org/historical-profile/gerhard-domagk

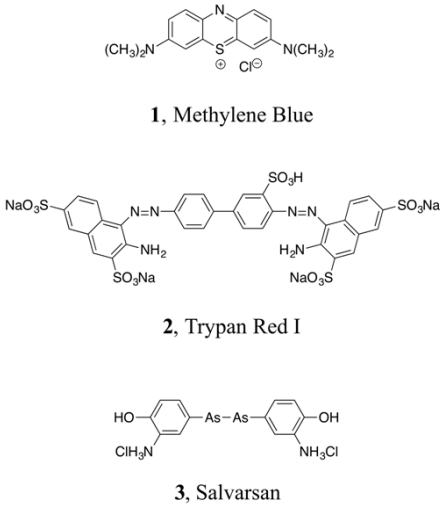
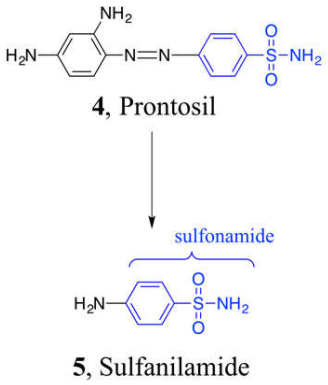



**Figure 1. First synthetic compounds used to treat infections and virus**

infection from a pin prick. Domagk decided to give her an oral dose of this dye, saving her life within a short time. In 1939, he was awarded the Nobel price in Medicine, because of his discovery of the first drug effective against bacterial infections. Since then,a new era in modern chemotherapy initiated. Prontosil, 4, was the first 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).




**Scheme 1. Metabolization of prontosil into sulfanilamide**

In 1936 it was found at the Pasteur Institute that Prontosil, 4, is metabolized in the human body to produce a compound namedsulfanilamide, 5, (*p*-aminobenzenesulfonamide) (Scheme 1), a colorless molecule, which is the active agent against Streptococci. Prontosil, 4, was reclassified as a pro-drug.[5] Prontalbin became the first oral version of sulfanilamide manufactured by Bayer. However, sulfanilamide itself, 5, 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, 6, used in the treatment of toxoplasmosis and in the prevention of rheumatic fever recurrence,[6]sulfathiazole, 7, used as an oral and topical antimicrobial,[7] sulfacetamide, 8, used for the treatment of acne and seborrheic dermatitits, it also has anti-inflamatory properties as a treatment of blepharitis or conjunctivitis, and sulfamethoxazole, 9, (Figure 2) used to treat urinary tract infections, bronchitis, prostatitis and also is effective against Gram (+) and Gram (-) bacteria.

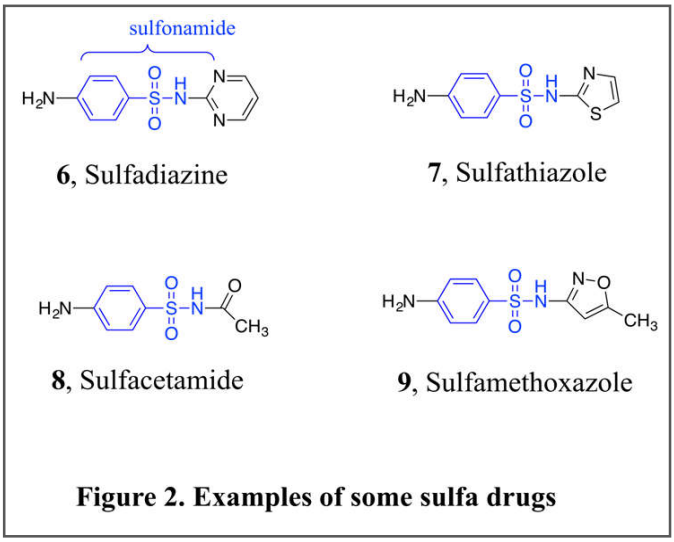



**Figure 2. Examples of some sulfa drugs**

In the mid 1040's and 1950's most of the sulfa drugs were replaced by penicillin and other antibacterials that proved to be more effective against more types of bacteria. Some sulfa drugs such as sulfamethoxazole, 9, 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.[8] We would like to report herein the synthesis of *N*-[3-(prop-1-yn-1-yl)phenyl] benzene sulfonamide, 10, a sulfanilamide derivative, and the determination of its antimicrobial activity against *Staphylococcus aureus* (Gram +) and *Escherichia coli*(Gram-).

## RESULTS AND DISCUSSION

***Synthesis****:* The synthesis of title compound 10 initiated by reaction of 3-iodoaniline, 11, with benzenesulfonyl chloride, 12, 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,[9] using CuI and $(Ph_3P)_2PdCl_2$as catalysts, according to Scheme 2. After purification by column chromatography, compound 10, was isolated in 70% yield. Total yield of the synthesis was 53%.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 in13, and an acetylene in 10, on theirbiological activity.

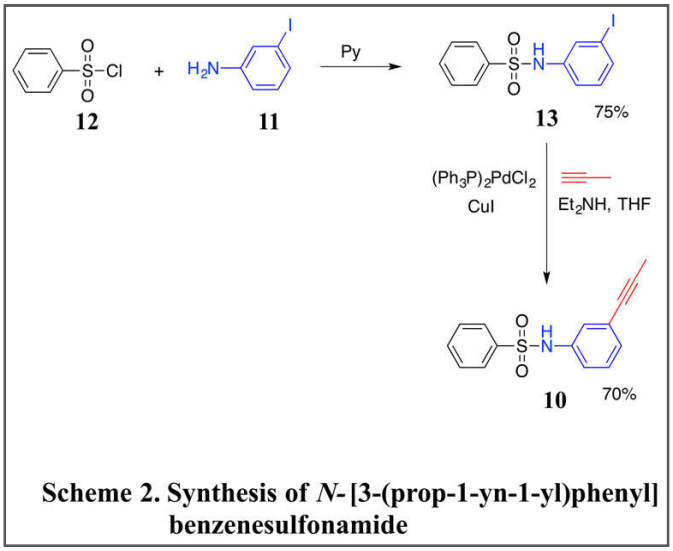
[5]J. et T. Tréfouël, F. Nitti et D. Bovet, "Activité du *p*-aminophénylsulfamide sur l'infection streptococcique expérimentale de la souris et du lapin", *C. R. Soc. Biol.*, **120**, 23 novembre 1935, p. 756

[6]*WHO Model Formulary 2008* (PDF). World Health Organization. 2009. pp. 126, 205. ISBN 9789241547659.

[7]A. Rouf, C. Tanyeli, (2015). Bioactive thiazole and benzothiazole derivatives. *European Journal of Medicinal Chemistry*. **97**: 911–927. doi:10.1016/j.ejmech.2014.10.058

[8]https://medlineplus.gov/druginfo/meds/a684026.html

[9]K. Sonogashira, Y. Tohda and N. Hagihara (1975). A convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes, and bromopyridines. Tetrahedron Lett. 50, pp 4467-4470.




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

***Antibacterial activity:*** The antibacterial activity of compounds13 and 10, against *Staphylococcus aureus*, a Gram-positive bacteria, and against *Escheriirichia coli*, and Gram-negative bacteria, was tested and the minimum inhibitory concentration (MIC) in µg/mL was determined(Table 1). Iodide 13 inhibited *S. aureus* and *E. coli*growth at a concentration of 256µg/mL and 125 µg/mL respectively (Table 1). Remarkably, when the iodide substituent in 13, was replaced by an acetylene group (propyne), in the title compound 10, the activity against *Staphylococcus aureus*increased more than 20 times (12.5 µg/mL), and the activity against *Escherichia coli* was 5 times as much as compound 13 (25 µg/mL, Table 1).

**Table 1. Determination of minimalinhibitory concentration (MIC) of compounds X and 10, against Gram-Positive and Gram-Negative Bacteria MIC (µg/mL)**

| Microorganism | 13 | 10 |
|---|---|---|
| *Staphylococcus aureus (G+)* | 256.0 | 12.5 |
| *Escherichia coli (G-)* | 125.0 | 25.0 |

These results show that, the presence of a triple bond, directly attached to the aniline aromatic ring in 10, enhances the activity of this sulfone significantly, against both *S. aureus* and *E. coli*. 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, 9. *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.[10] 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.[11] 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.[12]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.[13] 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.[14] The sulfone10, showed preliminary promising results, and further studies have to be done to determine its activity against some other strains. Also it is important to perform some other chemical changes in its structure in order to establish a structure-activity correlation.

## Conclusion

In this study the title sulfone, 10, was synthesized and its antibacterial activity tested against *Staphylococcus aureus*and *Escherichia coli*. The minimum inhibitory concentration (MIC) was determined and values of 12.5 µg/mL and 25.0 µg/mL were obtained. The introduction of an acetylene group in the aniline aromatic ring was essential to increase the activity of sulfone 10, when compare with the synthetic precursor 13 (Table 1). The MIC values obtained for this compound makes of them a suitable candidate for further investigations as a promising antibacterial agent.

## 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. High resolution mass were measured on a Waters Synapt HMDS G1, Q-TOF. 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

[10]P. D. Stapleton and P. W. Taylor (2002). Methicillin resistance in Staphylococcus aureus mechanisms and modulation. Sci Prog, vol 85, pp. 57-72. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2065735/

[11]Y. Genç, R. Özkanca and Y. Bekdemir (2008). Antimicrobial activity of some sulfonamide derivatives on clinical isolates of Staphylococcus aureus. Ann Clin Microbiol and Antimicrobials, vol7, pp 17-22.

[12]M. Rashhed, N. Thajuddin and K. Jami (2014). Antimicrobial drug resistance in strains of Escherichia coli isolated from food sources. Rev Inv Med Trop Sao Paulo, vol. 58, pp 341-346.

[13]J. Mazurek, E. Bok, M. Stosik and K. Baldy-Chudzik (2015). Antimicrobial resistance in commensal Escherichia coli from pigs during Metaphylactic Trimethoprim and sulfamethoxazole treatment an in the post-exposure period. Int. J. Environ. Res. Public Health, vol. 12, pp 2150-2163.

[14]W. Kaplan, R. Laing (2004). Priority Medicines for Europe and the World: A Public Health Approach to Innovation. World Health Organization, Geneva, Switzerland.

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.[15] 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.

***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) and *Escherichia coli* (ATCC 25922).

**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 x$10^8$ 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).

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**Conflicts of interest:** The authors declare that there is no conflict of interest.

[15]K. Sonogashira, Y. Tohda and N. Hagihara (1975). A convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes and bromopyridines. Tetrahedron Lett. No. 50, pp 4467-4470.

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