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Synthesis and antibacterial activity of Benzo-2-phenyl-1-thia-2, 3-diazolium bromide and its derivatives

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Abstract

We have synthesized a series of sulfenyl derivatives of o-*mercapto compound where S(II) atom is electrophilic in nature. The antibacterial activity of five sulfenyl derivatives of* o-*mercapto compound was studied against three pathogenic bacteria viz.* Escherichia coli, Klebsiella, Staphylococus aureus *and two nonpathogenic bacteria viz.* lactobacillus casei, bacillus cereus *by disk diffusion method. Amongst these sulfenyl compounds, three have distinct inhibitory properties against four strains of bacteria except* bacillus cereus. *The antibacterial activity of these sulfenyl compounds against* lactobacillus casei *was found to be more compared to the standard ceftriaxone, but was less against pathogenic bacterial strains. Benzo-2-phenyl-1-thia-2,3-diazolium bromide was found to have the highest activity against all bacterial strains. These sulfenyl compounds show cidal action against all tested bacterial strains. Further density functional studies (DFT) were performed to observe stability of BTD-salt and corresponding open structures.*

Keywords: *Sulfenyl compound,* o-*Mercapto compound, disk diffusion method, zone of inhibition, antibacterial activity.*

Introduction

Sulfenic acids (Ar-SOH) though unstable, have been recommended as key intermediates in many biochemical reactions and metabolic pathways [1,2]. Biologically the most important reaction of thiols (ArSH) is oxidation to sulfenic acids. This oxidation is believed to be essential for enzymatic activity of proteins. For example, in thiol proteinase, a free sulfhydryl (-SH) group is converted to sulfenic acid with mild oxidants under non denaturing conditions [3]. The highly reactive sulfenic acids remain unusually stable due to the steric restriction in the enzyme. On the other hand, some enzymes like papain and GAPDH lose their normal activity due to the oxidation of P-SH $\rightarrow$ P-SOH and the oxidized enzyme P-SOH can act as an enzyme for a different reaction e.g. GAPDH-SH on oxidation to GAPDH-SOH loses its normal dehydrogenase activity but the oxidized form of the enzyme GAPDH-SOH now acts as a deaminase enzyme to oxidize 1°-amines to aldehydes under anaerobic condition [4]. Sulfenic acid intermediate is believed to be involved in the irreversible enzyme inactivation of FAD–linked monooxygenase [5]. Milk and saliva possess antimicrobial activity which has been ascribed to lactoperoxidase peroxide thiocyanate system which oxidizes bacterial sulfhydryl group -SH to sulfenic acid [6-9]. The chemistry of penicillin sulfoxides is intimately related to the stability of 2-oxazetidine-4-sulfenic acid [10]. On the other hand, exogenous sources of sulfenic acids e.g. 6-thiopurine, an antineoplastic

agent undergoes metabolic activation by hepatic microsomal cytochrome P-450 to the corresponding sulfenic acid[11]. The sulfenic acid is capable of binding to the microsomal proteins. Thus, the bioactivity of endogenous or exogenous bivalent organosulfur compounds is somehow related to their *in vivo* metabolic activation to the corresponding sulfenic acids.

In the present study, we have synthesized five sulfenyl derivatives of *o*-mercapto compound namely as benzo-2-phenyl-1-thia-2,3-diazolium bromide (I), benzo-2-(22 -nitro-42 -methyl phenyl)-1-thia-2,3-diazolium bromide (II), benzo-2-phenyl-1-thia-2,3-diazolium thiocyanate (III), benzo-2-phenyl-1-thia-2,3-diazolium cyanide (IV) and benzo-2-phenyl-1-thia-2,3-diazolium iodide (V) where S (II) atom is electrophilic in nature as shown in Figure 1. We have chosen these sulfenyl compounds mainly for their unusual solubility in polar solvent water and their higher stability in water, properties which are related to their benzothiadiazolium salt like structure (BTD form). Sulfenyl compounds have been used as selective modifying reagent for a variety of amino acids in aqueous or aqueous alcoholic environment [12,13]. Sulfenium cation carriers play a role in oxidative phosphorylation [14]. The interaction of sulfenyl halides with penicillium carboxylesterase has been studied [15]. These sulfenyl compounds may be regarded as the derivatives of sulfenic acid and have the potential to be converted into the corresponding transient sulfenic acids *in vivo* and therefore, may have bioactivity against specific microorganisms as shown in Figure 1.

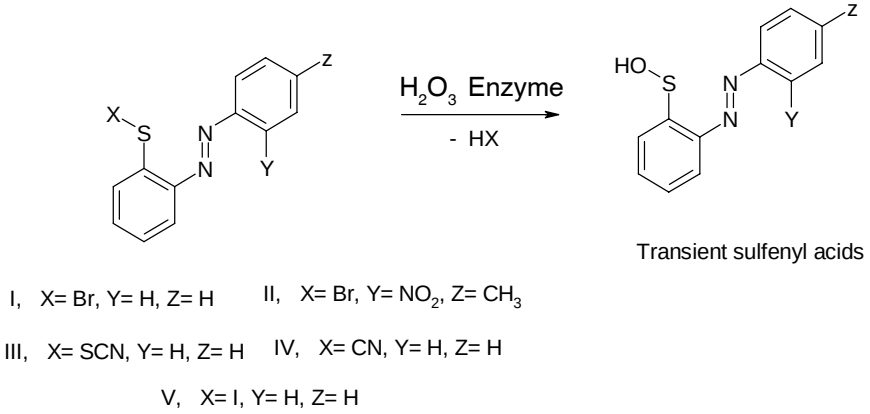


Fig. 1: Structure of sulfenyl compounds (I-V) and their hypothetical conversion to sulfenic acid in the enzyme.

Materials and Methods

Experimental

Melting points were recorded on a Veego melting point apparatus and are uncorrected. CHN analyses were recorded on Perkin Elmer Series II CHNS/O analyzer. IR spectra were recorded on IR affinity I FTIR Spectrometer SHIMADZU as KBr Pellet. ^{1}H and ^{13}C NMR spectra were recorded on Ultrasonic Brukar 300 MHz FT NMR Spectrometer, using TMS as internal standard and $CDCl_3$ as solvent. All the chemicals used were of Merck. Sulfenyl compounds (III-V) were synthesized as described by Burawoy A [19].

General procedure for debenzylation of o-azoarylbenzyl sulfide

To a magnetically stirred warm solution of *o*-azoarylbenzyl sulfide (1 mmol) in 10 mL acetic acid was added drop wise a solution TBATB (1mmol) in 15 mL acetic acid and was refluxed for 3 min. The colour of the solution changed immediately. The reaction mixture was allowed to cool and precipitation occurred. The precipitate was filtered off, dried and crystallized from water.

Benzo-2-phenyl-1-thia-2,3-diazolium bromide (I): yellow crystals of mp 220-222^{0}C (Lit. 224-226^{0}C). MS

(m/z, %): 213 (M-1, 100), 214 (M, 15), 215 (M+1, 8). UV-VIS Spectra ($\lambda_{max, nm}$ in ethanol): 336 (A=0.4514), 342 (A=0.5758). IR (KBr), υ_{max}, cm^{-1}: 2997.38 (C-H str), 1598.34 (N=N str.), 1365.60 (Ar. C-N str.), 763.81 (C-S str). ^{1}H NMR (300MHz, $CDCl_3$, TMS): δ 7.695(d, 3H, J=6.9Hz), 7.914-8.027 (m, 2H), 8.169 (d, 2H, J=6Hz), 8.565 (d, 2H, J=8.1Hz), 9.604 (d, 2H, J=7.8Hz). ^{13}CNMR (300MHz, $CDCl_3$, TMS): 122.54(2C), 125.71, 128.80, 130.62(2C), 130.87, 133.15, 134.19, 140.23, 151.14. Anal. Calcd. for $C_{12}H_9BrN_2S$: C, 49.16; H, 3.09; N, 9.55. Found: C, 49.0; H, 3.1; N, 9.4.

Benzo-2-(22 -nitro-42 -methylphenyl)-1-thia-2,3-diazolium bromide (II): yellow crystals of mp 226-227°C (Lit.228-290°C). MS (m/z, %): 215 (M). UV-VIS Spectra ($\lambda_{max, nm}$ in ethanol): 331 (A=0.1570), 448 (A= 0.0328). IR (KBr), υ_{max}, cm^{-1}: 3074.53 (C-H str), 1597.06 (N=N str.), 1539.20, 1357.89 (NO_2 str.), 763.81 (C-S str). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.658 (s, 3H, CH_3), 7.757-7.787 (m, 2H), 7.962-7.988 (m, 1H), 8.068-8.093 (m, 2H), 8.525 (d, 1H, J=8.1Hz), 9.597 (d, 1H, J=8.7Hz). ^{13}CNMR (300 MHz, $CDCl_3$, TMS): 21.58 (C of CH_3), 126.28, 127.15, 128.40, 1129.26, 131.09, 134.79, 134.90, 145.44. Anal. Calcd. for $C_{13}H_{10}BrN_3O_2S$: C, 44.3; H, 2.8; N, 11.9. Found: C, 44.1; H, 2.6; N, 11.7.

Benzo-2-phenyl-1-thia-2,3-diazolium thiocyanate (III): red crystals of mp 145-146°C (Lit. 147-148°C). UV-VIS Spectra ($\lambda_{max, nm}$ in ethanol): 342.8 (A =1.5942), 415.9 (A =0.5361). IR (KBr), υ_{max}, cm^{-1}: 2989.66 (C-H str), 2360.87, 2071.55 (CN), 1593.20 (N=N str.), 1361.74 (Ar. C-N str.), 763.81 (C-S str). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.742 (s, 3H), 7.986-8.049 (m, 2H), 8.309 (s, 2H), 8.726 (d, 1H, J=8.1 Hz), 8.879 (s, 1H). ^{13}CNMR (300 MHz, $CDCl_3$, TMS): 122.41 (C of SCN), 125.92, 126.27, 130.99, 131.27, 133.66, 134.97, 144.72, 151.49. Anal. Calcd. for $C_{13}H_9N_3S_2$: C, 57.54; H, 3.34; N, 15.48. Found: C, 57.6; H, 3.3; N, 15.4.

Benzo-2-phenyl-1-thia-2,3-diazolium cyanide (IV): yellow crystals of mp 98-99°C (Lit. 99-100°C). UV-VIS Spectra ($\lambda_{max, nm}$ in ethanol): 339.2 (A=1.4215), 396.6 (A=0.6012). IR (KBr), υ_{max}, cm^{-1}: 2927.94 (C-H str), 2360.87, 2337.72 (CN str.), 1593.20 (N=N str.), 1361.74 (Ar. C-N str.), 763.81 (C-S str). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.268-7.550 (m, 5H), 7.880-8.033 (m, 4H). ^{13}CNMR (300 MHz, $CDCl_3$, TMS): 111.51 (C of CN), 123.11, 124.12, 124.98, 127.99, 128.44, 129.39, 132.17, 132.29, 148.02, 151.21. Anal. calcd. for $C_{13}H_9N_3S$: C, 65.25; H, 3.79; N, 17.56. Found: C, 65.3; H, 3.8; N, 17.5.

Benzo-2-phenyl-1-thia-2,3-diazolium iodide (V): red crystals of mp 191-192°C (Lit. 192-193°C). UV-VIS Spectra ($\lambda_{max, nm}$ in ethanol): 342.8 (A=1.5942), 415.9 (A=0.5361). IR (KBr) $\upsilon_{max,}$ cm^{-1}: 2924.09 (C-H str), 1597.06 (N=N str.), 1357.89 (Ar. C-N str.), 771.53 (C-S str). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 7.717 (d, 3H, J=7.8), 7.790-8.182 (m, 4H), 8.565 (d, 1H, J=7.8 Hz), 9.612 (d, 1H, J=9 Hz). ^{13}CNMR (300 MHz, $CDCl_3$, TMS): 116.33 (3C), 119.40, 124.41(3C), 124.73, 126.97, 127.86. Anal. calcd. for $C_{12}H_9N_2SI$: C, 42.37; H, 2.67; N, 8.23. Found: C, 42.4; H, 2.7; N, 8.4.

Selected microorganism

Three pathogenic strains of bacteria namely *Escherichia coli*, *Klebsiella* and *Staphylococcus aureus* and two nonpathogenic strains of bacteria namely *Lactobacillus casei*, and *Bacillus cereus* were selected for bioactivity studies. *E.coli*, gram +ve bacteria cause diarrhea, *Staphylococus aureus*, gram +ve bacteria cause food poisoning by producing toxins and *Klebsiella*, gram – ve bacteria cause pneumonia. These pathogenic strains were collected from the Department of Microbiology, Gauhati Medical College, Guwahati, India. The experiment was conducted in the Department of Bio-Techolnology, Cotton College, Guwahati, India. The collected strains of bacteria were subcultured in Agar slants before use and then cultured in nutrient Agar.

Preparation of test samples

A 1% solution of the sulfenyl compounds (I-IV) was prepared by dissolving 0.05g in 5mL distilled water. Sulfenyl compound (V) being insoluble in water, was dissolved (0.05g) in 5mL in ethanol to test. During preparation of these solutions, a mechanical shaker was used for constant stirring. The stock solution was stored

at room temperature in an airtight container under sterile conditions. Serial dilution of 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4% and 0.1% solutions of the compounds were prepared in distilled water from the 1% stock solution.

Inoculum preparation

Nutrient agar plates containing pure culture of the microorganisms were taken. Four to five colonies of each organism were inoculated into sterile nutrient broth thoroughly. The inoculated test tubes were incubated overnight at 37°C for bacteria to grow. The observed frequency (OD) was observed at 600nm to determine the concentration of bacteria by the formula, OD of 1= 8.0×10^8 cells/mL. The calculated ODs of all the bacterial strains; *Escherichia coli, Klebsiella Staphylococcus aureus, Lactobacillus casie* and *Bacillus cereus* at 600nm were 0.770, .0754, 0.601 0.605 and 1.035 and bacterial concentrations were 6.16×10^8, 6.03×10^8, 4.81×10^8, 4.84×10^8 and 8.29×10^8 organisms/mL respectively.

Test

Disk diffusion method was employed for the test of bioactivity of compounds [23]. Eight Petri disc plates were taken and dried in a bio-safety cabinet. Sterile swabs were dipped into inoculums solution and were used to prepare lawn-culture on the MHA plates by spreading uniformly with 300 µL inoculum of the bacterial strains i.e *E.coli, Klebsiella, Staphylococus aureus, Lactobacillus casei* and *Bacillus cereus*. A filter paper of 5mm diameter was punched out and sterilized in autoclave for 15 minutes. 5µL of each 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4% and 0.1% solutions were pipetted into the filter paper and allowed to parch for 5 minutes. These filter papers were placed on the Petri plate containing culture medium of bacterial strains by numbering each for different concentrations. These plates were then incubated at 37°C for 48 hours. After incubation, the plates were taken out and observed for zones of inhibition (ZOI) for 24 hours and 48 hours. To confirm the cidal activity of the sulfenyl compounds, the plates were kept for 7 days. The plates showing the ZOI around the paper indicated the presence of antibacterial activity of the tested compounds. The diameters of ZOIs around the filter paper were measured in mm by normal scale and were recorded.

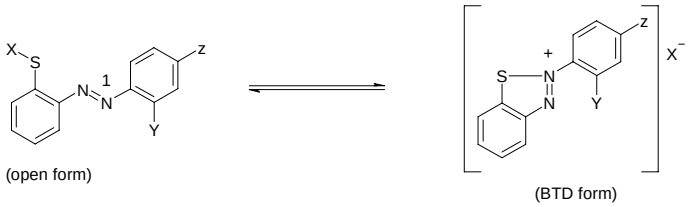
Computational details

The geometrical minima of both open form and BTD salt form of the sulfenyl compounds (I, II, IV and V) were optimized with 6-311++G(d,p) basis set with Becke three parameter exchange and Lee, Yang and Parr correlation functional, B3LYP and was confirmed by frequency calculations. Thereafter, on the optimized geometry of these compounds single point energy calculations were performed in same level of theory in the water and ethanol phases. To observe the consistency in results we repeated our calculations at MP2/6-311++G(d,p) level of theory on Gaussian09.

Results and Discussion

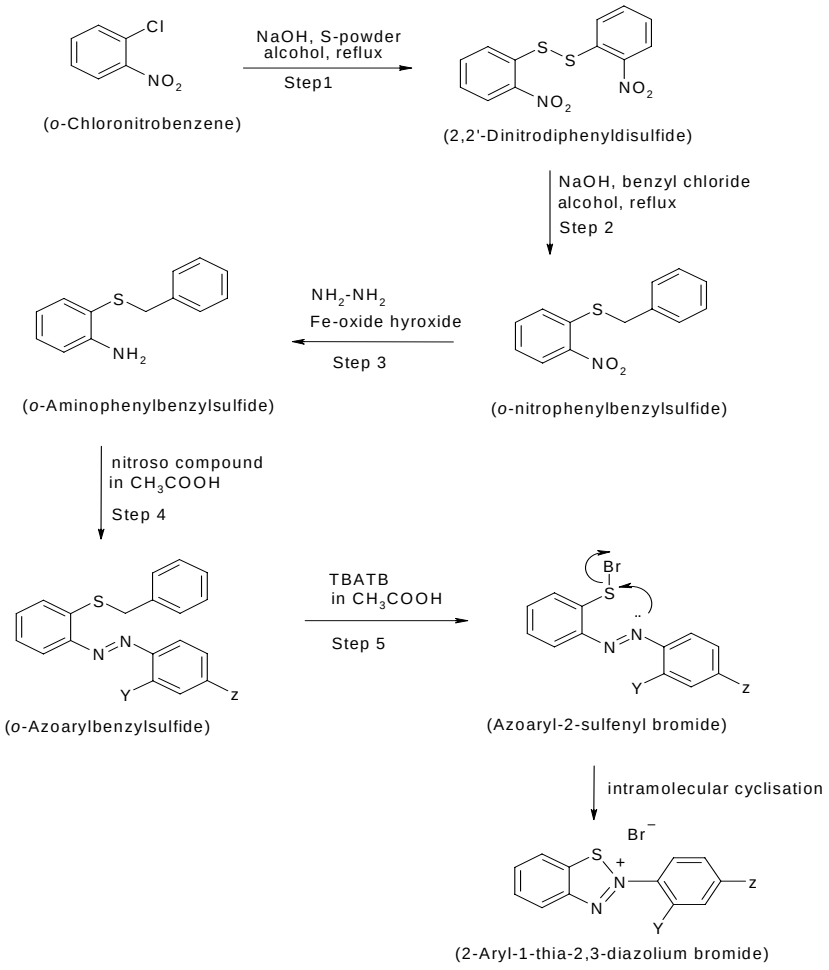
Aliphatic sulfenyl compounds, (X= Cl, Br) having α-H atom are very prone to Pummerer type rearrangement [16]. Aromatic sulfenyl compounds are more stable compared to aliphatic sulfenyl compounds due to absence of α-H atom and also due to the d-resonance of S atom with aromatic system [17]. When an *o*-arylazo group is introduced into the benzene ring, the properties of the *o*-mercapto compounds are dramatically changed [18]. Burawoy A. et al. (1954) synthesized azobenzene-2-sulfenyl bromide [benzo-2-aryl-1-thia-2,3-diazolium bromide (I)] and its derivatives [18-19]. We have synthesized the sulfenyl bromides (I and II) using different modified methodology for steps 3 and 5 as shown in Scheme 1. Theoretical calculations of these sulfenyl compounds (I-IV) at B3LYP/6-311++G(d,p) and MP2/6-311++G(d,p) levels of theory using Gaussian09[24] have shown that these sulfenyl compounds are more stable (stability defined as $\Delta E = E_{BTD} - E_{open}$) in BTD-salt structure than open structure and importantly, S-X (X = Br, SCN) bonds are longer than the experimental bonds whereas, in S-CN, it is reverse (Table 1).

Table 1: Energy difference as DE = E_{BTD}- E_{open} (in kcal/mol) in gas, ethanol and water phases at B3LYP and MP2 levels of theory. g ® gas, e ®ethanol, w ® water.



	B3LYP/ 6-311++G(d,p)			MP2/ 6-311++G(d,p)				
Sulfenyl compounds	ΔE_g	ΔE_e	ΔE_w	ΔE_g	ΔE_e	ΔE_w	Observed bond distance	*Experimental bond distance
I	-8.96	-12.47	-12.61	-10.47	-14.o2	-17.63	2.49	2.27
II	-9.11	-11.49	-11.56	-9.80	-11.51	-11.59	2.47	2.27
III	-4.38	-5.38	-5.41	-1.67	-2.46	-2.51	2.29	2.147
IV	-2.52	-1.86	-1.81	-.44	0.14	0.18	1.72	1.74

* Seninning Alexander, (Eds.) Sulfur in organic and inorganic chemistry, Marcel Dekker INC, New York, 1971.



Scheme 1: Synthesis of Sulfenyl compounds

In step 3, reduction of *o*-nitro phenyl benzylsulfide with hydrazine in presence of catalyst Fe-oxide hydroxide [20] gives better yield (95%) instead of 60% in case Fe/CH_3COOH reduction. Moreover, instead of using toxic molecular bromine, we are using green bromating agent tetrabutylammoimuntribromde (TBATB) [21] in step 5 which gives batter yield. Mass spectum of compound (I) reveals that it exists as BTD-salt structure. Molecular mass of the compound (I) is 214(M) without the Br-atom. Because of its BTD-salt structure, it becomes soluble in water and undergoes ion-exchange reaction with salt like KSCN, KCN, and KI in aqueous medium to give the corresponding thiocyanate (III), cyanide (IV) and iodide (V) by a single step nucleophilic substitution reaction. Benzo-2-(22 -nitro-42 -methylphenyl)-1-thia-2,3-diazolium bromide (II) has also been synthesized by the same methodology for benzo-2-phenyl-1-thia-2,3-diazolium bromide (I) by using 1-methyl-3-nitro-4-nitrosobenzene instead of nitrosobenzene.

The sulfenyl compounds I, II, III, IV and V reported here were tested for their antibacterial activity against three pathogenic viz. *Escherichia coli*, *Klebsiella*, *Staphylococcus aureus* and two nonpathogenic bacteria viz. *lactobacillus casei, bacillus cereus*. The antibacterial activity produced by sulfenyl compounds I, III and V against four bacterial strains *Eschericia coli, Klebsiella, Staphylococcus* and *Lactobecillus casei* are presented in Table 2.

Table 2. ZOIs produced by diffrent dilutions of sulfenyl compounds against *Eschericia coli (E.), Klebsiella (K.), Staphylococcus (S.) and Lactobecillus casei (L.).*

Sl. no.	Conc. (w/v)	Conc. (mcg/mL)	I				III				V			
			ZOI (mm)				ZOI (mm)				ZOI (mm)			
			E.	*K.*	*S.*	*L.*	*E.*	*K.*	*S.*	*L.*	*E.*	*K.*	*S.*	*L.*
1	1.0%	10,000	13	12	14	21	11	12	13	20	10	10	11	12
2	0.9%	9,000	12	11	14	18	10	9	12	17	10	10	9	11
3	0.8%	8,000	12	11	14	18	9	11	11	17	10	9	9	11
4	0.7%	7,000	11	10	13	17	8	11	13	16	10	9	9	10
5	0.6%	6,000	11	10	13	16	8	8	10	16	9	8	9	10
6	0.5%	5,000	11	9	13	15	8	8	9	16	9	8	8	10
7	0.4%	4,000	11	8	13	14	8	8	6	15	9	8	7	8
8	0.1%	1,000	8	6	8	7	7	6	6	7	6	7	7	7

It is observed from Table 1, that a concentration of 1% of each of the compounds was highly effective against all the bacterial strains. Compound I and III showed almost similar antibacterial activity against E., K., S. and L. with a ZOI of 13, 12, 14 and 21mm and 11, 12, 13 and 20mm. The differences in ZOI produced by sulfenyl compounds (I, III and V) against bacterial strains as well as ceftriaxone (CT) are shown in Figure 2.

The differences in ZOI produced by the sulfenyl compounds (I, III, V) against strains of bacteria were compared with ZOI produced by ceftriaxone (CT). The required concentration of the compounds to produce 1mm of ZOI by sulfenyl compound as well as ceftriaxone were calculated to make a comparison and also to find out the supremacy of antibacterial properties of the three sulfenyl compounds (I, III and V) as shown in Table 3.

Hence, 1mm ZOI =1.85 mcg of ceftriaxone = 3.33 mcg of compound I= 3.57 mcg of compound III= 4.65 mcg of compound V. In other word, 1 mcg of ceftriaxone = 1.8 mcg of compound I= 1.93 mcg of compound III= 2.51 mcg of compound V. Thus, the order of antibacterial activity is benzo-2-phenyl-1-thia-2, 3-diazolium bromide (I) > benzo-2-phenyl-1-thia-2, 3-diazolium thiocyanate (III) > benzo-2-phenyl-1-thia-2, 3-diazolium iodide (V). Similar findings were observed both in 24 hours and 48 hours of incubation.

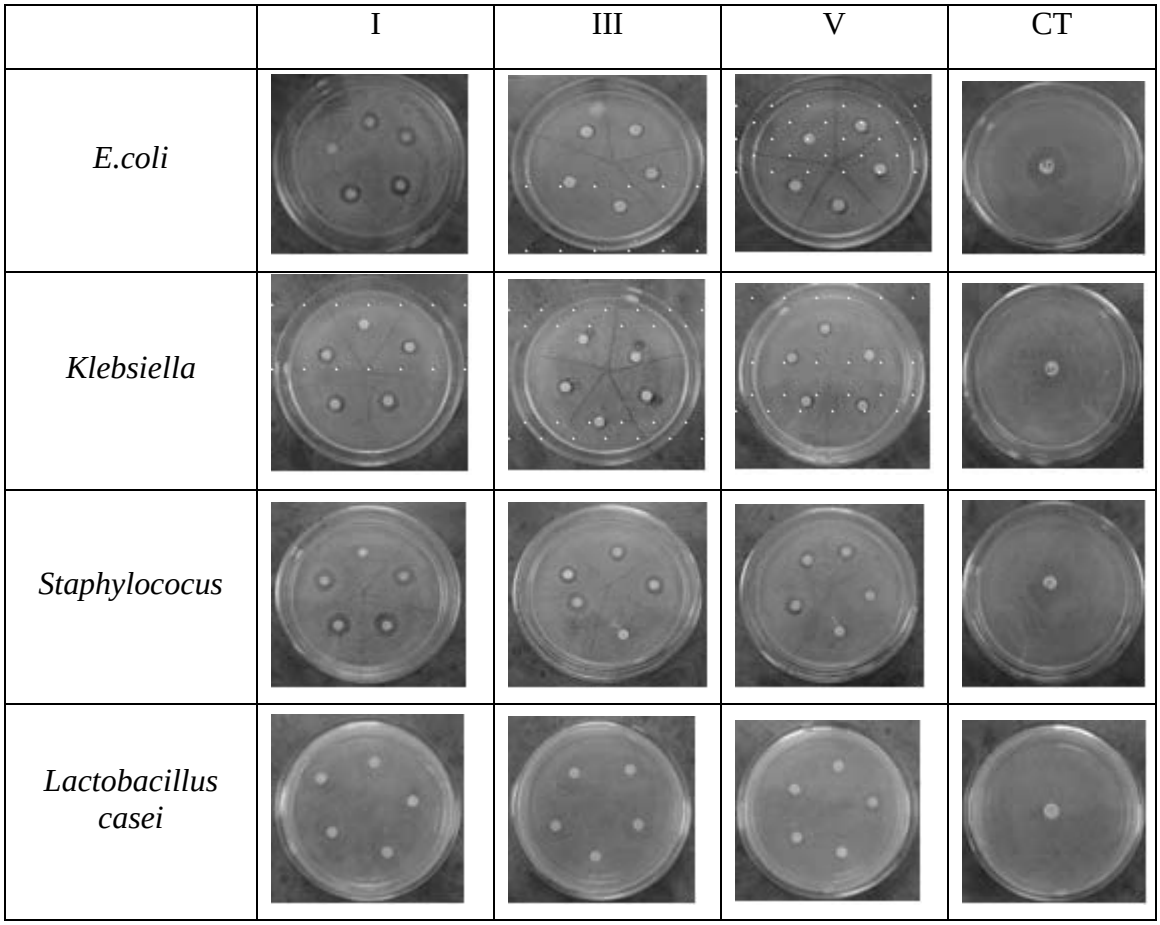


Fig. 2: ZOIs produced by sulfenyl compounds (I, III, V) and CT against the bacterial strains of *Escherichia coli*, Klebsiella, staphylococcus *aureus* and *Lactobecillus casei*. (Each plate contains 1%, 0.9%, 0.8%, 0.7% solutions along with distilled water, expect CT).

Table 3. The strength of antibacterial activity of sulfenyl compounds as compared to Ceftriaxone.

	I		III		V		CT	
Test Organism	Conc. (mcg)	ZOI (mm)	Conc. (mcg)	ZOI (mm)	Conc. (mcg)	ZOI (mm)	Conc. (mcg)	ZOI (mm)
E.coli	50	13	50	11	50	10	30	21
Klebsiella	50	12	50	12	50	10	30	22
Staphylococcus	50	14	50	13	50	11	30	14
Lactobacillus casei	50	21	50	20	50	12	30	8
Average	50	15	50	14	50	10.75	30	16.25
	1mm ZOI = 3.33 mcg		1mm ZOI = 3.57 mcg		1mm ZOI = 4.65 mcg		1mm ZOI = 1.85 mcg	

Earlier, Acharjee et al. studied the bioactivity of benzo-2-(42 -methylphenyl)-1-thia-2,3-diazolium bromide and its corresponding thiocyanate and cyanide [22]. The derivatives we synthesized were found to have superior antibacterial activity in comparison to established report. As seen from Tables 1 and 2, compound (I) showed very good antibacterial activity against all strains of bacteria especially against *Lactobacillus casei* with a ZOI of 16 mm at a concentration of 30 mcg. A similar concentration of the drug ceftriaxone exhibited a ZOI of 8 mm against the strains of *Lactobacillus casei.* This clearly suggested the superior antibacterial activity of the synthesized derivatives of sulfenyl compounds.

Conclusions

All activated sulfenyl compounds were found to be active against *lactobacillus casei*. It was noticed that all the tested sulfenyl compounds showed cidal action against all tested bacterial strains. Sensitivity of our sulfenyl compounds was found to be much lower as compared to the standard drug.

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