Anti-microbial activities of sulfonamides using disc diffusion method

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Abstract: Sulfonamides, being the member of the oldest anti-microbial group of compounds possess wide anti-microbial activities and are effective against pathogenic strains of gram-positive and gram-negative bacteria. They are widely used in the treatment of various infectious diseases e.g. malaria, urinary tract infections, respiratory tract infections etc. Based on their effectiveness against most of the bacteria, two novel sulfonamides (N-(2-methoxy phenyl)-4-methylbenzenesulfonamide and N-ethyl-4-methyl-N-(3-methyl phenyl)benzenesulfonamide) were synthesized. The compounds were characterized by FT-IR and elemental analyzer. Their anti-microbial activity was assessed and observed against gram-positive and gram-negative bacteria using disc diffusion method. They showed good anti-microbial activities.

Keywords: Sulfonamides, anti-microbial, disc diffusion, FT-IR, elemental analyzer.

INTRODUCTION

Sulfonamides were discovered by a German Bacteriologist, Gerhard Domagk (Mascaretti, 2003; Rogers, 2011; King and Brucker, 2011), and belong to the oldest group of anti-biotic compounds (Adams, 2011) but they are still in use because they are normally tolerated by the patients and are relatively less expensive (Connor, 1998).

Sulfonamides are the derivatives of sulfanilamide and differ in the radical group (R) which is attached to the amido group ($-SO_2NHR$) and sometimes also as the substituent on the amino group ($–NH_2$). Sulfonamides have different properties depending upon their R-group. Their degree of anti-microbial activity also varies accordingly (Giguere *et al*., 2006).

The synthetic drug, sulfonamides (Hirsh *et al*., 2004) have a broad range of activity against bacteria, protozoa, and toxoplasma. Gram-positive, gram-negative and even anaerobic bacterial growth is inhibited by them (Giguere *et al*., 2006; Finch, 2003; Buch, 2010; Hirsh *et al*., 2004; Yadav, 2008; Udaykumar, 2007; Buch, 2010; Boothe, 2001; King and Brucker, 2011). Due to their broad spectrum they are used for almost all bacterial diseases in fish (Brown, 2000).

All the sulfonamides basically follow the same mechanism of action. As they are bacteriostatic drug not the bacteriocidal so when incorporated into the bacterial cell, they stop the synthesis of Folic acid by competing with *para*-aminobenzoic acid (PABA) (Giguere *et al*., 2006; Finch, 2003; Adams, 2001; Buch, 2010; Hirsh *et al*., 2004; Udaykumar, 2007; Buch, 2010; Boothe, 2001; Yadav, 2008; King and Brucker, 2011). But when they are added in higher concentration to bacterial cells they act as bactericidal (Vaid *et al*., 2004). Since Folic acid is essentially required for the synthesis of Purine and DNA (Ahrens, 1996), therefore its inhibition results in the inhibition of the repairing or synthesis of nucleic acid (Maddison *et al*., 2008). Thus, bacterial growth is strongly inhibited by sulfonamides (Ahrens, 1996).

They have selective toxicity because mammalian cells, although having lost the ability to synthesize folic acid but can still absorb it from the intestine where bacteria might have synthesized it (Hirch *et al*., 2004).

Now bacterial strains are developing resistance against sulfonamides. The resistance can be due to chromosomal point mutation (Mayers, 2009) or it can be plasmid mediated resistance (Clark, 2005) or by acquiring the ability to produce more amount of PABA (*para*-aminobenzoic acid) (Nord, 2009).

In this research paper, two novel sulfonamides namely N-(2-methoxy phenyl)-4-methylbenzenesulfonamide and N-ethyl-4-methyl-N-(3-methyl phenyl)benzenesulfonamide are reported which were synthesized and characterized by single crystal X-ray diffraction, FT-IR and elemental analyzer. Their anti-microbial activity was observed against gram-positive and gram-negative bacteria using disc diffusion method.

MATERIALS AND METHODS

Materials

All synthetic reactions were carried out in normal environment at atmospheric conditions. The analytical grade materials and reagents were used in these reactions. *p*-toluene sulfonylchloride, o-anisidine and m-toulidine were used to prepare the desired compounds.

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Synthesis

1. N-(2-methoxy phenyl)-4-methylbenzenesulfonamide

p-toluene sulfonylchloride (10 m*M*) was dissolved in 20 mL distilled water and 10 m*M* of *o*-anisidine was added in it. pH of the reaction mixture was maintained at 8-10 using 3% Na_2CO_3 and was stirred for about 1-2 hours. The reaction was monitored using TLC plates. The product obtained was filtered and precipitates were washed with distilled water and recrystallized using methanol.

2.N-ethyl-4-methyl-N-(3-methyl phenyl)benzenesulfonamide

5 m*M* of *m*-toluidine was dissolved in 20mL of distilled water then 5 m*M* of ethyl iodide was added in it. The reaction mixture was stirred properly and 5 m*M* of *p*-toluenesulfonyl chloride was added. The mixture was stirred for about 1-2 hours and the pH was maintained 8-10 using Na_2CO_3 solution 3%. The progress of reaction was monitored by TLC. The product obtained was filtered and the precipitate was washed with distilled water, dried and recrystallized using methanol.

Characterization

Characterization of the synthesized novel compounds were carried out by Elemental Analyzer and Fourier Transform Infra-Red (FT-IR) Spectroscopy.

Elemental Analysis

Vario MICRO cube elemental analyzer was used. Approximately 1-3 mg of each product was placed in a tin packing separately and then twice the amount of tungsten oxide was added in each of the samples. Both the samples were then wrapped tightly ensuring that no opening or space was present in the packing and were placed in the labeled cells of the analyzer tray. Results were obtained in the form of percentage of Nitrogen, Carbon, Hydrogen and Sulphur present.

Fourier Transform Infra-Red (FT-IR) Spectroscopy

Infra red spectra were recorded on Thermo-Nicolet 200 USS instrument. The KBr pellets of each product were prepared and analyzed at the wave number range of 4000-400 cm^{-1}.

Microbial assay

Different methods are being used for the microbial assay. One of them is the disc diffusion method (Saify *et al*., 2000; Mojab *et al*., 2008; Qadrie *et al*., 2009) which was adapted for the study of anti-microbial activity of the two newly synthesized sulfonamides. Another method which is used for the determination of anti-microbial activities of a compound is plate hole diffusion method (Sarvanakumar *et al*., 2009) in which Minimum Inhibitory Concentration (MIC) of the compound can also be observed (Khan *et al*., 2008; Khalid *et al*., 1992).

Sulfonamide solution

1% solution of both compounds was prepared in the methanol.

Organisms used

Micro-organisms were obtained from the applied chemistry laboratory of the Chemistry Department, Govt. College University Lahore.

Gram-positive

Bacillus subtilis,
Bacillus licheniformis,
Bacillus subtilis (specific for amylase) are gram-positive bacteria.

Gram-negative

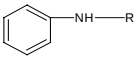
Streptococcus sp.
Escherichia coli I is gram-negative bacteria.

Anti-microbial assay

The inoculums of strains were developed in nutrient broth for 24 hours. The plates were prepared by nutrient agar and discs were soaked in the 1% solution of both compounds prepared in methanol. A control disc was also used. The agar plates were inoculated by the strains separately and discs were placed on each plate by slightly pressing them. The plates were kept at 37°C overnight. The zones developed are measured in "cm".

Analysis

N-(2-methoxy phenyl)-4-methyl benzene sulfonamide

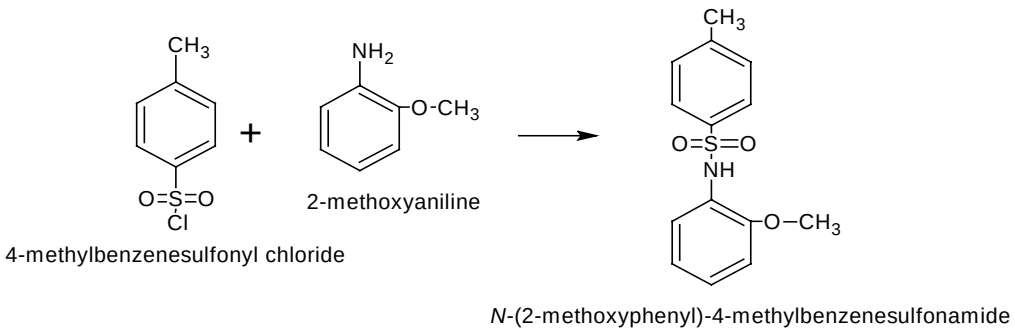
Analysis found: C 59.15, N 4.70, H 4.467, S 9.90% calculated for C 68.8, N 5.7, H 6.1, S 13.1%.
Wave number: *o*-disubstituted benzene ring 754.76 cm^{-1}, *p*-disubstituted benzene ring 823.23 cm^{-1},  ether group 1206.91 cm^{-1},  aromatic secondary amine 1595.61 cm^{-1}, O=S=O 1334.88 cm^{-1}.

N-ethyl-4-methyl-N-(3-methyl phenyl) benzene sulfonamide

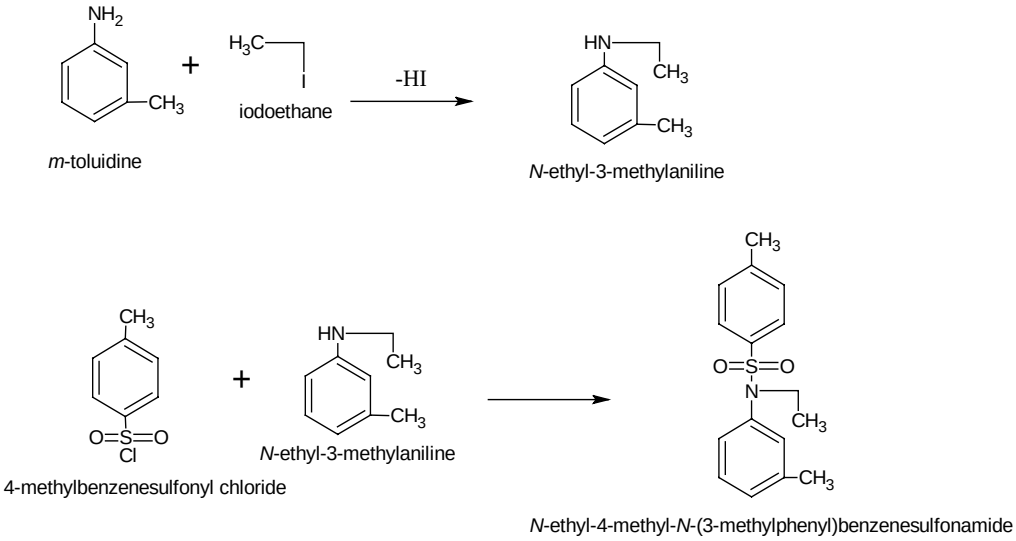
Analysis found: C 64.18, N 4.29, H 5.654, S 9.47%. calculated for C 67.4, N 4.9 H 6.67, S 11.2%.
Wave number: *m*-disubstituted benzene ring 796.91 cm^{-1}, *p*-disubstituted benzene ring 819.24 cm^{-1}, O=S=O 1341.99 cm^{-1}.

RESULTS

During the synthesis of sulfonamides, H and Cl were removed out and a bond was developed between "N" of amide and "S" of sulfonyl group. The chloride ion attacked on the hydrogen (partial positive) of amide group and removed it. As a result, nitrogen became negatively charged and sulphur got a positive charge. Thus an ionic



Scheme 1: synthesis of N-(2-methoxy phenyl)-4-methylbenzenesulfonamide



Scheme 2: synthesis of N-ethyl-4-methyl-N-(3-methyl phenyl)benzenesulfonamid

bond is developed between these two charged species present in the media. The alkali environment, where the reaction took place made the removal of hydrogen easier. It has been found from the elemental analysis and FT-IR that the compounds were without water of crystallization.

1. N-(2-methoxy phenyl)-4-methylbenzenesulfonamide

This compound was synthesized by reaction between *p*-toluene sulfonylchloride and *o*-anisidine as shown in scheme 1.

2. N-ethyl-4-methyl-N-(3-methyl phenyl)benzenesulfonamide

This was synthesized by reaction between *m*-toluidine and ethyl iodide in the first step then the reaction mixture was stirred with *p*-toluenesulfonyl chloride. Scheme 2 showed the synthesis of this product.

Fourier Transform Infra-Red (FT-IR) Spectroscopy

The FTIR results were reviewed. As the molecules are asymmetric in nature and have larger size so there are many vibrations which are difficult to describe as they involved the coupled movement of several parts of the groups attached. Only the fundamental modes of group vibrational concept and infra red spectrum were assigned (Bayari and Ide, 2002).

N-(2-methoxyphenyl)-4-methyl benzene sulfonamide

The IR spectra of N-(2-methoxyphenyl)-4-methyl benzene sulfonamide is given in the fig. 1. The standard value of *o*-disubstituted benzene ring is 770-735 cm^{-1} whereas in this molecule, the value was found to be 754 cm^{-1}. While *p*-disubstituted benzene ring has standard value of 860-800cm^{-1} and its group has the value within the range i.e. 823 cm^{-1}. The vibrations values of ether group present and aromatic secondary amine (as it has one hydrogen attached) are within the range of standard values (1250-1150 cm^{-1} and 1650-1550 cm^{-1} respectively) and can be observed at 1206 cm^{-1} and 1595 cm^{-1} respectively. The peak shown by sulfonyl group at 1334 cm^{-1} confirms that the compound prepared is a sulfonamide.

N-ethyl-4-methyl-N-(3-methyl phenyl) benzene sulfonamide

IR spectra of N-ethyl-4-methyl-N-(3-methyl phenyl) benzene sulfonamide is displayed in Figure 2. No deviations from the standard values were observed in this

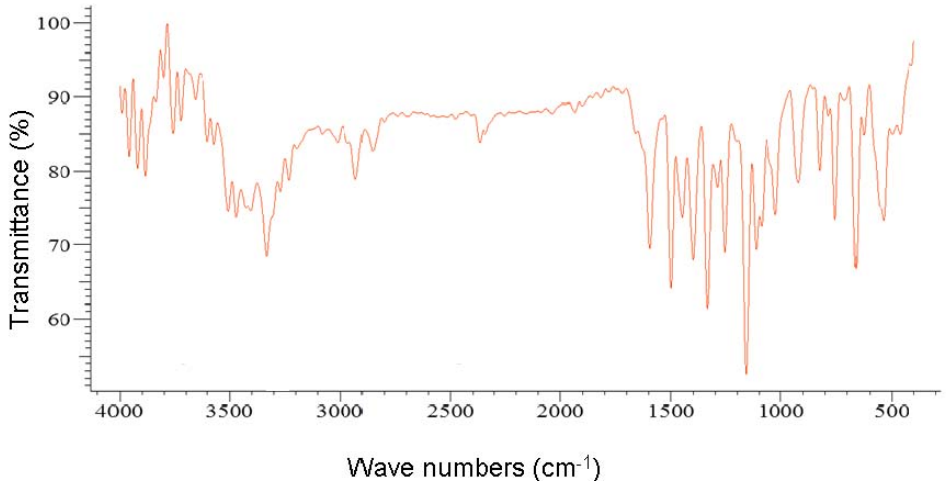


Fig. 1: FT-IR spectra of N-(2-methoxyphenyl)-4-methyl benzene sulfonamide

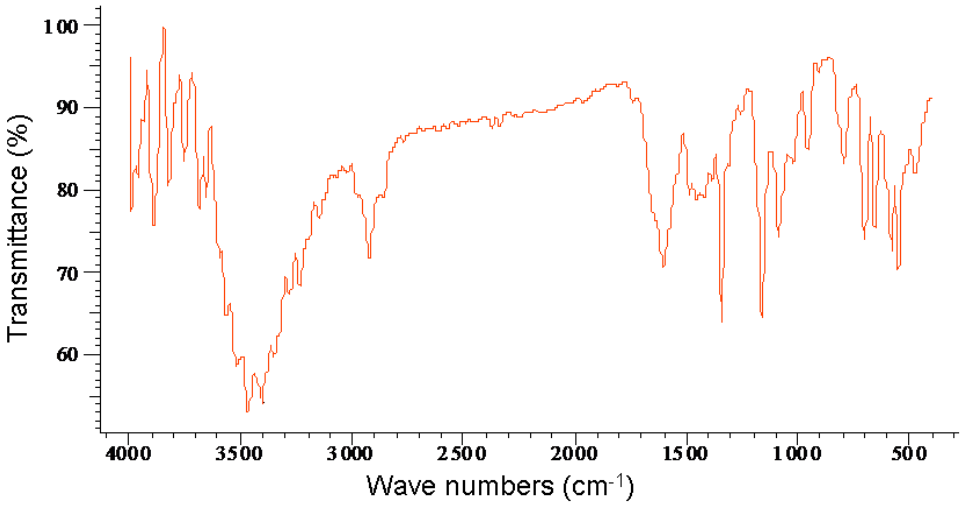


Fig. 2: IR spectra of N-ethyl-4-methyl-N-(3-methyl phenyl) benzene sulfonamide

spectrum. The standard values for *m*-disubstituted benzene ring are 810-750 cm^{-1} and 900-860 cm^{-1}, for *p*-disusbtituted benzene ring 860-800 cm^{-1} whereas the tertiary aromatic amine does not show any peak due to the absence of "H" atom. The values observed are 796 cm^{-1} for *m*-disubstituted benzene ring, 819 cm^{-1} for *p*-disubstituted benzene ring. The peak observed at 1341 cm^{-1} is for sulfonyl group which ensured that the compound is sulfonamide. Its structure has also been confirmed by single crystal X-ray analysis (Ahmad *et al*., 2011a). Single crystal data was collected on Bruker APEX2 CCD diffractometer (Bruker, 2007; Ahmad *et al.*, 2011b; Frayal *et al*., 2011)

DISCUSSION

The anti-microbial activities of both the synthesized compounds were studied against gram-positive and gram-negative bacteria. The disc diffusion method was adapted to observe the activities. The zones of inhibition were measured in "cm" and are shown in table 1.

The activity of sample N-(2-methoxyphenyl)-4-methylbenzenesulfonamide was found similar against *Bacillus licheniformis* and *bacillus subtilis* (amylase specific). The activity against *Bacillus subtilis* and *Streptococcus* (gram-ve) was found to be slightly more than the first two strains but it was still not so strong. There was no activity shown against *Escherichia coli I*. The molecule N-ethyl-4-methyl-N-(3-methylphenyl) benzenesulfonamide showed comparatively better results against these strains. Although there was no effect observed against *Bacillus subtilis* and *Escherichia coli I* yet a reasonably good anti-microbial activity is shown against *Bacillus licheniformis*. Growth of *Bacillus subtilis* and *Streptococcus* (gram-ve) was also reduced by this molecule and zones of inhibition were observed. On the whole, the compounds showed better activity against

Table 1: Zones of inhibition formed by sample 1 and 2 against different bacterial strains

Sample No.	Zone of inhibition in different micro-organism strains (cm)				
	Bacillus licheniformis	*Bacillus subtilis* (amylase specific)	*Bacillus subtilis*	*Streptococcus* (gram –ve)	*Escherichia coli I* (gram –ve)
1[a]	0.50	0.50	0.90	0.90	0.00*
2[b]	1.65	0.00	0.70	0.65	0.00*

*0.00 means no activity found.
[a]N-(2-methoxyphenyl)-4-methylbenzenesulfonamide, [b]N-ethyl-4-methyl-N-(3-methylphenyl)benzenesulfonamide

gram-positive bacteria than *gram-negative bacteria* which provides a good evidence of the structural differences between the cell walls of both type of bacteria. The activities may vary due to the number of cells present in innoculum. This is because, the bacterial cell is surrounded by cell wall which is present outside of the cytoplasmic membrane and composed with a material peptidoglycan (a polymer having composition of sugars and amino acids). Gram-positive and Gram-negative have two different types of cell walls present in bacteria which are differentiated on the basis of peptidoglycan. Former contains a thick wall with many layers of peptidoglycan while the later possess few layers of peptidoglycan. The antimicrobial activity of sulfonamides is the ability to kill bacteria by inhibiting a step in the synthesis of peptidoglycan and they are more efficient against Gram-positive than Gram-negative (Heijenoort, 2001., Koch, 2003).

CONCLUSION

In conclusion, both N-(2-methoxyphenyl)-4-methyl-benzenesulfonamide and N-ethyl-4-methyl-N-(3-methylphenyl)benzenesulfonamide showed good anti-microbial activity against gram-positive and gram-negative strains. N-ethyl-4-methyl-N-(3-methylphenyl) benzenesulfonamide was however found more efficient anti-microbial agent against gram-positive bacteria in general. Both sulfonamides showed no activity against the pathogenic stain of *E. coli I.*

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