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Synthesis, spectral (FT-IR, ^1H and ^{13}C NMR) and antimicrobial studies some of (E)-2-(2-((aryl)(phenyl)methylene)hydrazinyl)benzo[d]thiazole

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ABSTRACT

A series of (E)-2-(2-((aryl)(phenyl)methylene)hydrazinyl)benzo[d] thiazole compounds by condensation of substituted benzophenone with 2-hydrazinobenzothiazol in the presence acetic acid. The synthesized compounds **1-6** were characterized by elemental, FT-IR, ^1H and ^{13}C NMR spectral data. From the IR and NMR spectra, the characteristic frequencies were assigned and the data used for confirmation of the formation hydrazones **1-6**. All compounds were screened for their preliminary antibacterial and antifungal activities. The methoxy substituted compound **6** shows good antibacterial activity against their bacterial strains within the agreed mm of zone of inhibition. The methyl (**5**) and methoxy (**6**) substituted hydrazones show good antifungal activities against their fungal strains.

Keywords: Aryl hydrazone, IR and NMR spectra, antibacterial and antifungal activities

1. INTRODUCTION

Hydrazones, related to ketones and aldehydes belong to a class of organic compounds with the structure, $\text{R}_1\text{R}_2\text{C}=\text{NNH}$ [1]. The practice of medicinal chemistry is devoted to the discovery and development of new agents for treating disease. The process of establishing a

new drug is exceeding complex and involves talent of people from variety of disciplines [2]. The design of drug molecules arguably offers some of the greatest hopes for success in present and future era. Heterocyclic compounds are widely distributed in nature and are essential for life. There are vast numbers of pharmacologically active heterocyclic compounds many of which are in regular clinical use [3].

Thiazole belongs to a class of heterocyclic compounds having both a nitrogen atom and sulfur atom as part of the aromatic five-member ring. These compounds possess diverse biological and pharmacological properties such as antimicrobial, anti-inflammatory, analgesic, antifungal, anti-tubercular, antiviral, anticancer, antiplatelet, antimalarial, anticonvulsant, cardio protective, antihelmintic, antiprotozoal, anti-trypanosomal, antischistosomiasis etc. [4-13]. Owing to their biological properties, they are useful intermediate for the synthesis of heterocyclic compounds, pharmaceuticals, and materials [14]. Hydrazone-hydrazones compounds are not only intermediates but they are also very effective organic compounds in their own right. Many effective compounds, such as iproniazide and isocarboxazide, are synthesized by reduction of hydrazone-hydrazones.

Thiazole was first described by Hantzsch and Waber in 1887. Popp confirmed its structure in 1889. The substituted thiazoles compounds have number of characteristics features such as [15].

- 1) Relative stability and ease of starting material.
- 2) Built in biocidal unit.
- 3) Enhanced lipid solubility with hydrophilicity.
- 4) Easy metabolism of compounds.

It has been noticed continuously over the years that interesting biological activities, such as antiallergic [16], antioxidant [17], anti-HIV [18], analgesic [19], antimicrobial [20], and anti-inflammatory [21] were associated with thiazole derivatives. The emerging bacterial resistance causes a widespread problem for the treatment of various infections. Therefore, the search for antimicrobials is a never-ending task. Now-a-days a number of hydrazone derivatives have been developed and evaluated for their antibacterial activity. For several of these compounds, some prodrugs also received marketing approval (Nifuroxazide, Iproniazide, Isoniazid). In 1993, Willner et al. [22] synthesized the (6-maleimidocaproyl) hydrazone of doxorubicin (MC-DOXHZN) as a new derivative for the preparation of immunoconjugates of doxorubicin. Kratz et al. [23] have developed DOX-hydrazones and DOX amides to increase the therapeutic index of DOX. Lactosaminated human albumin (L-HSA) is a hepatotropic drug carrier safely used in humans [24]. The usefulness of aryl hydrazone has made their synthesis the subject of extensive research. In this article, we report the synthesis, elemental, ^1H , ^{13}C NMR, and antimicrobial studies of (E)-2-(2-((aryl)(phenyl)methylene)hydrazinyl)benzo[d]thiazole (**1-6**).

2. EXPERIMENTAL

2.1. Material and methods

All chemicals and solvents used were of AnalaR grade. The melting points were taken in open capillaries in an electrical apparatus and are uncorrected. Elemental analyses were carried out on VARIOMICRO V2.2.0 CHN analyser. The FT-IR spectrum of the synthesised compounds was taken in the range of $4000\text{-}400\text{ cm}^{-1}$ an AVATAR-330 FT-IR spectrometer

(ThermoNicolet) using KBr (pellet form). ^1H NMR was recorded on a Bruker 400 MHz NMR and ^{13}C NMR was recorded on a Bruker 100 MHz NMR using $\text{CDCl}_3/\text{DMSO}-d_6$ as solvent for all the compounds.

2. 2. Antimicrobial activities

The antimicrobial activities of title compounds were measured by disc diffusion method. In this experiment there are two each gram positive/ negative and two fungal strains were used for measuring the antibacterial and antifungal activities of synthesised compounds [25-27].

2. 2. 1. Antibacterial studies

The following Gram positive and Gram negative strains have been used for the study [28,29].

- 1) *Staphylococcus aureus* (Gram positive)
- 2) *Bacillus subtilis* (Gram positive)
- 3) *Vibrio cholerae* (Gram negative)
- 4) *Escherichia coli* (Gram negative)

Antibacterial activity by disc diffusion method

Nutrient agar plates were prepared under sterile conditions and incubated overnight to notice contamination. Regarding 0.2 ml of working stock culture was transferred into separate nutrient agar plates and spread thoroughly by a glass spreader. Whatmann No. 1 discs (6 mm in diameter) were impregnated with the test compounds dissolved in DMSO (200 mg/ml) for on the subject of half an hour. Commercially available drug disc (*Ciprofloxacin* 10 lg/disc) was used as positive reference standard. Negative controls were also prepared by impregnating the disc of same size in DMSO solvent. The discs were positioned on the inoculated agar plates and incubated at 37 ± 1 °C for about 18–24 h. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism.

2. 2. 2. Antifungal Studies

The following fungal strains were used for the study [28,29].

- 1) *Candida albicans*
- 2) *Aspergillus niger*
- 3) *Aspergillus flavus*
- 4) *Trichophyton mentagraphytes*

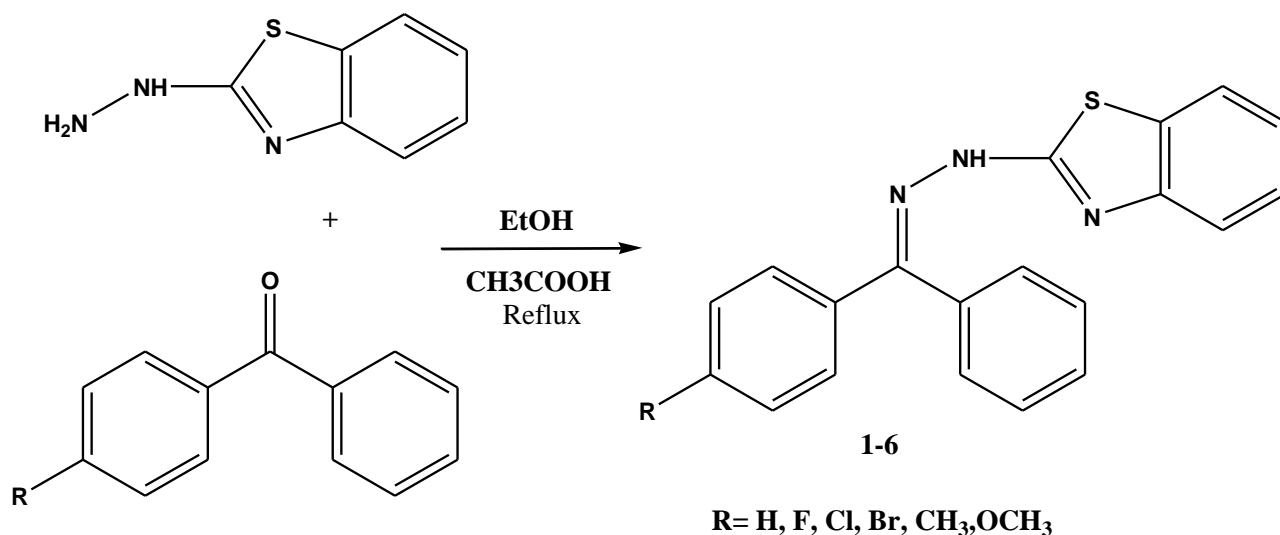
Antifungal activity by disc diffusion method

Sabouraud's dextrose agar (SDA) medium was used for the growth of fungi and testing was done in Sabouraud's dextrose broth (SDB) medium. The subculture and the viable count were carried out by the same procedure used in antibacterial studies except the temperature, which was maintained at 28 ± 1 °C for about 72 h. Similarly for disc diffusion method, the petridishes were incubated at 28 ± 1 °C for about 72 h. The same concentration of the test

compound, solvent (DMSO) and *Cetramazole* (standard) prepared previously were used for the antifungal studies.

2. 3. Synthesis of (E)-2-(2-((aryl)(phenyl)methylene)hydrazinyl)benzo[d] thiazole (1-6)

A mixture of substituted benzophenone (0.01mol) in ethanol, 2-hydrazinobenzothiazol (0.01 mol) and the catalytic quantify 1mL of acetic acid was added and then refluxed on a water bath for 3 h (Scheme 1). The completion of the reaction was monitored using TLC (Thin layer Chromatography). After cooling, the resulting mixture was poured in to the water and the solid was separated out. The separated solid was filtered, crystallized from ethanol to afforded yellow crystals.



Scheme 1. Synthetic route of compounds **1-6**

3. RESULTS AND DISCUSSION

Table 1. Physical constants, yields and analytical data of compounds **1-6**

Entry	X	M.F	M.W	Yield (%)	M.p (°C)	Found (Calcd.) (%)			
						C	H	N	S
1	H	C ₂₀ H ₁₅ N ₃ S	329.42	95	170-172	72.87 (72.92)	4.23 (4.59)	12.11 (12.76)	9.67 (9.73)
2	F	C ₂₀ H ₁₄ FN ₃ S	347.09	93	196-198	69.04 (69.14)	4.03 (4.06)	12.05 (12.10)	9.15 (9.23)

3	Cl	C ₂₀ H ₁₄ ClN ₃ S	363.86	91	204-206	68.96 (69.02)	3.85 (3.88)	11.50 (11.55)	8.72 (8.81)
4	Br	C ₂₀ H ₁₄ BrN ₃ S	408.31	90	196-198	68.96 (58.83)	3.85 (3.46)	11.50 (10.29)	7.70 (7.85)
5	CH ₃	C ₂₁ H ₁₇ N ₃ S	343.44	86	180-182	73.23 (73.44)	4.35 (4.49)	12.20 (12.23)	9.28 (9.34)
6	OCH ₃	C ₂₁ H ₁₇ N ₃ OS	359.44	80	176-178	70.03 (70.17)	4.65 (4.77)	11.54 (11.69)	8.80 (8.92)

The synthesized substituted hydrazones were characterized by their elemental analysis and FT-IR, NMR spectral data. The physical constants, analytical and elemental analyses data of title compounds are shown in **Table 1**.

3. 1. IR spectral analysis

The IR spectral data of compounds **1-6** are given in **Table 2**. The IR spectrum of compound **1** is shown in **Fig. 1**. Generally, secondary amines NH gives rise to a weak band in the range 3335-3310 cm⁻¹ [30]. The N-H stretches observed in the range of 3439.53-3462.47 cm⁻¹ of the compounds **1-6**. Aromatic compounds commonly exhibit multiple weak bands in the region 3150–2930 cm⁻¹ due to aromatic C-H stretching vibrations [31]. The band in the interval 3094.17-2925.93 cm⁻¹ is attributed to aromatic C-H stretching vibration. As seen from **Table 2**, the band at 2916.00 and 2922.59 are due to methyl group present in compounds **5** and **6**, respectively. The band ascribed to C=N stretching vibration appears in the region 1500-1900 cm⁻¹ [30].

In cyanopyridine compounds, the C=N stretching frequency is observed as a very strong band at ~1660 cm⁻¹ in IR [32]. Thirunarayanan et al. [33] assigned C=N stretching in the region 1548-1579 cm⁻¹ in phenazine derivatives. In this case, the carbonyl C=N stretching appeared in the region 1597.43-1657.14 cm⁻¹. The aromatic C=C stretches observed in the range of 1405.17-1602.43 cm⁻¹. The C-H in plane bending vibrations usually occurs in the region 1390–990 cm⁻¹ [34,35].

The aromatic C-H in- plane bending vibrations appear in the region 1247.87-1397.67 cm⁻¹. The bands in the range 1173.44-680.50 cm⁻¹ are due to the aromatic C-H out-of-plane bending vibrations.

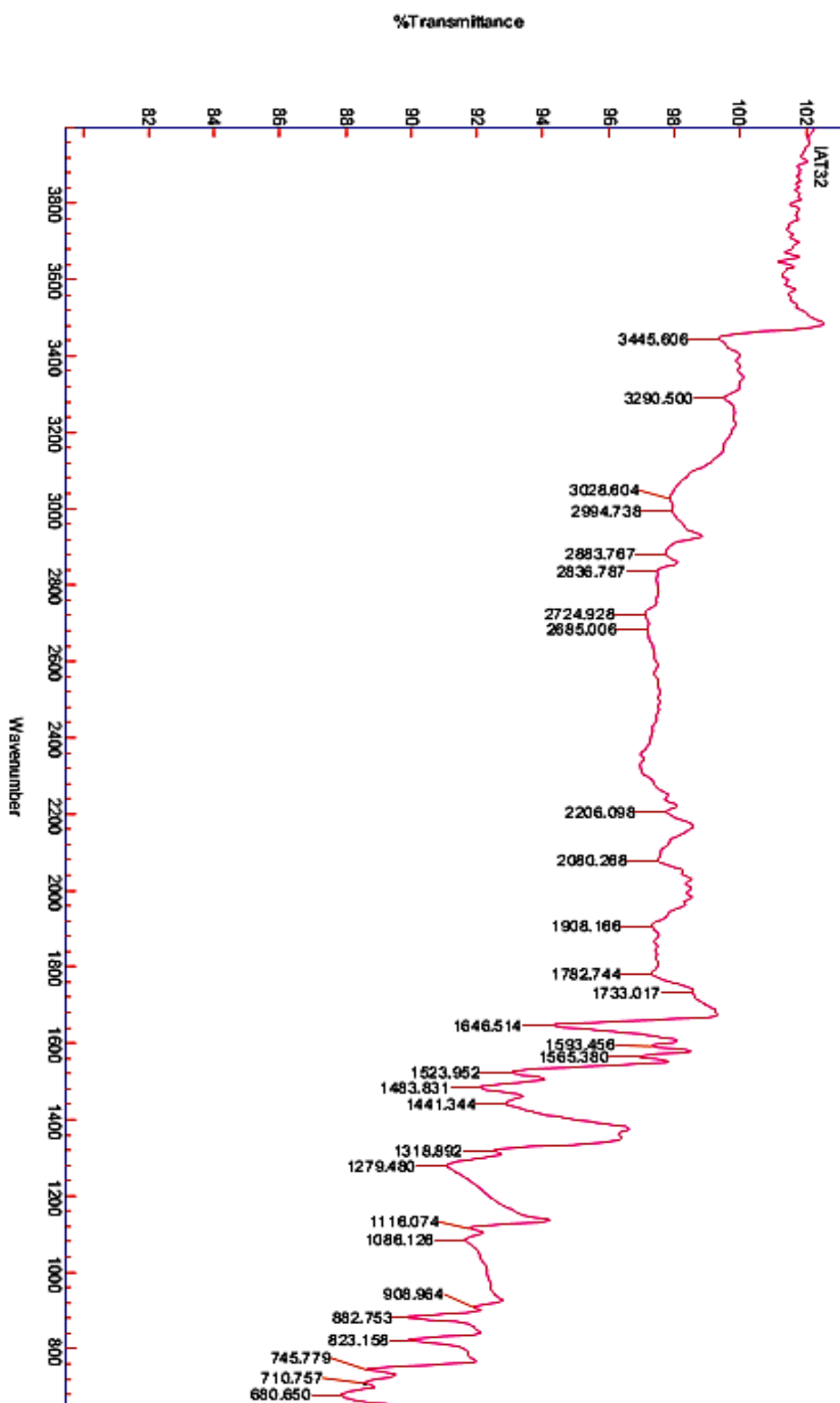


Fig. 1. FT-IR spectrum of compound 1.

Table 2. FT-IR spectral data of compounds **1-6**.

Assignments	1	2	3	4	5	6
	H	F	Cl	Br	CH₃	OCH₃
v_{N-H}	3445.61	3439.53	3461.10	3415.32	3456.49	3462.47
v_{ArC-H}	3028.60	3063.46	3059.69	3069.58	3094.17	3057.92
	2994.74	2925.93	2927.91	2950.38	-	-
v_{AlkC-H}	-	-	-	-	2916.00	2922.59
v_{C=N}	1646	1648.51	1657.14	1641.35	1597.43	1657.38
v_{C=C}	1523.95	1600.06	1601.83	1524.51	1524.98	1602.43
	1483.83	1552.99	1552.37	1489.59	1463.92	1443.94
	1441.34	1447.31	1444.34	1445.00	1390.37	1405.17

3. 2. NMR spectral analysis

The ¹H NMR spectrum of compound **1** is shown in **Fig. 2**. As seen from **Table 3**, the multiplet ranges from 7.07- 9.90 ppm are due to aromatic protons protons. In compound **6**, the methoxy proton signal appeared at 3.78 ppm. The up field signal at 2.35 ppm corresponds to methyl proton in compound **5**. The ¹H chemical shifts values of compounds **1-6** are given in **Table 3**.

Table 3. The chemical shifts of NMR (δ ppm) spectral values of compounds **1-6**

Entry	R	¹ H NMR			¹³ C NMR			
		CH ₃ / OCH ₃	N-H	Ar-H	C=N	<i>Cipso</i>	Ar-C	CH ₃ / OCH ₃
1	H	-	9.09	7.18- 8.97	169.03	151.77	116.54-148.12	-
2	F	-	7.79	7.07- 7.77	168.62	153.77	115.39-146.76	-
3	Cl	-	7.70	7.16- 7.69	-	138.94	118.52-137.25	-
4	Br	-	8.93	7.01- 8.91	163.76	142.85	111.27-140.06	-
5	CH ₃	2.35	7.70	7.10- 7.62	167.57	151.83	118.93-151.46	21.49
6	OCH ₃	3.78	7.68	6.79- 7.66	167.49	163.26	113.59-160.95	55.51

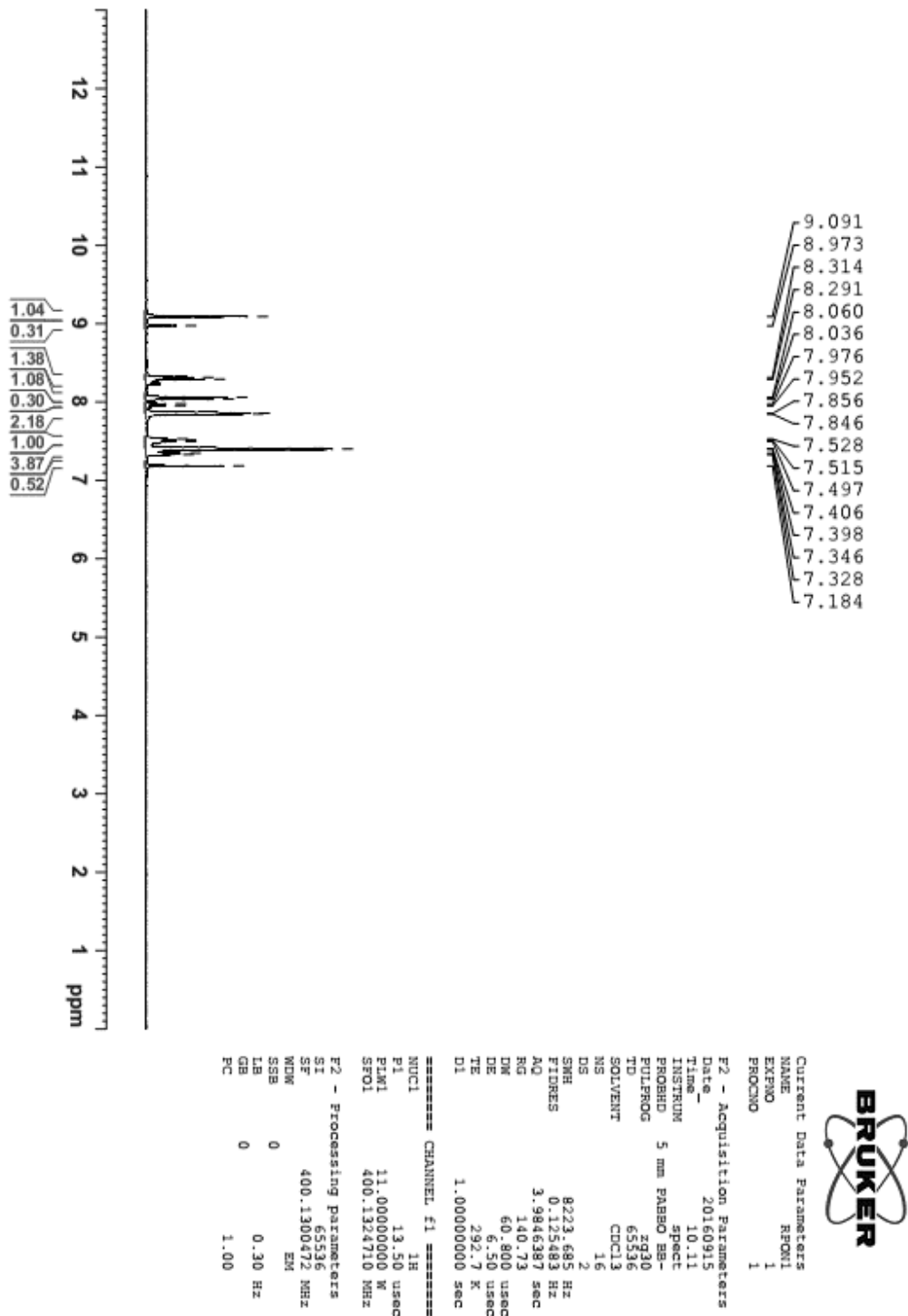


Fig. 2. ¹H NMR spectrum of compound 1.

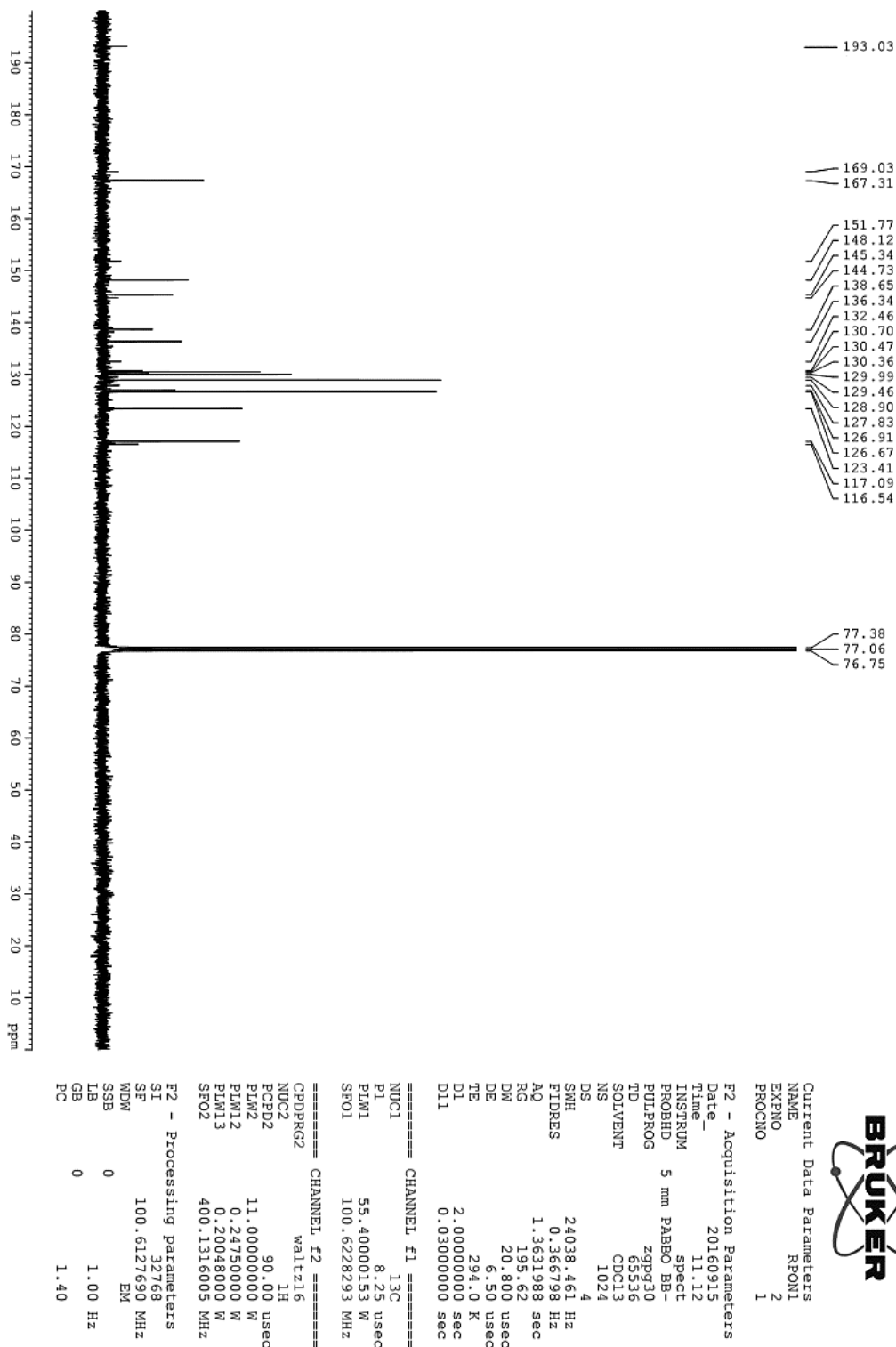


Fig. 3. ¹³C NMR spectrum of compound 1.

The representative ^{13}C NMR spectrum of compound **1** is shown in **Fig. 3**. In the ^{13}C NMR spectrum of **1-6**, the weak signal around 169.03 ppm is due to C=N of azomethine unit. The *ipso* attached carbon appeared in the region 151.77 ppm. The signals in the region 116.54-148.12 ppm are assigned to aromatic carbons compounds **1-6**. The up field signal at 21.49 and 55.51 ppm are assigned to methyl and methoxy carbon in compound **5** and **6**, respectively. The chemical shifts values are reproduced in **Table 3**.

3. 3. Antimicrobial studies of compounds 1-6

The preliminary antimicrobial activity of compounds **1-6** was examined using disc diffusion method method [36].

3. 3. 1. Antibacterial studies

The bacterial strains *viz.*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Vibrio cholerae* were used for this study. *Ciprofloxacin* is used as standard drug for bacterial strain. The Zone of inhibition values of compounds **1-6** along with the standard drug for comparison is furnished in **Table 4** and the statistical bacterial activity column chart was shown in **Fig. 4**. The representative photographs for the disc diffusion method are reproduced in **Figs. 5-8**.

Table 4. Antibacterial activities of compounds **1-6** by disc diffusion method

Compound	Diameter of zone of Inhibition (mm)				
	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>V. cholerae</i>	Total Inhibition %
1	10	15	20	09	54% Shows
2	14	23	24	13	74% Satisfied
3	16	20	28	16	80% Very good
4	18	22	23	14	77% Good
5	15	25	24	11	75% Good
6	20	26	24	16	86% Very good
Ciprofloxacin	24	27	30	28	

The zone of inhibition values of compounds **1-6** are given in **Table 4**, which indicate that all the tested compounds exhibited range 10-28 mm. The hydrazone derivatives **6** and **4** show excellent antibacterial activity against *E. coli* strain within 18& 20 mm of zone of inhibition.

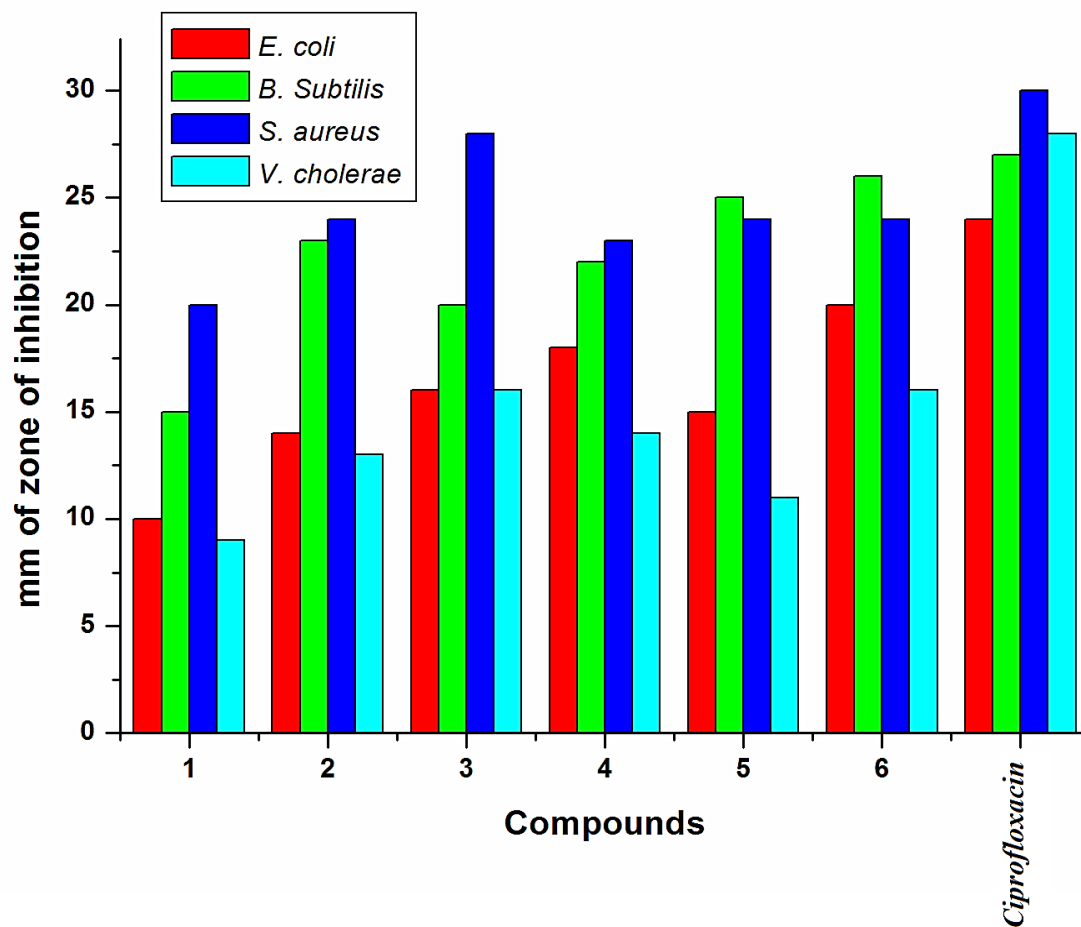


Fig. 4. The chart representation of the antibacterial activity of hydrazones **1-6**

The compounds **3** and **5** show satisfactory activity within 15 & 16 mm of zone of inhibition. The compounds **1** and **2** show moderate activity within 10-14 mm of zone of inhibition. The unsubstituted phenyl groups in compound **1** showed moderate activity against *B. subtilis* strain, whereas the compounds **2-6** exhibited good activity against *B. subtilis* within 20-26 mm of zone of inhibition. The hydrazone **1** shows good antibacterial activity against *S. aureus* strain. The introduction of different substituent in compounds (compounds **2-6**) in the phenyl group gave significant activity against the *S. aureus* bacterial strain at concentration 23-28 mm. Compound **1** and **5** show poor antibacterial activity against *V. cholerae* strain with 09-11 mm of zone of inhibition.

The compounds **2** and **4** showed good activity against *V. cholerae*. But the incorporation of chloro and methoxy substituents, enhanced the activity against *V. cholerae* with 16 mm of zone of inhibition.

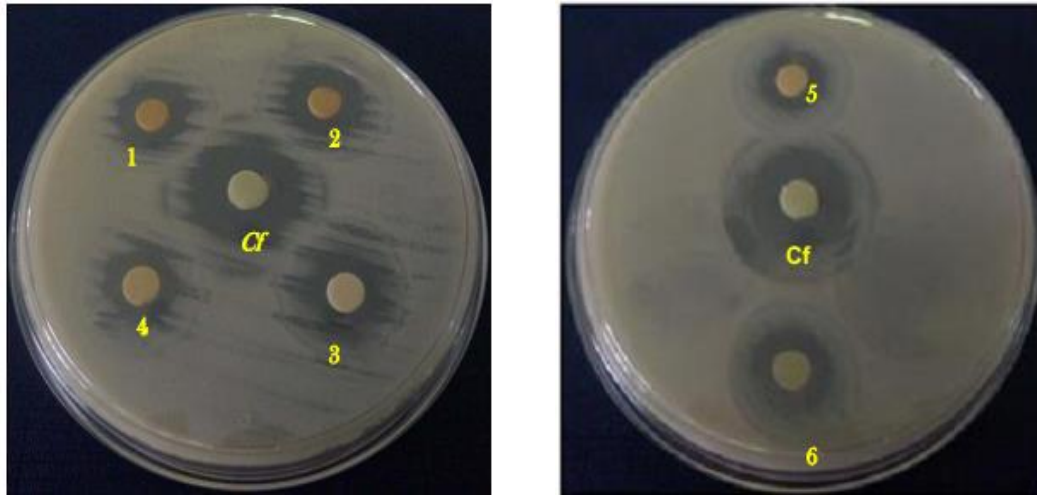


Fig. 5. Zone of inhibition of compounds 1-6 against *E. coli*

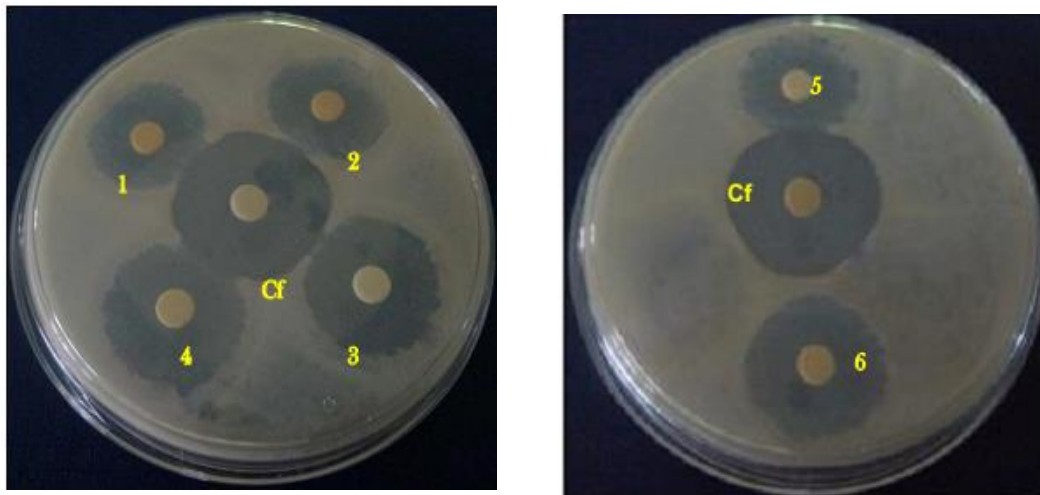


Fig. 6. Zone of inhibition of compounds 1-6 against *B. Subtilis*

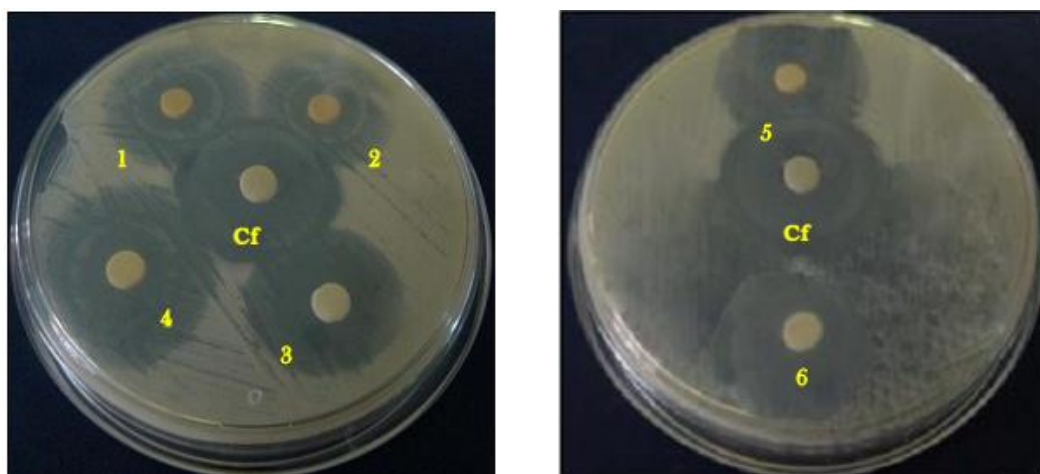


Fig. 7. Zone of inhibition of compounds 1-6 against *S. aureus*

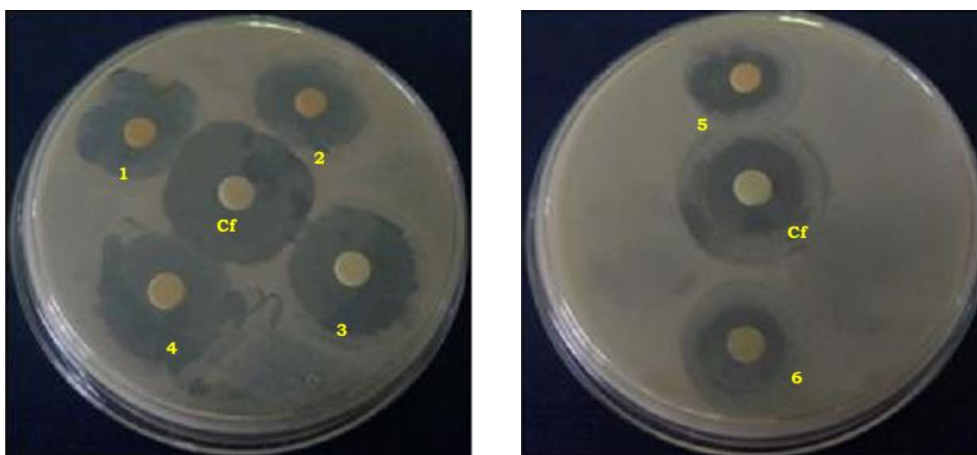


Fig. 8. Zone of inhibition of compounds **1-6** against *V. cholerae*

3. 3. 2. Antifungal activity of compounds 1-6

The measured antifungal activities of the hydrazones **1-6** by means of measurement of mm of zone of inhibition with respective fungal microbes and compared with *Amphotericin-B* using as the standard drug. The mm of zone of inhibition of antifungal activities of these hydrazones are presented in **Table 5** and the statistical clustered column chart was shown in **Fig. 9**. The synthesized compounds **1-6** were screened for antimicrobial studies against antibacterial and antifungal activity by disc diffusion method. The representative photographs for the disc diffusion method are reproduced in **Fig. 10-13**.

Table 5. Antifungal activities of compounds **1-6** by disc diffusion method

<i>Compound</i>	<i>Diameter of zone of Inhibition (mm)</i>				
	<i>A. niger</i>	<i>C. albicans</i>	<i>A. flavus</i>	<i>T. mentagrophytes</i>	<i>Total Inhibition %</i>
1	15	24	16	15	70% Good
2	13	30	10	12	65% Satisfied
3	10	33	15	10	68% Satisfied
4	12	32	13	14	71% Good
5	10	34	14	16	74% Good
6	10	30	16	14	70% Good
<i>Cetramazole</i>	18	35	30	33	100% Excellent

From the **Table 5**, the hydrazones **1**, **2** and **4** showed moderate antifungal activity against *A. niger* strain, whereas compounds **2**, **5** and **6** showed poor activity against *A. niger* strain. The compounds **2-6** had excellent antifungal activity (30-34mm) against *C. albicans* strain, but phenyl substituted hydrazone **1** showed less activity compared other hydrazones.

The title compounds **1**, **3** and **6** showed good antifungal activity against *A. flavus* strain within 15 & 16 mm of zone of inhibition whereas compounds **2**, **4** and **5** showed poor activity against *A. niger* strain in the range 10-14 mm of zone of inhibition. The compounds **2**, **3**, **4** and **6** show poor activity against *T. mentagrophytes* within 10-14 mm of zone of inhibition.

The hydrazone derivatives **1** and **5** show satisfactory antifungal activity against *T. mentagrophytes* strain within 15 & 16 mm of zone of inhibition.

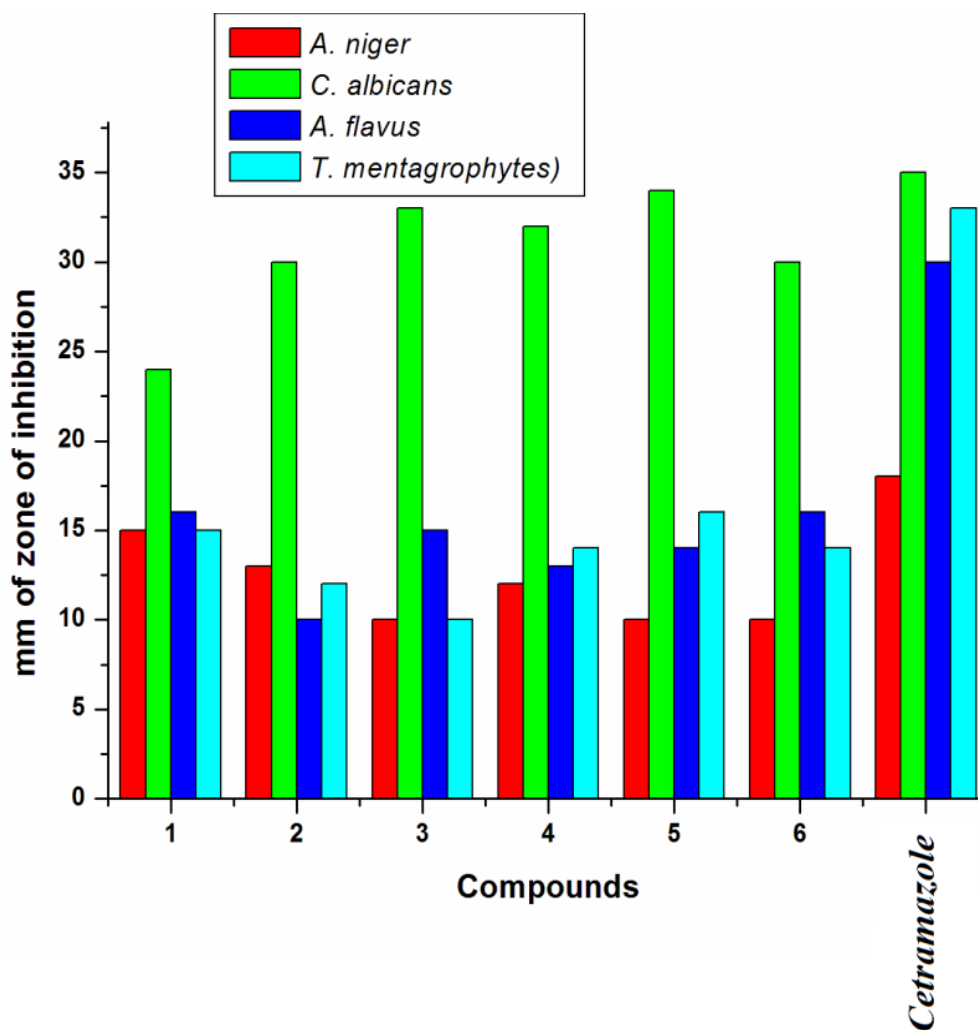


Fig. 9. The chart representation of the antifungal activity of hydrazones **1-6**



Fig. 10. Zone of inhibition of 1-6 against *A. niger*

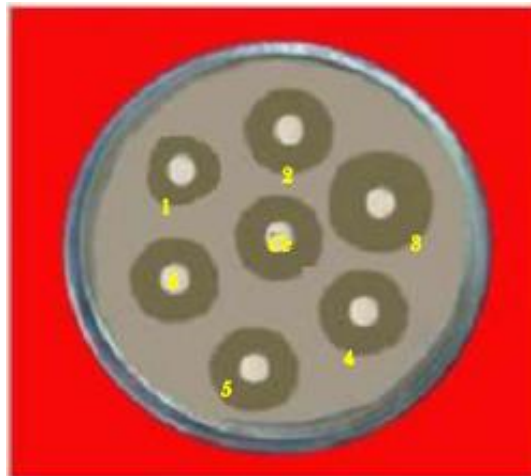


Fig. 11. Zone of inhibition of 1-6 against *C. albicans*

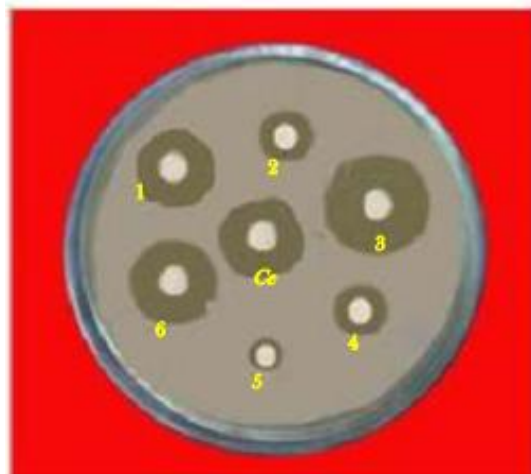


Fig. 12. Zone of inhibition of 1-6 against *A. flavus*

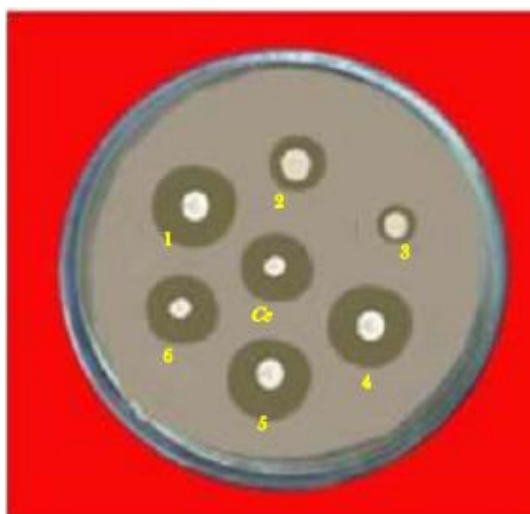


Fig. 13. Zone of inhibition of 1-6 against *T. mentagrophytes*

4. CONCLUSIONS

Overall, we synthesised a series of (E)-2-(2-((aryl)(phenyl)methylene)hydrazinyl) benzo[d] thiazole (**1-6**). The elemental, FT-IR, ^1H and ^{13}C NMR spectral data of all the synthesized compounds (**1-6**) suggest that the formation of compounds. Most of the compounds show good antibacterial and antifungal activities. However, antibacterial and antifungal activities are significantly influenced by the substituents in the phenyl ring. Among the synthesized compounds methyl and methoxy substituted hydrazones exhibit better activity against all the antibacterial and antifungal strains.

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