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SYNTHESIS AND CHARACTERIZATION OF NOVEL BENZALDEHYDE THIOSEMICARBAZONES AND EVALUATION OF THEIR ANTIBACTERIAL, ANTIFUNGAL AND ANTIOXIDANT ACTIVITY

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Keywords:

Benzaldehyde Thiosemicarbazones, Antibacterial Activity, Antifungal Activity, Antioxidant Activity

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ABSTRACT: In the present study 17 novel benzaldehyde thiosemicarbazone derivatives were synthesised using 2-methyl-3-thiosemicarbazide as the main reactant and different aromatic aldehydes. Reactions were monitored using thin layer chromatography technique and the newly synthesised derivatives were characterized by Elemental analysis, ATR and ^{1}HNMR techniques. For antibacterial activity four bacterial strains were selected namely *Bacillus subtilis* (gram +ve), *Staphylococcus aureus* (gram +ve), *Escherichia coli* (gram –ve), and *Pseudomonas aeruginosa* (gram -ve).*Candida albicans* was used to ascertain the antifungal activity of compounds. Antimicrobial assay was performed using nutrient agar media for the bacterial strains and Sabouraud agar for the fungus, zone of inhibition of all the compounds which could inhibit visible growth after incubation were calculated. Four different dilutions of the test compounds were taken and compared against ciprofloxacin and fluconazole as the standard drugs. Antimicrobial assay was performed in duplicate. The test compounds were active against both gram negative as well as gram positive bacteria. Considerable anti-fungal was shown by the compounds. Antioxidant assay was performed using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS method and 2,2-diphenyl-1-picrylhydrazyl or DPPH method.

INTRODUCTION: Thiosemicarbazones are a class of compounds containing important hetero atoms like sulphur and nitrogen. Structural simplicity, easy synthetic procedure, shorter course of reaction and wide pharmacological profile make them a class extensively studied and explored. They offer a wide spectrum of pharmacological activities like analgesic and anti-inflammatory [1], antibacterial [2], anticancer [3], anticonvulsant [4], antifungal [5], anti-HIV [6], anti leishmaniac [7], anti-malaria [8], neurotropic [9], anti trypanosomal [10], antitubercular [11], anti-viral [12], insulin like [13] and human and rat cholinesterase[14] activities. Metal complexes of thiosemicarbazones have also been found to be useful in radiopharmacy for therapeutic as well as diagnostic purposes [15].

Anti-cancer properties of theses derivatives have been studied widely.

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Many pharmacologically active moieties have been fused to the thiosemicarbazone system to enhance or widen the pharmacological potential of both. Fusion of 3-aminopyridine-2-carboxaldehyde to thiosemicarbazone system lead to development of Triapine, which is under process to be developed as an anticancer drug for cervical cancer [16]. Similarly fusion of isatin system to thiosemicarbazone backbone lead to the development of Methisazone, which was used to treat small pox and Herpes simplex virus [17]. Thioacetazone which is also a thiosemicarbazone analogue was once a useful drug in the combination therapy of tuberculosis. It was proposed that thioacetazone acted by inhibiting the synthesis of mycolic acid [18]. The decline in the use was seen due to emergence of resistance and a few instances of cross resistance were also reported to other drugs [19].

MATERIALS AND METHODS: All the chemicals used were obtained from Sigma Aldrich.

Synthetic Procedure: Ethanolic solution of 2-Methyl-3-thiosemicarbazide and the aldehyde were mixed in the molar ratio of 1:1. The resulting reaction mixture was refluxed with stirring for 2 hours or more depending on the course of reaction. The progress of the reaction was monitored by TLC. Diluted reaction mixture at equal intervals was taken and was run on silica gel GF coated TLC plates using n-Hexane: ethylacetate in the ratio 2:3 as the mobile phase. All the compounds (except TSC-1, TSC-8 and TSC-9) separated as precipitates in the reaction mixture after the completion of reaction. The precipitate was separated washed with cold ethanol and were recrystallized by slow evaporation of DMF (dimethylformamide) solution. TSC-1, TSC-8 and TSC-9 after completion of reaction were obtained by adding water to the reaction mixture. The precipitate so obtained was washed with water and the product was recrystallized from ethanol.

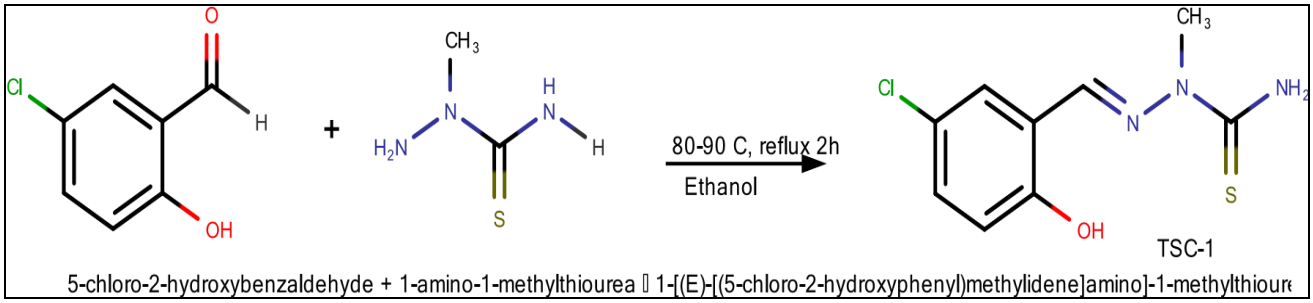


FIG. 1: REACTION SCHEME

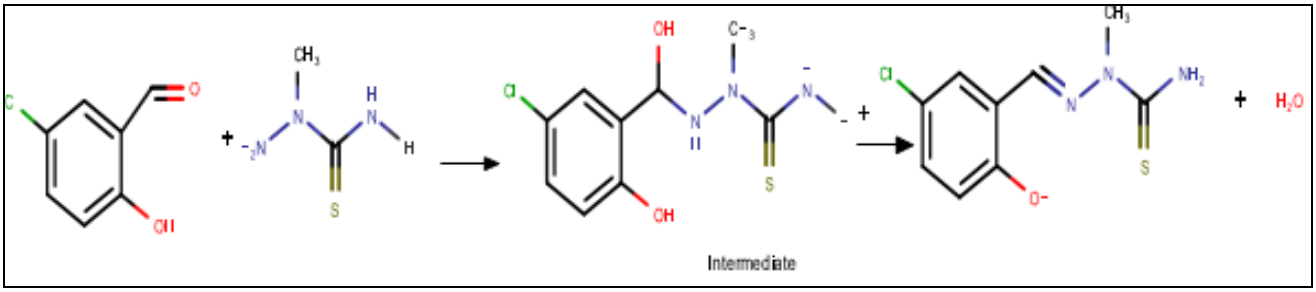
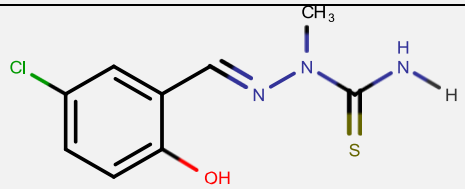
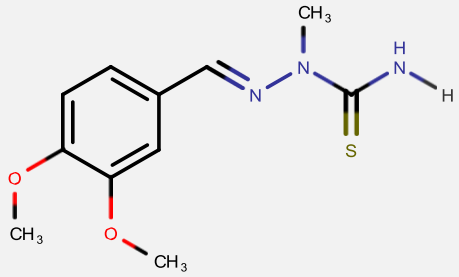
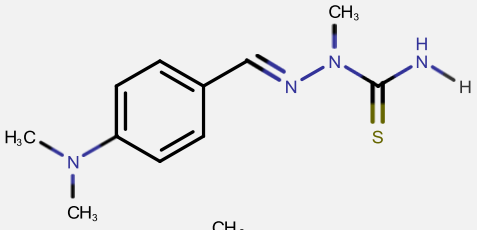
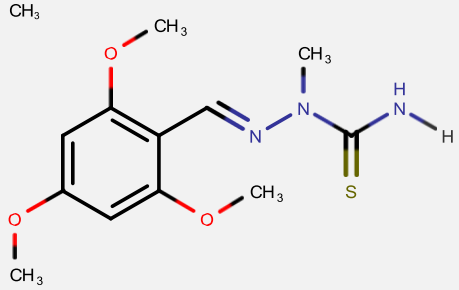
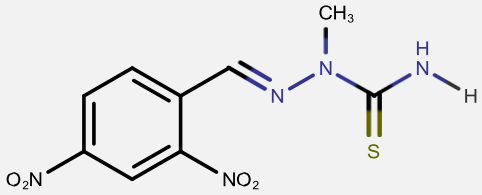
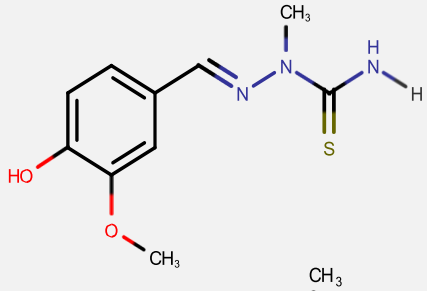
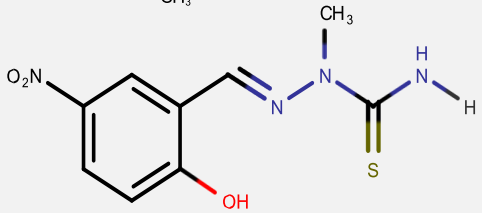
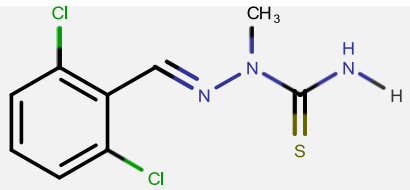
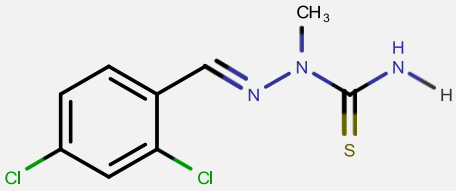
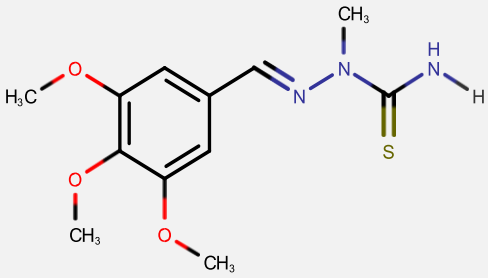
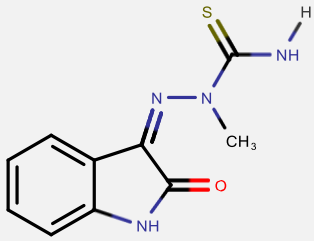
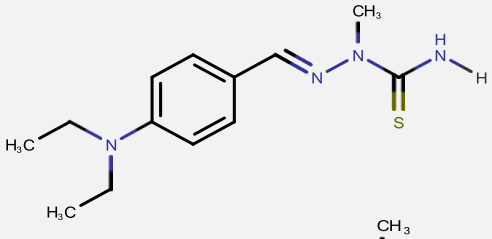
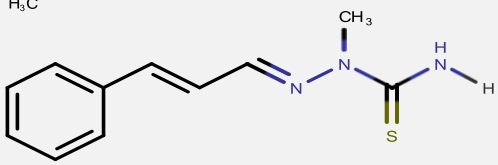
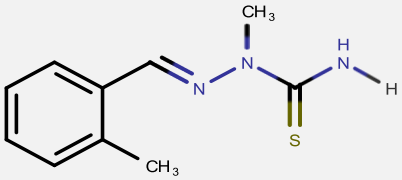
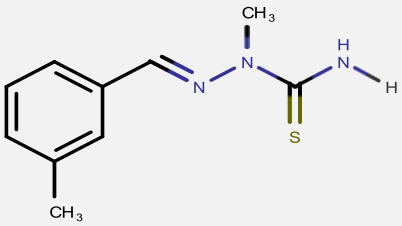


FIG. 2: MECHANISM OF REACTION

Melting point ranges of newly synthesized compounds were determined by open glass tube using visual melting apparatus and are uncorrected. The temperature at which compound started melting to the temperature at which it completely melted was taken as the melting point range. All the new derivatives were subjected to elemental detection for elements like Chlorine, Nitrogen and Sulphur. Qualitative assessment for the presence of these atoms was done by preparing Lassaign extract of the compounds. IR spectrum of compounds was recorded on an ATR Spectrophotometer. ^{1}HNMR spectrum of newly synthesised compounds was recorded on NMR spectrometer at 400MHz in Chloroform and DMSO (Dimethyl sulfoxide) using TMS (Tetramethylsilane) as internal standard. Chemical shift, delta δ in ppm was recorded.

TABLE 1: COMPOUNDS SYNTHESISED

Name	Structure	IUPAC Names
TSC-1		1-[(E)-[(5-chloro-2-hydroxyphenyl)methylidene]amino]-1methylthiourea
TSC-2		1-[(E)-[(3,4-dimethoxyphenyl)methylidene]amino]1-methylthiourea
TSC-3		1-[(E)-{[4-(dimethylamino)phenyl]methylidene}a mino]-1-methylthiourea
TSC-4		1-methyl-1-[(E)-[(2,4,6-trimethoxyphenyl)methylidene]amino]t hiourea
TSC-5		1-[(E)-[(2,4-dinitrophenyl)methylidene]amino]-1methylthiourea
TSC-6		1-[(E)-[(4-hydroxy-3-methoxyphenyl)methylidene]amino]-1methylthiourea
TSC-7		1-[(E)-[(2-hydroxy-5-nitrophenyl)methylidene]amino]-1methylthiourea

TSC-8		1-[(E)-[(2,6-dichlorophenyl)methylidene]amino]-1methylthiourea
TSC-9		1-[(E)-[(2,4-dichlorophenyl)methylidene]amino]-1methylthiourea
TSC-10		1-methyl-1-[(E)-[(3,4,5-trimethoxyphenyl)methylidene]amino]t hiourea
TSC-11		1-methyl-1-{[(3Z)-2-oxo-2,3-dihydro-1H-indol-3-ylidene]amino}thiourea
TSC-12		1-[(E)-{[4-(diethylamino)phenyl]methylidene}ami no]-1-methylthiourea
TSC-13		1-methyl-1-[(E)-[(2E)-3-phenylprop-2en-1-ylidene]amino]thiourea
TSC-14		1-methyl-1-[(E)-[(2-methylphenyl)methylidene]amino]thiou rea
TSC-15		1-methyl-1-[(E)-[(3-methylphenyl)methylidene]amino]thiou rea

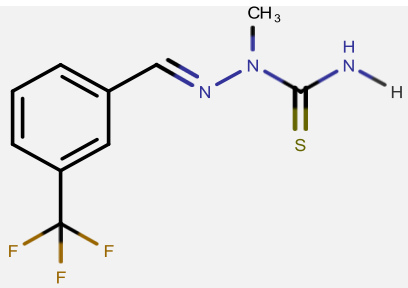
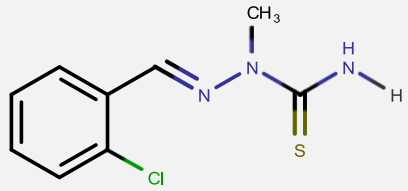
TSC-16		1-methyl-1-[(E)-{[3-(trifluoromethyl)phenyl]methylidene}amino]thiourea
TSC-17		1-[(E)-[(2-chlorophenyl)methylidene]amino]-1methylthiourea

TABLE 2: PHYSICAL PROPERTIES OF COMPOUNDS

Name	Molecular Formula	Molecular Weight	Yield (%)	Melting Point (°C)	Solubility
TSC-1	$C_9H_{10}ClN_3OS$	243.71	80.8%	192-194°C	DMSO, Ethanol, Methanol
TSC-2	C11H15N3O2S	253.32	71.03 %	221-223°C	Chloroform, DMSO, Methanol
TSC-3	C11H16N4S	236.34	80.2%	246-247°C	DMSO, Methanol
TSC-4	C12H17N3O3S	283.35	66.5%	225-227°C	DMSO, Methanol
TSC-5	C9H9N5O4S	283.26	69.8%	267-269°C	DMSO, Methanol
TSC-6	C10H13N3O2S	239.29	76.02%	210-212°C	DMSO, Methanol
TSC-7	C9H10N4O3S	254.26	67.6%	246-248°C	DMSO, Methanol
TSC-8	C9H9Cl2N3S	262.15	69.9%	184-188°C	Chloroform, DMSO, Ethanol, Methanol
TSC-9	C9H9Cl2N3S	262.15	73.76 %	228-230°C	Chloroform, DMSO, Ethanol, Methanol
TSC-10	C12H17N3O3S	283.35	78.8%	225-227°C	Chloroform, DMSO, Methanol
TSC-11	C10H10N4OS	234.28	79.4%	233-235°C	DMSO, Methanol
TSC-12	C13H20N4S	264.29	83.6%	194-196°C	Chloroform, DMSO, Methanol
TSC-13	C11H13N3S	219.31	78.08 %	204-206°C	DMSO, Methanol
TSC-14	C10H13N3S	207.30	89.8%	153-155°C	Chloroform, DMSO, Methanol
TSC-15	C10H13N3S	207.30	69.0%	183-185°C	Chloroform, DMSO, Methanol
TSC-16	C10H10F3N3S	261.27	67.5%	154-156°C	Chloroform, DMSO, Methanol
TSC-17	C9H10ClN3S	227.71	79.1%	198-200°C	Chloroform, DMSO, Methanol

Biological evaluation: All synthesised compounds were screened for *in vitro* antibacterial activity against two gram positive strain of bacteria, *Staphylococcus aureus* and *Bacillus subtilis* and two gram negative strains of bacteria *Escherichia coli* and *Pseudomonas aeruginosa* by Paper disc diffusion method. Ciprofloxacin was used as the reference antibacterial drug. For antifungal assay *Candida albicans* was used to test the activity of compounds. Fluconazole was kept as the standard drug.

Antimicrobial Assay: The nutrient agar medium was prepared by dissolving nutrient agar 23g in 1000ml distilled water. The nutrient agar so prepared was allowed to boil. After that, it was autoclaved at 121°C, 15 psig for 30 minutes and cooled to 45-50 ˚C. The nutrient agar medium was inoculated aseptically with 0.5 ml of strains of *S.aureus, B.subtilis, P.aeruginosa* and *E.coli* at room temperature. For *C. albicans* Sabouraud agar was used to prepare the plates and was inoculated in the same way as described for bacterial strains. Into each sterile petridish about 15 ml of inoculated molten agar medium was poured. The plates were left at room temperature for solidification. After solidification, paper discs of 6mm diameter impregnated with respective solutions were placed on to the petri dishes and the petri dishes were labelled.

All the synthesised compounds and reference were dissolved in DMSO to get required concentration of 40µg/mL, 60µg/mL, 80µg/mL and 100µg/mL. Paper discs impregnated with solutions of each compound, reference and a control (DMSO) were placed on the inoculated petri dishes. For antibacterial assay, the plates were kept undisturbed for about 24 hours at room temperature. After incubation period of 24 hours the diameter of zone of inhibition was measured with the help of antibiotic zone reader. Whereas for anti-fungal assay the plates were incubated for a period of 48 hours.

Antioxidant Assay: The antioxidant assay was performed by the method of Mensor *et al* [20]. 0.01mM solution of DPPH was prepared in methanol. Methanolic solutions of all the compounds in the following concentration ranges were prepared 40µg/mL, 60µg/mL, 80µg/mL and 100µg/mL. Similarly solutions of ascorbic acid in the same concentration ranges were prepared.1mL of DPPH solution was added to 1mL of sample solution and to ascorbic acid as well and the volume was finally made upto 3mL using methanol. The test tubes containing the assay mixture were incubated in a dark and cool place for 30 minutes. Immediately after incubation the absorbance of the DPPH solution, Blank (methanol), Samples and ascorbic acid containing DPPH reagent were recorded at 517nm on a UV-VIS spectrophotometer. The antioxidant activity was measured using the formula,

$$\% \text{ scavenging or inhibition} = [(A_o - A_s)/A_o]*100$$

A_o = Absorbance of DPPH solution without the sample
A_s = Absorbance of DPPH solution with the sample

ABTS Assay: 7mM ABTS stock solution in water was prepared. To it 2.45mM solution of potassium persulfate was added in 1:1 ratio (volume/volume). This reaction mixture was left undisturbed in a dark place for about 16 hours for generation of the radicals [21]. This solution was further diluted using Methanol such that it has a stable absorbance of 0.700± 0.05 at 734nm.Methanolic solutions of all the compounds as well as ascorbic acid were prepared in the concentration ranges 40µg/mL. 60µg/mL, 80µg/mL and 100µg/mL. To 0.1mL of the test solutions 1mL of diluted ABTS solution was added. Absorbance of the test and standard solutions were taken immediately at 734nm.

The anti-oxidant activity of the samples were determined using the equation

$\% E = [(A_c - A_t)/A_c]*100$, where
E= Anti-oxidant activity
Ac= Absorbance of the ABTS stock solution
At= Absorbance of the tested compounds

RESULTS AND DISCUSSIONS:

TABLE 3: SPECTRAL FEATURES OF COMPOUNDS

Compounds	ATR	^{1}HNMR
TSC-1	3443,3158ν(NH2), 3328,1336,1209ν(Ar-OH), 1589ν(C=N), 1434ν(C=CAr), 1259,816ν(C=S), 1013ν(N-N), 1094ν(C-N), 766,724ν(C-Cl)	DMSO:δ10.324(1H, Ar-OH), δ8.207(1H, CH=N), δ7.899(1H, *o*-Ar-H), δ7.185(1H, *p*-Ar-H), δ6.858(1H, *m*-Ar-H), δ3.345(3H, N-CH_3)
TSC-2	3468,3281,3163ν(NH2), 1565ν(C=N), 1421ν(C=C), 1349,847ν(C=S), 1142ν(N-N), 1090ν(C-N), 1059ν(O-CH_3)	$CDCl_3$:δ7.677(1H,CH=N), δ7.179-6.432(3H, Ar-H), δ3.861(3H, N-CH_3), δ3.916-3.908(6H, (O-CH_3)$_2$)
TSC-3	3430,3260ν(NH_2), 1603ν(C=N), 1432ν(C=C), 1358,814ν(C=S), 1178ν(N-N), 1060ν(C-N)	DMSO:δ8.169(1H, CH=N), δ7.751-7.691(2H, *o*-Ar-H), δ6.688-6.666(2H, *m*-Ar-H), δ2.932 (6H, N-(CH_3)$_2$), δ3.332 (3H, N-CH_3)
TSC-4	3466,3231,3151ν(NH_2), 1599ν(C=N), 1421ν(C=C), 1339,837ν(C=S), 1122ν(N-N), 1001ν(O-CH_3), 930ν(C-N)	DMSO: δ8.275(1H, CH=N), δ6.289-6.280 (2H, Ar-H), δ3.802-3.606 (9H, (O-CH_3)$_3$), δ3.337 (3H, N-CH_3)
TSC-5	3433,3271,3154ν(NH_2), 1598ν(C=N), 1572,1338ν(N=O), 1433ν(C=C), 1305,860ν(C=S), 1098ν(C-N), 999ν(N-N)	DMSO: δ8.810(1H, CH=N), δ8.740-8.705(2H, *m*-Ar-H), δ8.113(1H, *o*-Ar-H), δ3.349(3H, N-CH_3)
TSC-6	3422,3276,3137ν(NH_2), 3357,1357,1198ν(Ar-OH), 1598ν(C=N), 1431ν(C=C), 1270,847ν(C=S), 1123ν(N-N), 1090ν(C-N), 1034ν(O-CH_3)	DMSO: δ9.444(1H, Ar-OH), δ8.276-8.223(1H, CH=N), δ7.767(2H, NH_2), δ 3.807(3H, O-CH_3), δ3.719 (3H, N-CH_3)

TSC-7	3466,3068ν(NH_2), 3350,1336,1216ν(Ar-OH), 1585ν(C=N), 1550,1336ν (N=O), 1420ν(C=C), 1285,838ν(C=S), 1123ν(N-N), 1085ν(C-N)	DMSO: δ8.878(1H, Ar-OH), δ8.520(1H, *o*-Ar-H), δ8.475(1H, CH=N), δ8.085(1H, *p* Ar-H), δ7.056-7.034(1H, *m*-Ar-H), δ3.747(3H, N-CH_3)
TSC-8	3403,3254,3131ν(NH_2), 1585ν(C=N), 1411ν(C=C), 1347,883ν(C=S), 994ν(N-N), 770,729ν(C-Cl), 1085ν(C-N)	$CDCl_3$: δ8.789(1H, CH=N), δ7.390-7.370(2H, *m*-Ar-H), δ7.247-7.206(1H, *p*-Ar-H), δ3.878(3H, N-CH_3)
TSC-9	3423,3240,3133ν(NH_2), 1583ν(C=N), 1433ν(C=C), 1341,814ν(C=S), 995ν(N-N), 1098ν(C-N), 785,723ν(C-Cl)	CDCl3: δ7.957(1H, CH=N), δ7.436-7.431(1H, *o*-Ar-H), δ7.290-7.246 (1H,*p*- Ar-H), δ6.493(1H, *m*-Ar-H), δ3.890(3H, N-CH_3)
TSC-10	1610ν(C=N), 1424ν(C=C), 1334,831ν(C=S), 1125ν(N-N), 1087ν(C-N), 996ν(O-CH_3)	CDCl3: δ8.268(1H, CH=N), δ7.672-6.871(2H, *o*-Ar-H), δ3.869(12H, (O-CH_3)$_3$, N-CH_3)
TSC-11	3440,3290,3164ν(NH_2), 1725ν(C=O), 1600ν(C=N), 1409ν(C=C), 1350, 813ν(C=S), 1098ν(N-N), 1065ν(C-N)	DMSO: δ10.922(1H, N-H), δ 7.752-6.992(4H,C-H), δ3.539(3H, N-CH_3)
TSC-12	3465,3242,3135ν(NH_2), 1628ν(C=N), 1434ν(C=C), 1340,844ν(C=S), 1131ν(N-N), 1080ν(C-N)	$CDCl_3$: δ9.958(1H, Ar-OH), δ7.839(1H, CH=N), δ7.246-6.164(3H, Ar-H), δ3.396-3.342(4H, CH_2-CH_3), δ3.269(3H, N-CH_3), δ1.196-1.160(6H, CH_2-CH_3)
TSC-13	1560ν(C=N), 1420ν(C=C), 1353,867ν(C=S), 994ν(N-N), 1132 ν(C-N)	DMSO: δ8.372-8.057(1H CH=N), δ7.710(1H, *p*-Ar-H), δ7.279-7.524(6H, Ar-H *o,m* NH_2), δ7.080(1H, Ar-CH=CH), δ6.969(1H, Ar-CH=CH), δ3.668(3H, N-CH_3)
TSC-14	3413,3245,3125ν(NH_2), 1580ν(C=N), 1426ν(C=C), 1356,872ν(C=S), 996ν(N-N), 1071ν(C-N)	$CDCl_3$: δ7.884-7.715(1H, CH=N), δ7.319-7.203(5H, Ar-H), δ3.880(3H, N-CH3), δ2.485(3H, Ar-CH3)
TSC-15	3434,3251,3125ν(NH_2), 1577ν(C=N), 1433ν(C=C), 1365,811ν(C=S), 1011ν(N-N), 1090 ν(C-N)	$CDCl_3$: δ7.753(1H, CH=N), δ7.633-7.203(5H, Ar-H), δ3.854(3H, N-CH_3), δ2.735(3H, Ar-CH_3)
TSC-16	3438,3266,3142ν(NH_2), 1588ν(C=N), 1433ν(C=C), 1372,754ν(C=S), 1331ν(C-F), 1119ν(N-N), 1068ν(C-N)	$CDCl_3$: δ8.342(1H, CH=N), δ7.896-7.247(5H, Ar-H), δ3.887(3H, N-CH3)
TSC-17	3427,3246,3129ν(NH_2), 1581ν(C=N), 1425ν(C=C), 1333,783ν(C=S), 1001ν(N-N), 1071 ν(C-N)	$CDCl_3$: δ8.027-7.848(1H, CH=N), δ7.732-6.570(5H, Ar-H), δ3.883 (3H, N-CH_3)

The ATR of the compounds was found to be in accordance with the data reported in literature [22]. The major peaks were recorded at 1628-1560 cm^{-1} for C=N group, at 1365-1305 $cm^{-1}$and 880-754$cm^{-1}$for C=S group, 1178-994cm^{-1} for N-N group. Absorption peaks for other functional groups were also observed in the respective derivatives. No sharp peak was obtained at 1740-1720cm^{-}[1]indicating that the aldehyde has been consumed in the reaction and condensation has taken place.[1]HNMR: NMR peak for CH=N was found to occur at δ7.67-8.78 ppm. NMR peak for N-CH_3 group was found at δ3.26-3.88ppm. The NMR peak for aromatic hydrogens Ar-H was found to be in the range δ6.28-8.74ppm.

Antimicrobial assay:

TABLE 4: DIAMETER OF ZONE OF INHIBITION (MM) OF COMPOUNDS AGAINST *STAPHYLOCOCCUS AUREUS*

Compound	Dilutions µg/mL			
	40µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
TSC-1	-	-	12	14
TSC-2	-	-	08	12
TSC-3	-	-	-	12
TSC-4	-	-	08	10
TSC-5	-	-	06	10
TSC-6	-	-	12	1
TSC-7	10	14	18	24
TSC-8	-	10	16	20

TSC-9	06	16	20	24
TSC-10	-	-	12	16
TSC-11	10	14	20	24
TSC-12	-	12	14	18
TSC-13	-	12	18	26
TSC-14	-	10	16	20
TSC-15	12	18	20	24
TSC-16	12	16	24	26
TSC-17	-	12	14	18
Ciprofloxacin	16	20	26	32

TABLE 5: DIAMETER OF ZONE OF INHIBITION (MM) AGAINST BACILLUS SUBTILIS

Compounds	Dilutions µg/Ml			
	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
TSC-1	-	12	18	22
TSC-2	-	-	-	16
TSC-3	-	-	12	14
TSC-4	-	-	12	16
TSC-5	-	10	14	22
TSC-6	-	-	14	24
TSC-7	08	12	18	22
TSC-8	-	12	20	24
TSC-9	10	18	22	24
TSC-10	-	-	12	14
TSC-11	-	12	22	24
TSC-12	08	10	12	20
TSC-13	08	12	18	22
TSC-14	-	-	16	18
TSC-15	12	14	16	22
TSC-16	14	16	24	26
TSC-17	-	12	16	20
Ciprofloxacin	16	20	26	30

TABLE 6: DIAMETER OF ZONE OF INHIBITION OF COMPOUNDS AGAINST *ESCHERICHIA COLI*

Compounds	Dilutions (µg/mL)			
	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
TSC-1	-	-	-	10
TSC-2	-	-	6	12
TSC-3	-	-	6	8
TSC-4	-	-	8	12
TSC-5	-	-	10	12
TSC-6	-	-	12	14
TSC-7	10	14	20	26
TSC-8	-	-	16	18
TSC-9	10	16	22	24
TSC-10	-	-	14	18
TSC-11	-	-	14	20
TSC-12	-	-	20	24
TSC-13	-	12	18	26
TSC-14	-	-	12	18
TSC-15	10	10	14	22
TSC-16	8	12	16	24
TSC-17	-	10	14	22
Ciprofloxacin	18	20	24	28

TABLE 7: DIAMETER OF ZONE OF INHIBITION (IN MM) OF COMPOUNDS AGAINST *PSEUDOMONAS AERUGINOSA*

Compounds	Dilutions (µg/mL)			
	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
TSC-1	-	-	-	12
TSC-2	-	-	6	10
TSC-3	-	-	6	8
TSC-4	-	-	8	12
TSC-5	-	-	10	16
TSC-6	-	-	12	14
TSC-7	-	12	16	20
TSC-8	-	-	14	20
TSC-9	10	16	18	24
TSC-10	-	-	15	19
TSC-11	-	-	13	16
TSC-12	-	-	14	20
TSC-13	8	12	16	22
TSC-14	-	-	14	18
TSC-15	6	10	14	16
TSC-16	8	12	14	20
TSC-17	-	10	15	18
Ciprofloxacin	15	22	26	28

TABLE 8: ZONE OF INHIBITION OF COMPOUNDS AGAINST *CANDIDA ALBICANS*

Compounds	Dilutions (µg/mL)			
	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
TSC-1	-	-	-	08
TSC-2	-	-	-	10
TSC-3	-	-	09	11
TSC-4	-	-	10	12
TSC-5	-	10	14	18
TSC-6	-	-	12	16
TSC-7	08	12	18	22
TSC-8	-	10	15	18
TSC-9	10	14	16	20
TSC-10	-	-	10	12
TSC-11	10	18	22	28
TSC-12	-	08	15	20
TSC-13	-	-	12	16
TSC-14	-	-	14	17
TSC-15	-	06	10	14
TSC-16	-	10	16	20
TSC-17	-	10	16	22
Fluconazole	16	24	34	40

TSC-7, TSC-9, TSC-11, TSC-13, TSC-15, TSC-16 were found to be active against *S. aureus*. *S. aureus* was found to be resistant to the derivatives at concentration of 40µg/mL. TSC-16 was found to be the most active derivative against *S. aureus*. *B. subtilis* was found to be the most susceptible strain towards the derivatives synthesised. TSC-1, TSC-5, TSC-6, TSC-7, TSC-8, TSC-9, TSC-11, TSC-13, TSC-15 and TSC-16 were found to inhibit *B.subtils* considerably. TSC-16 was the most active among all the derivatives against *B.subtilis*. The thiosemicarbazones were found to be less active against gram negative strains than the gram positive strains. TSC-7, TSC-9, TSC-12, TSC-13, TSC-15, TSC-16 and TSC-17 were found to be active against *E.coli*. TSC-13 was found to possess the greatest activity against *E.coli* among all other derivatives. Only TSC-9 and TSC-13 were found to significantly inhibit *Pseudomonas aeruginosa*. TSC-9 was found to possess greatest activity against this strain. This strain was found to be the least susceptible. The compounds synthesised were also tested for their antifungal activity against *Candida albicans*, taking fluconazole as the standard drug. TSC-11 was found to be most active against *C.albicans*. The fungus was also susceptible to TSC-7 and TSC-17.

Antioxidant assay:

TABLE 9: PERCENTAGE SCAVENGING OF DPPH BY COMPOUNDS

Compounds	Dilutions (µg/mL)			
	40µg/mL	60µg/mL	80µg/mL	100µg/mL
TSC-1	36.2%	46.6%	54.9%	72.53%
TSC-2	35.2%	41.45%	58.5%	62.69%
TSC-3	25.9%	39.37%	56.99%	73.57%
TSC-4	37.8%	47.66%	62.17%	83.93%
TSC-5	49.18%	56.99%	68.39%	79.79%
TSC-6	51.81%	62.69%	75.12%	88.08%
TSC-7	39.37%	52.84%	63.73%	74.61%
TSC-8	15.54%	27.97%	51.81%	62.69%
TSC-9	46.63%	59.06%	69.43%	82.63%
TSC-10	10.36%	26.42%	43%	59.58%
TSC-11	31.60%	49.22%	60.62%	78.23%
TSC-12	36.78%	58.03%	72.53%	87.11%
TSC-13	19.17%	36.26%	47.66%	59.06%
TSC-14	8.80%	21.76%	39.37%	43.77%
TSC-15	30.56%	42.48%	58.03%	72.53%
TSC-16	52.84%	67.87%	79.79%	84.19%
TSC-17	36.78%	58.03%	73.57%	81.34%
Ascorbic acid	65.75%	72.41%	86.52%	92.22%

TABLE 10: PERCENTAGE SCAVENGING OF ABTS RADICAL BY COMPOUNDS AND ASCORBIC ACID

Compounds	Dilutions (µg/mL)			
	40µg/mL	60µg/mL	80µg/mL	100µg/mL
TSC-1	25.28%	38.14%	54.71%	63.85%
TSC-2	25%	40.71%	49.71%	59.14%
TSC-3	22.42%	35.14%	45.2%	56.14%
TSC-4	25.71%	43.42%	58.71%	69%
TSC-5	32.57%	49.28%	64.71%	80.14%
TSC-6	35.5%	52.8%	68.57%	84.42%
TSC-7	28.42%	40.85%	59.14%	64.4%
TSC-8	19.57%	31.2%	42.85%	53.57%
TSC-9	22.14%	32.42%	48.31%	58.52%
TSC-10	18.16%	30.88%	45.71%	53.02%
TSC-11	28%	43.42%	60.57%	80.71%
TSC-12	30.38%	41.2%	61.71%	82.71%
TSC-13	20.57%	33.57%	47%	60.78%
TSC-14	10.71%	18.4%	30.25%	41.64%
TSC-15	18.71%	30.2%	39.5%	48.8%
TSC-16	39.8%	48.2%	65.85%	78.2%
TSC-17	29.71%	42.57%	59.8%	70.28%
Ascorbic acid	41.2%	53.27%	70.28%	89.74%

DPPH assay: Antioxidant activity of compounds was determined using neutral DPPH radical following a standard protocol. The antioxidant capacity of compounds was evaluated against ascorbic acid as the standard. TSC-6 and TSC-12 were found to possess antioxidant activity comparable to ascorbic acid. ABTS assay: ABTS radical cation was also used to determine the radical scavenging activity of thiosemicarbazone derivatives. TSC-6 was found to scavenge the radical nearly to the same extent as the standard ascorbic acid.

CONCLUSION: This work was aimed to establish the antimicrobial and antioxidant activities of benzaldehyde thiosemicarbazones. Emergence of resistance often complicate the treatment of various bacterial and fungal infections, thus the need to develop better and newer antimicrobial agents will always be there. Five very common and opportunistic pathogenic microbial strains which cause mild to severe infections in immunocompromised as well as healthy individuals were selected to determine the antimicrobial potential of the synthesised compounds. The compounds showed good inhibition of the growth of bacterial strains when compared against the standard drug ciprofloxacin. Gram positive strains of bacteria were more susceptible than the gram negative strains. Considerable inhibition in the growth of the fungus *Candida albicans* was also seen. Since thiosemicarbazones are known chelators thus chelation of any of the metalloenzyme or metal dependent enzyme might be the possible mode of inhibition of growth of the microbes. These compounds also showed free radical scavenging activity nearly to the same extent as the standard ascorbic acid. This implies that these derivatives can be very useful in those pathological ailments where the condition is further deteriorated due to involvement of some free radical mechanisms.

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