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Original Research

Sulfonyl thiosemicarbazones: synthesis, characterization and antibacterial activity

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Abstract: The reaction of sulfonyl ketones (**1-3**) with thiosemicarbazide under reflux conditions yielded the sulfonyl thiosemicarbazones (**4-6**) in good to potential amounts (72-80%). The structure of new compounds (**4-6**) was confirmed by different spectral (IR, ^{1}H NMR, ^{13}C NMR, MS) and analytical techniques. The investigation of antibacterial screening data revealed that all the tested compounds (**4-6**) exhibited moderate to decent antibacterial activity against *S. pyogenes*, *S. aureus* and *E. coli* strains. The compounds **4-6** depicted zones of inhibition, *i.e.*, 20.3 mm, 21.2 mm and 19.6 mm against *S. pyogenes* strain, respectively.

Keywords: antibacterial activity; disk diffusion method; sulfones; thiosemicarbazones

1. Introduction

Sulfones are the particular class of heterocyclic organosulfur compounds, represented by general structural formula R-S(O)$_2$-R', where R and R' are hydrocarbon groups [1]. The sulfone chemistry has been discovered because of their prime importance as synthetic intermediates to produce several chemically and biologically potential molecules [2, 3]. Several drugs containing sulfone groups treat many diseases like leprosy, dermatitis, herpetiformis, and tuberculosis. Biological chemists have checked one of the sulfones containing compound, which depicted antimicrobial behaviour against different microbial strains during which it got declared that sulfones can be used as anti-infectious agents [4]. Literature reveals that there are many thiosemicarbazone derivatives known to show the potential antibacterial and antifungal behaviour. Namiecinska *et al.* studied the antimicrobial behaviour of specific metal ions coordinates with thiosemicarbazide motif and related heterocyclic compounds during which the compounds depicted better antimicrobial behaviour [5]. They also studied the relationship between the structure and biological activity of the new compounds. Pahontu *et al.* also studied antimicrobial behaviour as well as antileukemia activity of thiosemicarbazone derivatives during the biological screening; they depicted the potential biological behaviour against different microbial strains [6]. Shebl *et al.* prepared different binary and ternary metal complexes of thiocarbohydrazone ligand, and after synthesis, they screened them against various bacterial and fungal strains, during which the compounds showed excellent antimicrobial behaviour [7]. Contemplating the biological applications of sulfones [4] and during the progress of usual work [8-11], we here depict the synthesis of sulfone thiosemicarbazones and simultaneously study their antibacterial behaviour.

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2. Experimental

The Kofler apparatus was used to check the melting points. Perkin Elmer RXI Spectrophotometer was used to check the IR spectra using KBr pellets and values given in cm^{-1}. JEOL Eclipse (400 MHz) was used to check ^{1}H, and ^{13}C NMR spectra in $CDCl_3$ with TMS as internal standard and values are given in ppm (δ). JEOL SX 102/DA-6000 Mass Spectrometer was used to record the Mass spectra. The homogeneity and the purity of the reaction were checked by thin-layer chromatography (TLC). Petroleum ether refers to a fraction of boiling point 60-80 °C. The drying agent used was anhydrous sodium sulfate. The sources of chemicals were Merck India and were used only after distillation.

2.1 Synthesis of 2-substituted sulfonyl-1-(4-nitrophenyl)-thiosemicarbazones (4-6)

The equimolar solution of compounds (**1-3**) [4] (1 mmol) and thiosemicarbazide in absolute ethanol (20 mL) was refluxed for 3-4 h. The continuity and endpoint of the reaction were checked using TLC. After the reaction got

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completed, the extra solvent was removed using rota vapour. Then diethyl ether and water were added to the reaction mixture so that the organic compound was separated and dried over anhydrous sodium sulphate followed by the solvent evaporation and recrystallization from methanol that gave products (**4-6**) with 72-80% yield.

2-Benzenesulfonyl-1-(4-nitrophenyl)-ethanone thiosemicarbazone (4)

Off white crystals, yield=76%, m.p. 155 °C, IR (KBr, cm^{-1}): 3320 (NH), 1643 (C=N), 1269 (C=S), 1025 (C-N), 1620 (C=C arom.), 1400 (S=O), 1510 (N=O); ^{1}H NMR ($CDCl_3$), δ: 8.34-7.91 (m, 4H, arom), 7.81-7.51 (m, 5H, arom.), 7.1 (s, 1H, N*H*, exchangeable with D_2O), 4.77 (s, 2H, CH_2), 2.3 (s, 2H, NH_2, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$), δ: 180 (C=S), 156.8 (C=N), 124- 140 (aromatic carbons), 64.0 (CH_2). Anal. Calcd for $C_{15}H_{14}N_4O_4S_2$: C, 47.61, H, 3.73, N, 14.81 found: C, 46.24, H, 3.47, N, 14.67; ESI MS: *m/z* 378.4123 [$M^{+\cdot}$].

1-(4-Nitrophenyl)-2-(toluene-4-sulfonyl)-ethanone thiosemicarbazone (5)

Yellow crystals, yield=72%, m.p. 159 °C, IR (KBr, cm^{-1}): 3320 (NH), 1643 (C=N), 1269 (C=S), 1025 (C-N), 1620 (C=C arom), 1400 (S=O), 1510 (N=O); ^{1}H NMR ($CDCl_3$), δ: 8.34-8.15 (m, 4H, arom.), 7.61-7.37 (m, 4H, arom.), 7.0 (s, 1H, NH, exchangeable with D_2O), 4.74 (s, 2H, CH_2), 2.47 (s, 3H, CH_3), 2.1 (s, 2H, NH_2, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$), δ: 182 (C=S), 159.8 (C=N), 21.7 (CH_3), 64.2 (CH_2), 124.0 (2CH), 128.5 (2CH), 130.0 (2CH), 130.5 (2CH), 135.4 (2C), 140.0 (C), 145.9 (C), 107 (C-N). Anal. Calcd for $C_{16}H_{16}N_4O_4S_2$: C, 48.97, H, 4.11, N, 14.28 found: C, 48.84, H, 3.97, N, 14.07; ESI MS: *m/z* 392.2215 [$M^{+\cdot}$].

***2-(4-Nitrobenzenesulfonyl)-1-(4-nitrophenyl)-ethanone thiosemicarbazone* (*6*)**

Pale yellow crystals, yield = 80%, m.p. 150 °C, IR (KBr, cm^{-1}): 3320 (NH), 1643 (C=N), 1269 (C=S), 1025 (C-N), 1620 (C=C arom), 1400 (S=O), 1510 (N=O); ^{1}H NMR ($CDCl_3$), δ: 8.45-8.14 (m, 4H), 8.0-7.7 (m, 4H, arom.), 8.14 (m, 4H), 7.2 (s, 1H, NH, exchangeable with D_2O), 4.84 (s, 2H), 2.4 (s, 2H, NH_2, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$), δ: 186.5 (C=S), 154 (C=N), 124.2 (2CH), 124.5 (2CH), 130.3 (2CH), 130.4 (2CH), 139.5 (2C), 143.6 (2C), 100 (C-N), 63.4 (CH_2). Anal. Calcd for $C_{15}H_{13}N_5O_6S_2$: C, 42.55; H, 3.09; N, 16.54. Found: C, 42.31; H, 3.01; N, 16.19. ESI MS: *m/z* 423.1148 [$M^{+\cdot}$].

2.2 Antibacterial activity

The Disk Diffusion Method [12, 13] was employed to screen the compounds (**4-6**) against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (MRSA +ve) (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* (ATCC 19615) and *Klebsiella pneumoniae* (clinical isolate). Standard inoculums (1-2 × 10^7 c.f.u. / ml 0.5 McFarland standards) were introduced on the sterile agar plate surface, and for even distribution of the inoculums, a sterile glass spreader was used. The Whatman no. 1 filter paper was used to prepare discs (6 mm) and was sterilized at 140 °C for one h. The sterile discs were placed in nutrient agar medium after being already soaked with a known concentration (50 mg/mL) of the test compounds. Solvent and growth controls were kept. The positive control taken was Ciprofloxacin (30 µg), while the negative control selected was DMSO. The plates were inverted and incubated for 24 h at 37 °C. The potential bioactivity was checked in terms of the diameter of the zone of inhibition against all strains. Inhibition zones were measured and

Table 1: Showing the zone of inhibition of compounds (**4-6**) with given bacterial strains

Compounds	Diameter of zone of inhibition (mm)				
	Gram-positive bacteria			Gram-negative bacteria	
	S. pyogenes	*MRSA**	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
4	20.3±0.2	17.6±0.4	13.4±0.6	14.1±0.4	15.7±0.4
5	21.2±0.3	16.6±0.2	17.3±0.4	16.2±0.3	21.6±0.4
6	19.6±0.4	18.9±0.5	16.8±0.2	15.7±0.2	15.7±0.2
Standard	23.0±0.2	22.0±0.2	32.0±0.3	19.0±0.2	27.0±0.2
DMSO	-	-	-	-	-

Positive control (standard); ciprofloxacin and negative control (DMSO)

Table 2: Showing the MICs and MBCs of compound **4-6** with given bacterial strains

Compounds	Gram-positive bacteria						Gram-negative bacteria			
	S. pyogenes		***MRSA****		***P. aeruginosa***		***K. pneumoniae***		***E. coli***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
4	12.5	25	25	50	25	50	25	50	25	50
5	25	50	25	50	25	50	25	100	50	100
6	25	100	25	100	50	100	50	100	50	100
Standard	12.5	12.5	6.25	12.5	12.5	25	6.25	25	6.25	12.5

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compared with the controls. The bacterial zones of inhibition values are given in **table 1**.

The broth dilution technique was used to find the minimum inhibitory concentrations (MICs). The growth of cultures was monitored visually and spectrophotometrically after being incubated for 24 h at 37 °C. The minimum inhibitory concentration (MIC) is the lowest concentration (highest dilution) required to arrest bacterial growth. To obtain the minimum bacterial concentration (MBC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 18-24 h of incubation at 35 °C. The lowest concentration of compound at which 99.9% of the inoculums were killed is actually MBC. The MIC and MBC are given in **table 2**.

3. Results and discussion

3.1 Chemistry

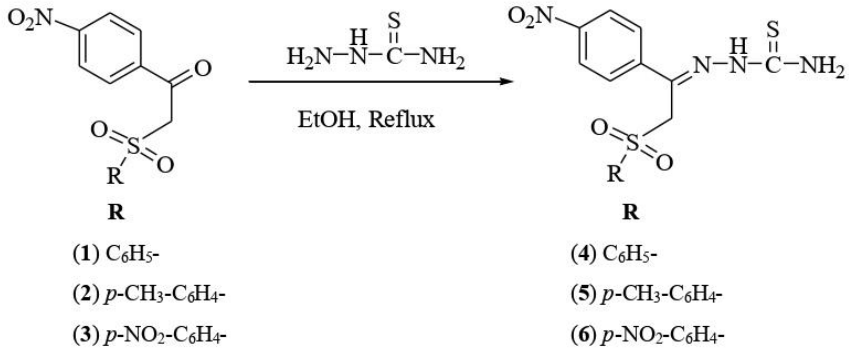
The synthesis of sulfonyl thiosemicarbazone derivatives (**4-6**) was carried out from corresponding sulfonyl ketones (**1-3**) and thiosemicarbazide in absolute ethanol, as shown in **scheme 1**. The reaction was conducted under reflux conditions for 3-4 hours.

The formation of sulfone thiosemicarbazone was shown by IR absorption bands at 3320 cm^{-1} (N-H), 1269 cm^{-1} (C=S), 1510 cm^{-1} (S=O), 1400 cm^{-1} (N=O) and 1643 cm^{-1} (C=N) in the compounds **4-6**. The formation of sulfone thiosemicarbazone was further established based on ^{1}H NMR spectra. The assignment of signals is based on the chemical shift (δ) and intensity pattern. The ^{1}H NMR spectra depicted a singlet (exchangeable with D_2O) for a single proton (N*H*) at δ 7.0-7.2 and another singlet (exchangeable with D_2O) for two protons (NH_2) at δ 2.1-2.4. It also predicts singlet for two protons at δ 4.74-4.84 suggested the presence of -CH_2-S. ^{13}C NMR signals are in good agreement with the anticipated structure of synthesized compounds. Finally, the mass spectra exhibited distinct molecular ion peak [M^+] at m/z: 378.4123, 392.2215 and 423.1148, respectively.

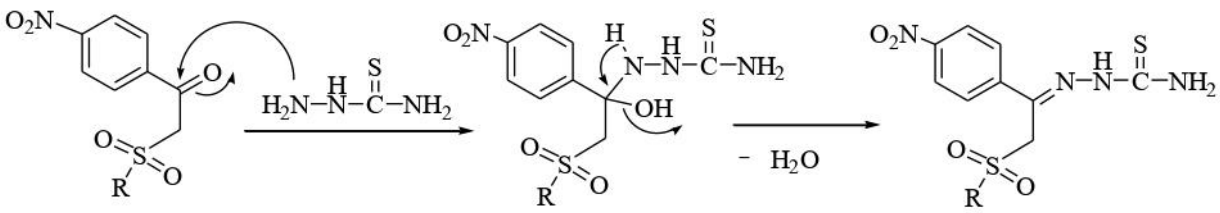
The formation of these thiosemicarbazones (**4-6**) under consideration and as per the known literature [14] can be shown according to the most probable mechanism (**Scheme 2**). The proposed mechanism involves the nucleophilic attack of nitrogen of thiosemicarbazide on carbonyl group (C=O) of sulfone because the availability of lone pair of electrons on nitrogen makes it potentially nucleophilic in nature, and once it has undergone reaction with the carbonyl group, it attacks the carbon of C=O, forms one linkage with nitrogen, i.e. (C-NH) and other with oxygen, i.e. (C-OH) which in the later stage involves the removal of the water molecule which paves the path for the formation of corresponding products (**4-6**). The novelty in the synthesis of these sulfonyl thiosemicarbazones is that there is an active methylene group between the carbonyl and sulfonyl groups. It was expected that the active methylene group may react with thiosemicarbazide and may form normal amine linkage (CH-NH), but once reaction got completed, after characterization, it was observed that only the carbonyl group undergone reaction with thiosemicarbazide moiety and result in the formation of imine linkage (C=N) as shown in scheme 2.

3.2 Antibacterial activity

The investigation of antibacterial screening data revealed that all the tested compounds (**4-6**) showed moderate to good antibacterial activity against *S. pyogenes*, *S. aureus* and *E. coli* species. The compounds **4**, **5** and **6** depicted zones of inhibition, i.e., 20.3 mm, 21.2 mm and 19.6 mm, against *S. pyogenes* strain. In general, all the compounds (**4-**



Scheme 1: Synthesis of sulfonyl thiosemicarbazones



Scheme 2: Proposed mechanism for the formation of sulfonyl thiosemicarbazones (**4-6**)

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6) were more effective against Gram-positive bacteria as compared to Gram-negative bacteria.

Figure 1 represents the pictures of the strains showing maximum inhibition zones during the antibacterial screening. Among the Gram-negative bacteria, compound **4** showed a maximum zone of inhibition of 20.3 ± 0.2 against the *S. pyogenes* strain, while compound **6** showed a large inhibition zone of inhibition of 18.9 ± 0.5 against *S. aureus* strain. Among the Gram-negative bacterial strains, compound **5** showed a maximum zone of inhibition 21.6 ± 0.4 against the *E. coli* strain. It is clear from **fig. 1** that these compounds (**4-6**) showed large zones of inhibition against Gram-positive bacteria as compared to Gram-negative bacteria.

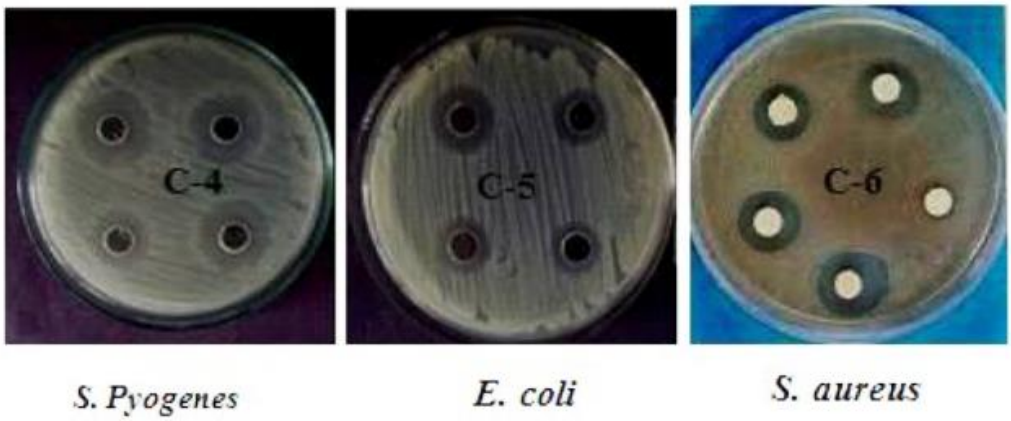

Figure 1: Showing the zones of inhibition against bacterial strains

It has been reported in the literature that the biologically active potentialities of the nitrogen-based heterocycles depend on the certain bio-pathways that lead to the formation of a highly reactive intermediate via reduction of the nitrogen-containing functional group, which is responsible not only for antimicrobial activity but also for cytotoxic and mutagenic properties [15]. The literature has also reported that the hydrocarbon skeleton functions as a lipophilic group to drive these organic compounds through the semipermeable membrane of the cell, i.e., enhanced penetration of these heterocycles into lipid membranes blocking the binding sites of the enzymes of the micro-organism [16]. The MIC of almost all three compounds was found to be two-fold or four-fold higher than the MIC of the standard drug, as shown in **table 2**. This also depicts that the compounds are potential antibacterial agents.

Literature reveals that many thiosemicarbazone derivatives showed potential antimicrobial activities. Recently, Božic *et al*. explored the antimicrobial activity of many mono- and bisthiocarbohydrazones derived from 2-acetylpyridine and related carbonyl compounds against four different strains of Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus, Clostridium sporogenes* and *Kocuria rhizophila*) and four strains of Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus hauseri* and *Salmonella enterica* subsp.) during which the compounds depicted better antimicrobial behaviour [17]. Avaji *et al.* also depicted the antimicrobial activities of the new thiosemicarbazone derivatives and their La (III) and Th (IV) complexes [18]. The ligand was highly active against *A. niger* and *A. fumigates* (fungi), whereas its La (III) complex shows high activity against *S. aureus* (bacteria), *A. niger*, and *A. fumigates* (fungi). On the other hand, the Th (IV) complex shows more activity against *P. auregonosa* (bacteria), *A. niger*, and *A. fumigates* (fungi) [18]. Bacchi *et al.* also studied thiosemicarbazone derivatives of isatin and N-methylisatin organotin (IV) complexes exhibit the highest antibacterial activity against Gram-positive bacteria, and some of the butyl-tin compounds are broad-spectrum agents as they are active also towards Gram-negative bacteria [19]. Moreover, the antibacterial properties of these complexes are coupled with a lack of mutagenicity.

4. Conclusion

In summary, the convenient and operationally simple protocol for the synthesis of sulfonyl thiosemicarbazone was successful. The reaction completed in a moderate time period and, potential yields (72-80%) were obtained on completion. This strategy offered a very straight forward and efficient method for access to sulfonyl thiosemicarbazone. During the biological screening against different bacterial strains, compounds showed moderate to good antibacterial activity against *S. pyogenes*, *S. aureus* and *E. coli* species. This protocol paves the path for synthesizing other thiosemicarbazones to be actively involved in different biologically active reactions.

Declarations

Author Contribution: AMD has contributed to the conception of the presented idea for the article; PY and PK did literature search and data analysis; and all the authors have contributed in preparing the manuscript.

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Conflict of Interest: No potential conflict of interest is being reported by the authors.

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