

Synthesis and *in vitro* Antibacterial Activity of Novel Substituted N-1, 3-Benzoxazol-2-yl Benzene Sulfonamides

Gajanan Shanbhag¹, H. M. Vagdevi²

Department of PG Studies and Research in Industrial chemistry, Jnana Sahyadri, Kuvempu University, Shimoga 577 451, India

Abstract: From many centuries human beings have been exposed to pathogens. In current modern medicine microbial infections are a major growing problem in which only a few antimicrobial agents are used in medical practice. Though several antimicrobial agents are currently available, there is a critical need for the development of new and specific antimicrobial agents. Amongst them benzoxazole derivatives constitute much importance in medicinal field due to their broad range of biological activity. Two novel N-1,3-Benzoxazol-2-yl benzene sulfonamides have been synthesized by cyclizing four different amino alcohols with Cyanogen bromide to get 1,3-benzoxazol-2-amine which upon treating with substituted phenyl sulfonyl chlorides gave novel N-1,3-Benzoxazol-2-yl benzene sulfonamides. After structural confirmation, the title compounds were screened for their antimicrobial and anti oxidant activity. The obtained compounds were tested for antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* at 30ppm and 300ppm and subjected the same compound for molecular docking studies with respect to antimicrobial activity. Both the compounds 3a (N-(7-bromo-1, 3-benzoxazol-2-yl)-4-methylbenzene-1-sulfonamide) and 3b (4-bromo-N-(7-methyl-1, 3-benzoxazol-2-yl) benzene-1-sulfonamide) showed pronounced antimicrobial activity.

Keywords: antibacterial activity, sulfonamides, benzoxazole

1. Introduction

From many centuries human beings have been exposed to pathogens. In current modern medicine microbial infections are a major growing problem in which only a few antimicrobial agents are used in medical practice. Though several antimicrobial agents are currently available, there is a critical need for the development of new and specific antimicrobial agents. Sulfonamides are known to be the first effectively synthesized selectively toxic antimicrobial drugs (Neu, et al., 1996; Van Meter et al., 2016). It represents the large group of drugs with the antimicrobial activity which can be classified as oral sulfonamides in absorbable or non absorbable form and topical sulfonamides. Based on duration of action and half-life, absorbable oral sulfonamides can be further divided into short- acting (3-8 h), Intermediate-acting (8-18 h) and Long-acting sulfonamides (>35 h) (Varagić et al., 2009 and Struller 1968). Sulfonamides are antimicrobial drugs with a broad spectrum of action, effective against Gram-positive and certain Gram-negative bacteria, such as intestinal bacteria *Escherichia coli*, *Klebsiella*, *Salmonella*, *Shigella* and *Enterobacter* species (Struller 1968) and also possess antifungal (*Pneumocystis carinii*) and anti- protozoan activity (*Toxoplasma gondii*).

Recent studies suggest that substituted benzoxazoles constitute much importance in medicinal field due to their broad range of pharmaceutical activity. They are structural isosteres of natural nucleotides and effortlessly intermingle with biopolymers. Benzoxazole characterize the key structure feature of an ample number of pharmaceutical compounds with antibacterial and antifungal activity (AbuMohsen 2014). Many reports also suggests that targets containing benzoxazole moiety have remarkable biological activities like antibacterial, antihistaminic, antiparasitics,

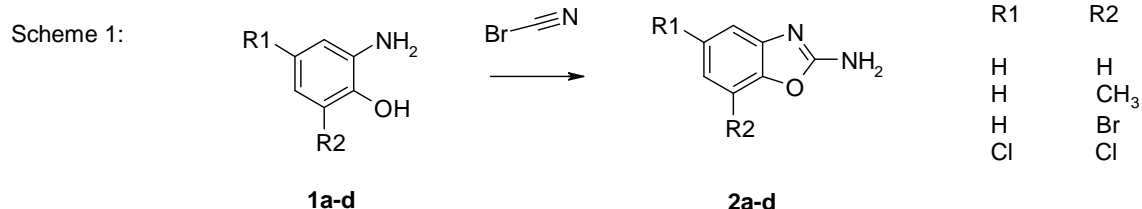
antiviral and antifungal activity (Kumar and Kumar 2007; Katsura et al., 1992; Haugwitz et al., 1982; Paget et al., 1969; Ozlem et al., 2008). In the present study two novel N-1,3-Benzoxazol-2-yl benzene sulfonamides have been synthesized, characterized and the designated compounds were screened for their antimicrobial activity.

2. Materials and Methods

All reagents and solvents were used as purchased without further purification. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Purity of the compounds was determined by thin layer chromatography (TLC). IR spectra were recorded using PerkinElmer BX serried FTIR 5000 spectrometer using KBr pellet (Vector-22, Bruker, France). H-NMR spectra were recorded on a Varian EM-390 spectrophotometer (chemical shift in δ ppm). Mass spectra were recorded on 70 eV on VG-Micromass 7070H spectrometer and elemental analysis were carried out using FLASH EA 1112 CHN analyzer (Thermo Finnigen, Italy).

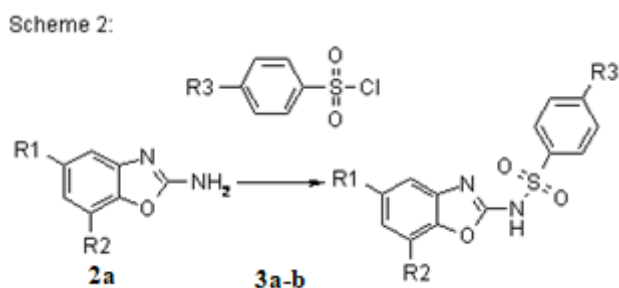
2.1 Synthesis of 1, 3-benzoxazol-2-amines

To substituted amino phenol (1mmol), 20ml of ethanol and 3mmol Cyanogen bromide was added. This reaction mixture was warmed at 60°C for 12 hours. TLC was performed to know the non-appearance of starting material (SM) and appearance of new spot of reaction mixture (RXN). The RXN was evaporated and the residue was treated with aqueous sodium bicarbonate solution (20ml) to obtain the pH of 8 to obtain the solid filtrate which was washed well with water and crystallized using ethanol. This reaction is shown in scheme 1. The yield was 70% and melting point was 111-113°C.

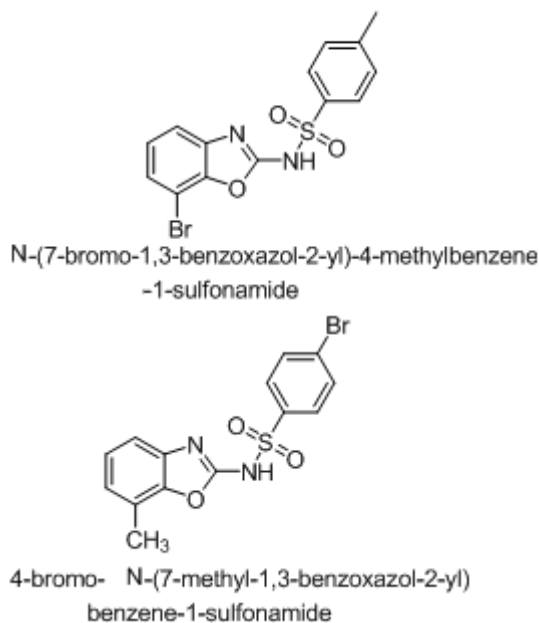


2.2 Synthesis of N-1, 3-Benzoxazol-2yl benzene sulfonamides

In 5ml of anhydrous pyridine amine (1mmol) was suspended. Meanwhile steadily sulfonyl chloride (1.1 mmol) was added. The reaction mixture was warmed at 60°C for 1-4 hours. This mixture was then poured into ice water and acidified with 1N HCl. This solid product was filtered, washed well with water, and crystallized using ethanol. This reaction was shown in Scheme 2. The yield was 58% and melting point was 181°C-185°C. The



Where 3a and 3b are as follows:



2.3 In vitro antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*

Antimicrobial activity was performed in vitro by the disc diffusion method (Bauer et al., 1966). Bacterial inoculums of *Staphylococcus aureus* and *Escherichia coli* were prepared from overnight grown cultures in nutrient broth and turbidity was adjusted equivalent to 0.5 McFarland units (approximately 10⁸ cfu/ml). On nutrient agar, 100 µL aliquots of inoculums were spread over the surface with a sterile L-

glass spreader. Sterilized paper discs (Oxoid, 6mm diameter) were wetted with 10 µL of a solution of each compound to be tested, in the concentration of 0.02 gr/ml (200 µg per disc) in DMSO. Meanwhile rifampicin and ampicillin were used as standards and DMSO as control. The plates were then incubated 24 h at 37 °C and the zone of complete growth inhibition was measured. The values are reported at Table 1 as a mean of three replicates.

3. Result and Discussion

A two novel N-1, 3-Benzoxazol-2yl benzene sulfonamides (3a-b) have been synthesized by cyclizing different amino alcohols with Cyanogen bromide to get 1, 3-benzoxazol-2-amine which upon treating with substituted phenyl sulfonyl chlorides gave novel N-1, 3-Benzoxazol-2yl benzene sulfonamides. All reactions of N-1, 3-Benzoxazol-2yl benzene sulfonamides were prepared with satisfactory yield and accompanied with intensive change in color of the reaction mixture to gray solid. On completion of the reactions the precipitated crystals were of the synthesized compounds **3a** and **3b** purified with ether. The chemical structures were elucidated on the basis of elemental analysis, IR, and mass spectroscopy. The results of elemental analyses (C, H, and N estimation) were found to be within ±0.4% of the theoretical values. IR data also confirmed the presence of specific functional groups present in the final synthesized compounds. The mass spectra of new compounds were in conformity with the assigned structure. The melting points and the thermal stability of the compounds were taken from TG and DSC methods.

The compounds 3a and 3b groups are historically well-known sulfonamide drugs and have been extensively studied (Alsughayer et al., 2011; Reeves 1975), but for most of this group of compounds microorganisms have often shown resistance against them (Cummings et al., 2013). All synthesized Compounds were tested for antimicrobial activity by the disc diffusion method. In general, these results indicated good antimicrobial activities for all compounds (Table 1). Both the compounds showed good mean inhibition zone (MZI) for both the strains of bacteria which indicates that these groups of compounds have pharmaceutical properties which can be effective against human pathogens. These synthesized compounds, were found to possess significant antibacterial activity when compared to standard antibacterial drugs.

4. Conclusion

The newly synthesized two compounds 3a (N-(7-bromo-1, 3-benzoxazol-2yl)-4-methylbenzene-1-sulfonamide) and 3b (4-bromo-N-(7-methyl-1, 3-benzoxazol-2-yl) benzene-1-sulfonamide) showed good potency against different bacterial strains. Both 3a and 3b showed prominent

antibacterial activity. These new data of the molecules might be helpful in the future development of sulfanilamide analogues as novel antimicrobial agents and provides a scope for extending this series in future with some more molecules. Further work will be carried out to know the site of action of these molecules through molecular docking studies against *E. coli* and *S. aureus*.

Table 1: The *in vitro* antimicrobial activity of the synthesized compounds and the control drugs (1mg/mL).

Compound number	Microorganisms, zone of inhibition (diameter, in mm)	
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
3a	20	21
3b	21	20
Rifampicin	25	22
Ampicillin	22	23
DMSO	—	—

Shown are mean values of triplicate tests; “—” indicates no significant inhibitory effect (<6 mm).

References

- [1] Abu Mohsen U (2014). Synthesis and antimicrobial activity of novel substituted benzoxazole derivatives. *World journal of pharmacy and pharmaceutical sciences* 3, 1362-1368.
- [2] Alsughayer A., Elassar, A. Mustafa, S. and Sagheer, F. (2011). Synthesis, structure analysis and antibacterial activity of new potent sulfonamide derivatives. *Journal of Biomaterials and Nano biotechnology*, 2, 144–149.
- [3] Bauer, A.W., Kirby, W.M.M. Sherris J.C. Turck, M., (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 45: 493-496.
- [4] Cummings K. J., Perkins G. A., Khatibzadeh S. M, L. D. Warnick, and Altier, C. (2013) Antimicrobial resistance trends among *Salmonella* isolates obtained from dairy cattle in the northeastern United States, 2004–2011,” *Foodborne Pathogenes Disease*, 10,353–361.
- [5] Haugwitz R.D., Angel R.G., Jacobs G.A., Maurer, B.V., Narayanan, V.L., Cruthers, L.R. and Szanto J. (1982). Synthesis and anthelmintic activities of novel 2-heteroaromatic-substituted isothiocyanato benzoxazoles and benzothiazoles. *J Med Chem.* 25:969-974.
- [6] Katsura Y, Inoue Y, Nishino S, Tomoi M, Itoh H and Takasugi H.(1992). Synthesis and antiulcer activities of imidazo [1, 2- a] pyridinyl ethyl-benzoxazoles and related compounds. A novel class of histamine H2-receptor antagonists. *Chem Pharm Bull.* 40:1424-1438.
- [7] Kumar A and Kumar D.2007. Synthesis and antimicrobial activity of metal complexes from 2-(10/20- hydroxynaphthyl) benzoxazoles. *Arxivoc.* 117-125.
- [8] Neu, H. C., Gootz T. D., in Medical Microbiology, S. Baron Ed., The University of Texas Medical Branch at Galveston, Galveston 1996.
- [9] Ozlem T.A., Ilkay Y and Semih O. (2008). Synthesis and biological activity of some new benzoxazoles. *Eur J Med. Chem.* 43:1423-1431.
- [10] Paget C.J., Kisner K., Stone R.L., and DeLong D.C. (1969) Immunosuppressive and antiviral activity of benzothiazole and benzoxazole ureas. *J Med Chem.* 1969; 12: 1016-1018.
- [11] Reeves D. S., (1975). “Laboratory and clinical studies with sulfametopyrazine as a treatment for bacteriuria in pregnancy,” *Journal of Antimicrobial Chemotherapy*, 1. 171–186.
- [12] Struller T., in Antibiotics and Chemotherapy, O. Gsell Ed., Karger, Basel 1968, p. 179-215.
- [13] Van Meter K. C. and Hubert, R. J. Microbiology for the Healthcare Professional, Elsevier, China, 2016, p. 214-232.
- [14] Varagić V. M., Milošević M. P., Farmakologija, Elitmedica, Beograd, 2009, p. 622-627.