International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2024; 12(5): 99-104

Received: 03-06-2024
Accepted: 08-07-2024

Mittal M Patel
Department of Biogas Research and Microbiology, Faculty of Science and Applied Science, Gujarat Vidyapith, Ahmedabad, Gujarat, India

Mayur C Shah
Department of Biogas Research and Microbiology, Faculty of Science and Applied Science, Gujarat Vidyapith, Ahmedabad, Gujarat, India

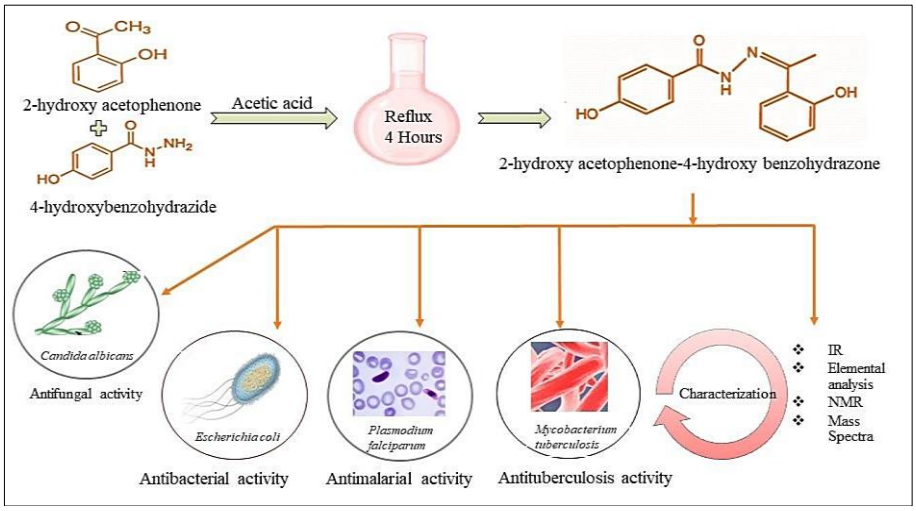
Corresponding Author:
Mayur C Shah
Department of Biogas Research and Microbiology, Faculty of Science and Applied Science, Gujarat Vidyapith, Ahmedabad, Gujarat, India

Synthesis and characterization of hydrazone derivative and its antibacterial, antifungal, antimalarial and antituberculosis activity studies

Mittal M Patel and Mayur C Shah

Abstract
In this study, 2-hydroxyacetophenone-4-hydroxy benzohydrazone (HAHBH) was synthesized by the condensation reaction of 2-hydroxy acetophenone and 4-hydroxy benzohydrazide in the presence of glacial acetic acid. This hydrazone compound was characterized through IR, 1H-NMR and 13C-NMR and Mass spectroscopic measurements and Elemental analysis. The synthesized compound has shown Antibacterial, Antifungal, Antimalarial and Antituberculosis Activity.

Graphical abstract



Keywords: 2-hyroxy acetophenone, 4-hydroxy benzohydrazide, Hydrazone, Antimalaria activity, Antitubercular activity

Introduction
In Schiff-base family hydrazones are very special organic compounds and it is more substantial reagent in different organic reactions such as hydrazone iodination, Shapiro and Bamford-Stevens reaction [1] to vinyl compound. Hydrazones are intermediates in the Wolff-Kishner reduction and used as a good spectrophotometric reagent for the determination of metal ions in spectrophometric study [2]. Generally, hydrazones are prepared by the condensation of the aldehyde or ketone and hydrazide in suitable solvents with the general structure R1R2C=N-NH2 [3].

Hydrazones are useful in many ways like analytically, catalytically in pharmacology, toxicology, pharmaceutical science etc. and it is also present in bioactive heterocyclic compounds, but it provided wide range of applications in biological and pharmaceutical fields [4]. In biological field they are also act as antitubercular [5], antitumor [6], antimicrobial [7], antimalarial, analgesic, antiinflammatory and antiplatelet, antidepressant [8], antimycobacterial, antiviral, anticonvulsant [9], antifungal, anticancer [10], antioxidant [11] agent.

Experimental

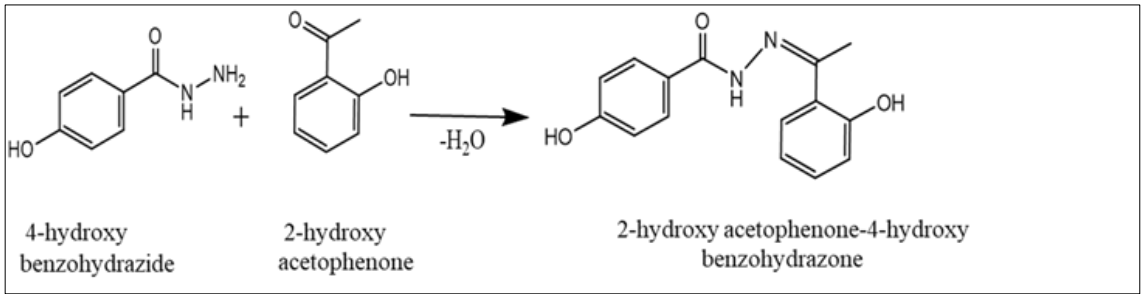
Materials and Methods

In this work all the reagents and chemicals are used analytical grade (AR). Double distilled water was used throughout experiment. Shimadzu (Model Name: IRAFFINITY-1S) FT-IR spectrometer is used for functional group identification. Mass spectra was recorded by using an Electron Multiplier Detector in Mass Spectrophotometer System AB SCIEX and QTRAP-4500 Model with standard accessories. 1H and 13C NMR spectra was recorded in Bruker AVANCE NEO 500 MHz NMR spectrometer.

The molecular formula of synthesized compound C15H14N2O3 was confirmed by elemental analysis and biological activity like antimicrobial, antimalarial and antitubercular activity were also done.

Synthesis of 2-hydroxyacetophenone-4-hydroxy benzohydrazone (HAHBH)

Solution of 2-hydroxy acetophenone (3.40 g, 25 mmol) in 50 mL ethanol and 4-hydroxy benzohydrazide (3.80 g, 25 mmol) in 50 mL ethanol were prepared. These two solutions were mixed in 250 mL round bottom flask. 5 mL glacial acetic acid was added as a catalyst. This mixture was refluxed for 4 hours in water bath and after refluxing light yellow-colored solid was appeared and then progress of the reaction was checked by TLC technique. Resulting reaction mixture was cooled at room temperature and then reaction mixture was poured in ice. Shiny light yellow colored precipitates were formed. The precipitates were filtered, washed with cold ethanol and dried in oven for 2 hours at 95 ℃. Percentage of yield was found 90.84%. The product was recrystallized from ethanol and melting point of HAHBH was found to be 249-250 ℃.



Results and Discussion

Characterization of compound

IR Spectra: The infrared (IR) spectrum of the compound is presented in figure 1. The formation of the Schiff base was confirmed by following band frequencies of particular functional group. The peak was observed at 3305 cm^{-1} is assigned to -OH group [12] and peak at 3238 cm^{-1} is assigned to -NH group [13, 14] and peak at 1643 cm^{-1} indicates -C=O group. A peak at 1606 cm-1 and 1595 cm-1 are assigned -C=N [12, 15] group and this is indicating the absence of -NH2 in structure of compound. The band at 1033 cm−1 is identified the presence of N-N group [15, 16]. A weak peak at 3057 cm-1 is due to aromatic -C−H bonds.

Table 1: FTIR data of hydrazone reagent

FTIR Bands (cm-1)					
Sample	**-NH**	**-OH**	**-C=O**	**-N-N**	**-C=N**
HAHBH	3238	3305	1643	1033	1606

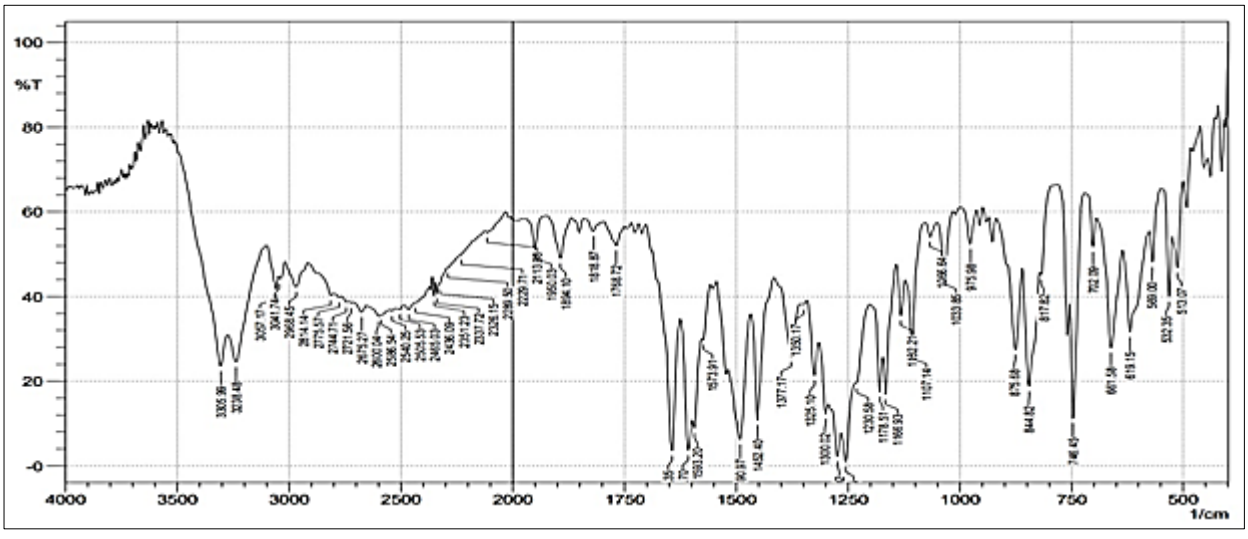


Fig 1: IR Spectra of HAHBH

1H NMR Spectra: 1H-NMR Spectra of compound HAHBH is recorded in d6-DMSO solvent and it is presented in figure 2. In the low field region proton spectra show one singlet peak of azomethine proton at 10.22ppm. The hydroxyl proton(C-OH) of 2-hydroxy acetophenone and 4-hydroxy benzohydrazide show singlet at 13.51ppm and 11.11ppm respectively. In high field region compound show one singlet at δ (2.499) ppm, which is belongs to methyl hydrogens (-CH3). The all aromatic hydrogens show peak in low field region, among them two hydrogens are showing triplet at δ (6.88-6.91) ppm, δ (7.28-7.31) ppm, two hydrogens are showing doublet at δ (6.61-7.62) ppm, δ (7.89-7.91) ppm and four hydrogens of 4-hydoxy benzohydrazide show one doublet at δ (6.94-6.95) ppm.

Table 2: [1]H- NMR data of HAHBH

δ value in ppm	Assignments
2.4998 (s,3H)	C-CH3
10.22(s,1H)	>N-H
10.22(s,1H)	>N-H
11.11(s,1H)	C-OH (4-hydroxy benzohydrazide)
13.51(s,1H)	C-OH (2-hydroxy acetophenone)
6.88-6.91(t,1H)	
7.28-7.31(t,1H)	
6.61-7.62 (d,1H)	Aromatic hydrogens
7.89-7.91(d,1H)	
6.94-6.95(d,4H)	

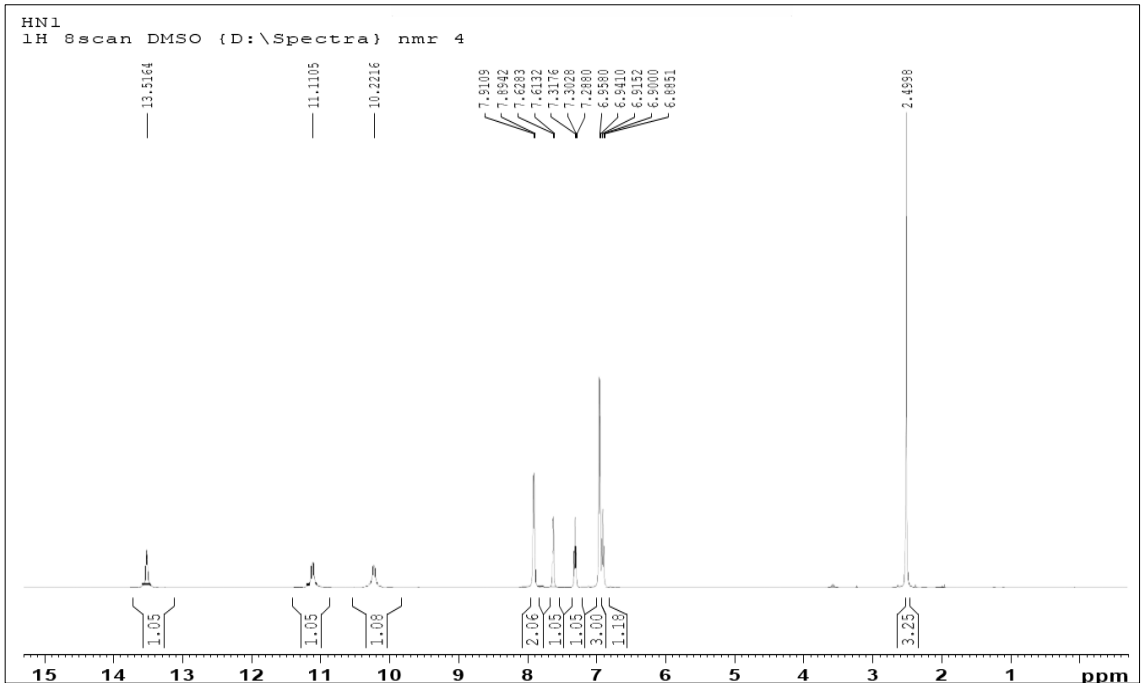
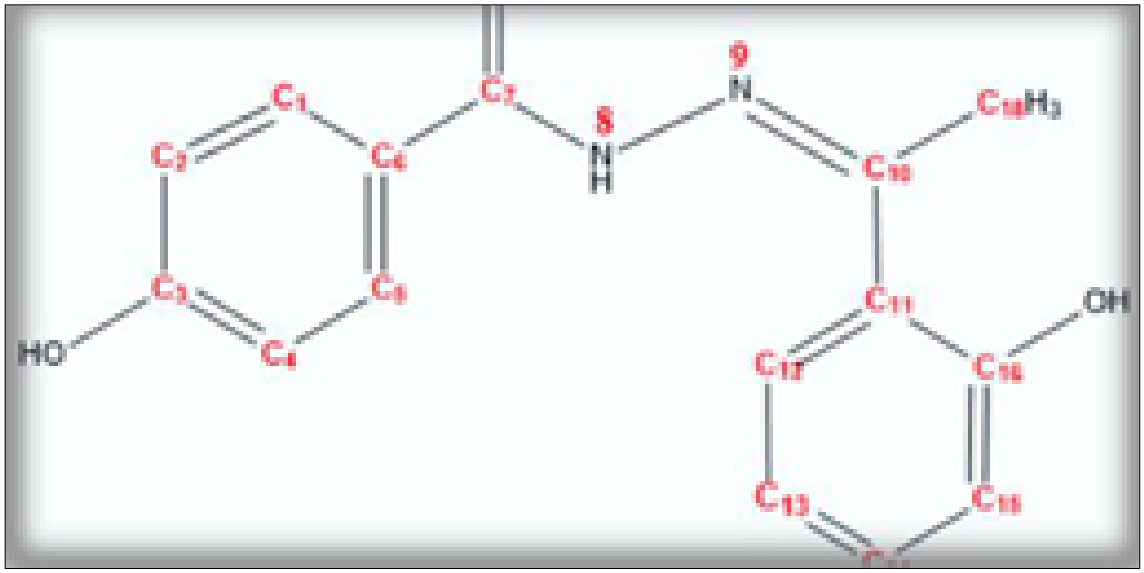


Fig 2: [1]H- NMR Spectra of HAHBH

13C-NMR spectra: - 13C-NMR spectra for ligand (500Hz, d6 -DMSO) δ(ppm): 13.83 (C18); 39.08-39.75 (DMSO-d6); 115.04,117.27, 118.42, 119.51,123.35,128.28,130.24,131.02 (C1, C2, C5, C6, C12, C13, C14, C15) Aromatic carbons [17, 19]; C10 and C11 (157.01) [17, 18]; 158.74 (C4); 161.01(C3 and C16) [19]; 163.97 (C7) [18]. [Figure 4]



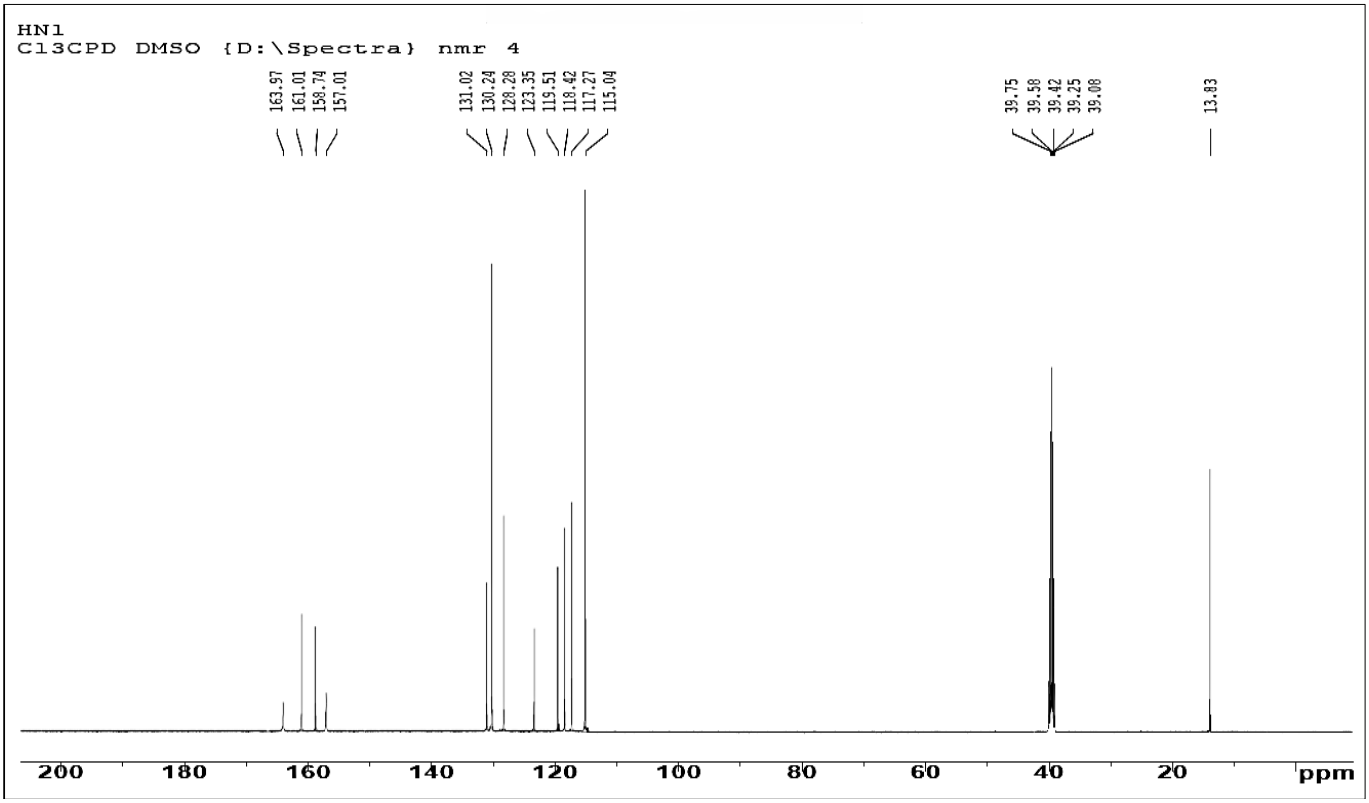


Fig 3: ^{13}C- NMR Spectra of HAHBH

Mass Spectra: Mass spectra are shown in Figure 5. The compound shows m/z value of molecular ion peak at 271-1=270. This is corresponding to it's the molecular formula (Molecular Mass =270) C15H14N2O3.

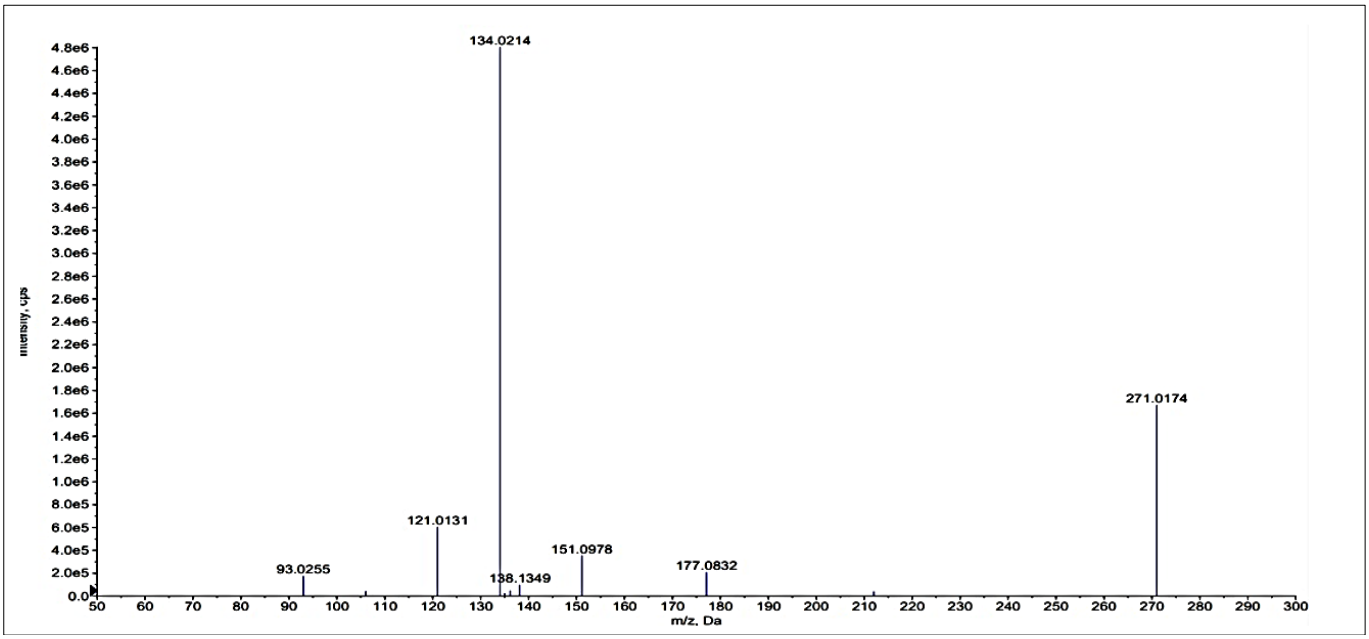


Fig 4: Mass Spectra of HAHBH

Table 3: Analytical and physical data of synthesized compound (HAHBH)

Compound	Molecular Weight	Color	Melting Point	% of Yield	% Found by analysis (From calculation)		
					C	H	N
HAHBH	270.3 g mol-1	Light Yellow	249-250 ℃	90.84	66.575 (66.6)	4.938 (5.18)	10.372 (10.36)

Biological Activity of HAHBH

Antibacterial and Antifungal Activity: The *in vitro* antibacterial and antifungal activity of newly synthesized hydrazone derivative was tested against the *Staphylococcus aureus* (MTCC 96) and *Streptococcus pyogenes* (MTCC 442) from gram positive and *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 1688) from gram negative group of bacteria and *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus* from group of fungi. The evaluation of the antibacterial activity we have to used Microbroth Dilution

Method and DMSO was used as diluents/solvent to get desired concentration of drugs to test upon standard bacterial strains. The strains were procured from Institute of Microbial Technology, Chandigarh. Results shows the comparison of MIC (µg/mL) value of the synthesized compound with standard drugs that are presented in following chart. Chart shows that synthesized compound gave equal MIC value that of Chloromphenicol standard drug against *E. Coli* (MTCC 443) bacteria and HAHBH shows higher MIC value than that of Ciprofloxacin against *S. Aureus*, *P. Aeruginosa*, *S. Pyogenes*. HAHBH gives equal MIC value that of Greseofulvin against *C. Albicans* (MTCC 227) fungi and compound shows higher MIC value than that of Nystatin against *A. Niger* and *A. Clavatus*

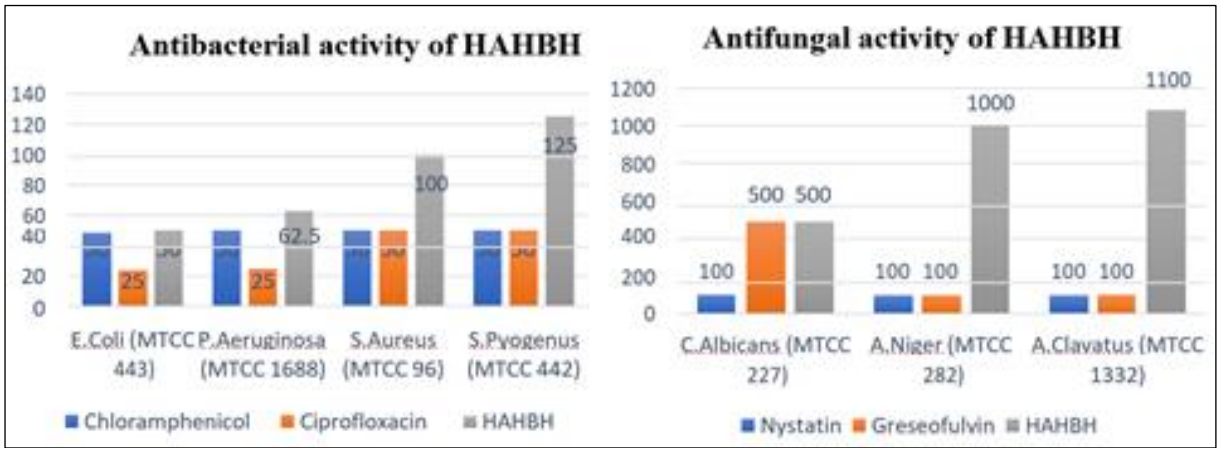


Fig 5: a) Antibacterial activity of HAHBH and b) Antifungal activity of HAHBH

Antimalarial Activity: *In vitro* antimalarial screening was carried out well microlitre plates according to the micro screening procedure of Rieckmann and Co-workers [20] with minor changes. The 3D7 strain of *Plasmodium falciparum* culture was maintained in RPMI 1640 medium added with 25 mM HEPES, 0.23% sodium bicarbonate, 1% D-glucose and 10% heat inactivated human serum. After the D- sorbitol treatments the asynchronous parasites of *P. falciparum* were synchronized to obtain only for the ring stage parasitized cells. For screening an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µl of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to evaluate the rings and uniformally maintained with 50% RBCs (O+). The stock solution of 5 mg/mL of each of the test sample are prepared in DMSO and consequent dilutions were prepared by culture medium. The 20 µL solution of diluted sample were added to the test wells so as to final concentration are obtained range between 0.4 µg/mL to 100 µg/mL in duplicate well containing parasitized cell preparation. In a candle jar the culture plates was incubated at 37℃ temperatures. Thin blood smears were prepared from each well and stained with JSB stain after 36-40 hours. These slides were observed by microscope for the record maturation of ring stage parasites in the presence of different concentrations of the test agents like trophozoites and schizonts. The inhibition of the test concentration of complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Chloroquine was used as a standard drug. After 38 hours incubation the mean number of rings, schizonts and trophozoites was recorded per 100 parasites from duplicate wells and percent maturation inhibition with respect to control group.

Compound shows antimalarial activity with IC50 value 0.92 µg/mL against 3D7 strain of *Plasmodium falciparum*. Following table 3 shows IC50 value of synthesized compound which were found to be active [21, 22].

Table 3 Antimalarial Activity

Minimal inhibition concentration		
Sr. No.	**Compound**	**Mean IC50 value a**
1	HAHBH	0.92 µg/mL
Standard drug (Chloroquine)		0.020 µg/mL

a: Mean values in representative screening. Experiments were performed in duplicate.

Antituberculosis Activity

Isoniazid and Rifampicin is one type of drug which is used in the treatment of tuberculosis and it prevents the growth of the microorganisms that cause the infection. So, in this study isoniazid was used as a standard drug to evaluated the antituberculosis activity of newly synthesized compound. This tuberculosis screening data indicated that the newly synthesized compound gives MIC value 250 µg/mL, it's not equivalent with standard drug Isoniazid but it was act as antitubercular agent. The MIC (µg/mL) values of compound against H37RV bacteria using L. J. Medium [Conventional Method] are shown in table 4

Table 4: Antituberculosis Activity

Method		**L. J. Medium [Conventional Method]**
Bacteria		H37RV
Standard Drug with MIC value(µg/mL)		Isoniazid = 0.20 µg/mL
Sr. No.	**Sample name**	**MIC value (µg/mL)**
1	HAHBH	250

Conclusion

HAHBH is synthesized successfully by the condensation reaction of 2-hydroxy acetophenone and 4-hydroxy benzohydrazide in the presence of glacial acetic acid. IR, NMR, Mass spectroscopy and elemental analysis data revealed that HAHBH was synthesized. Compound shows

potential antibacterial activity against *E. Coli* with reference to Chloromphenicol standard drug and also shows potential antifungal activity against C. *Albicans* with reference to Greseofulvin standard drug. Compound shows less antimalarial and antituberculosis activity with reference to standard drugs Chloroquine and Isoniazid respectively.

References

1. Ansari TM, Shah Gilani MRH, Xu G, Liang G, Luque R, Alsaiari M, Jalalah M. Facile and straightforward synthesis of Hydrazone derivatives. Journal of Nanomaterials; c2022. p. 1-6. DOI: https://doi.org/10.1155/2022/3945810
2. Singh RB, Jai P, Singh RP. Hydrazones as analytical reagents: a review. Talanta. 1982;29(2):77-84. DOI: https://doi.org/10.1016/0039-9140(82)80024-6
3. Gawande PU, Mandlik PR, Aswar AS. Synthesis and characterization of Cr (III), Mn (III), Fe (III), VO (IV), Zr (IV) and UO2 (VI) complexes of schiff base derived from isonicotinoyl hydrazone. Indian Journal of Pharmaceutical Sciences. 2015;77(4):376. DOI: https://doi.org/10.4103%2F0250-474x.164779
4. Verma G, Marella A, Shaquiquzzaman M, Akhtar M, Ali MR, Alam MM. A review exploring biological activities of hydrazones. Journal of Pharmacy Bioallied Science. 2014;6(2):69-80. DOI: 10.4103/0975-7406.129170
5. Mallikarjuna P, Mastanaiah T, Raffiyuddin M, Thippanna G, Rao VS. Direct spectrophotometric determination of ferrous iron using Diacetyl monoxime isonicotinoyl hydrazone. World Journal of Pharmaceutical Research. 2012;1(5):1320-1329. DOI: 10.9790/5736-0212025
6. Mallikarjuna P, Sreenivasulu K, Prasad Gupta MVNV, Thippanna G, Mastanaiah T, Rao VS. Simultaneous second order derivative spectrophotometric determination of Gallium (III) and Aluminium (III) using Diacetyl monoxime isonicotinoyl hydrazone. IOSR Journal of Applied Chemistry. 2012;2(1):20-25. DOI: 10.9790/5736-0212025
7. Singh S, Agnihotri N, Rathi P, Agnihotri R, Kumar V. Molecular dynamics, biological study and extractive spectrophotometric determination of vanadium (V)-2-methyl-8-quinolinol complex. Iranian Journal of Chemistry and Chemical Engineering (IJCCE). 2021;40(1):207-214. DOI: https://doi.org/10.30492/ijcce.2020.93050.3273
8. Rollas S, Küçükgüzel ŞG. Biological activities of hydrazone derivatives. Molecules. 2007;12(8):1910-1939. DOI: https://doi.org/10.3390/12081910
9. Kumari D, Bansal H. Benzohydrazides: as potential bio-active agents. The Pharma Innovation Journal. 2018;7:543-550. Available from: http://www.thepharmajournal.com/archives/2018/vol7issue8/PartI/7-7-164-286.pdf
10. Kumar P, Narasimhan B, Ramasamy K, Mani V, Mishra RK, Abdul Majeed AB. Synthesis, antimicrobial, anticancer evaluation and QSAR studies of 2/3-bromo-N'-(substituted benzylidene/3-phenylallylidene) benzohydrazides. Arabian Journal of Chemistry, 2017, 10. DOI: https://doi.org/10.1016/j.arabjc.2014.05.010
11. Randhawa H, Kamboj A, Saluja AK. Synthesis, pharmacological evaluation and computational studies of some novel hydrazine derivatives of thiophene chalcone as antimicrobial and antioxidant agents. World Journal of Pharmaceutical Research. 2014;3:3146-3159. Available from: https://www.semanticscholar.org
12. Deshmukh SD, Mandlik PR. Synthesis, spectral characterization, thermal studies and antimicrobial activity of metal (II) complexes of 2,4-dihydroxybenzophenone salicyloyl hydrazone. Vidyabharati International Interdisciplinary Research Journal; c2020. p. 406-413. Available from: https://www.researchgate.net/publication/351748026
13. Al-Daher AGM, Mohammed AH. Preparation, characterization and study of ethyl pyruvate aroyl hydrazone metal complexes. Rafidain Journal of Science. 2018;27(3):100-112. Available from: https://www.iasj.net/iasj/download/fadf79f2569a989d
14. Vidyasagar Babu S, Eswaramma S, Krishna Rao KSV. Synthesis, characterization, luminescence and biological activities of lanthanide complexes with a hydrazone ligand. Main Group Chemistry. 2018;17(1):99-110. DOI: 10.3233/MGC-180251
15. Omer DA, Al-Daher AGM. New tridentate hydrazone metal complexes derived from 2-hydroxy-4-methoxyacetophenone and some acid hydrazides: synthesis, characterization and antibacterial activity evaluation. Rafidain Journal of Science. 2019;28(2):100-111. Available from: https://www.iasj.net/iasj/download/032c347b9dee53b3
16. Aldabagh AA, Al-Daher AGM. Synthesis and characterization of new Co (II), Ni (II), Cu (II) and Zn (II) with bis-hydrazones complexes. Rafidain Journal of Science. 2019;28(2):112-119. Available from: https://www.iasj.net/iasj/download/e85bb68b21c4a83d
17. Hassan AU, Sumrra SH, Zafar MN, Nazar MF, Mughal EU, Zafar MN, Iqbal M. New organosulfur metallic compounds as potent drugs: synthesis, molecular modeling, spectral, antimicrobial, drug likeness and DFT analysis. Molecular Diversity. 2022;26(1):51-72. DOI: https://link.springer.com/article/10.1007/s11030-020-10157-4
18. Ammar YA, Abd El-Hafez SM, Hessein SA, Ali AM, Askar AA, Ragab A. One-pot strategy for thiazole tethered 7-ethoxy quinoline hybrids: synthesis and potential antimicrobial agents as dihydrofolate reductase (DHFR) inhibitors with molecular docking study. Journal of Molecular Structure. 2021;1242:130748. DOI: https://doi.org/10.1016/j.molstruc.2021.130748
19. Devi J, Pachwania S, Kumar D, Jindal DK, Jan S, Dash AK. Diorganotin (IV) complexes derived from thiazole Schiff bases: synthesis, characterization, antimicrobial and cytotoxic studies. Research on Chemical Intermediates. 2022;48(1):267-289. DOI: https://sci-hub.hkvisa.net/10.1007/s11164-021-04557
20. Rieckmann KH, Campbell GH, Sax LJ, Mrema JE. Drug sensitivity of *Plasmodium falciparum*, an *in vitro* microtechnique. Lancet. 1978;1:221-223. DOI: https://doi.org/10.1016/s0140-6736(78)90365-3
21. Verma G, Chashoo G, Ali A, Khan MF, Akhtar W, Ali I, et al. Synthesis of pyrazole acrylic acid based oxadiazole and amide derivatives as antimalarial and anticancer agents. Bioorganic Chemistry. 2018;77:106-124. DOI: https://doi.org/10.1016/j.bioorg.2018.01.007
22. Altamimi AS, El-Azab AS, Abdelhamid SG, Alamri MA, Bayoumi AH, Alqahtani SM, et al. Synthesis, anticancer screening of some novel trimethoxy quinazolines and VEGFR2, EGFR tyrosine kinase inhibitors assay; molecular docking studies. Molecules. 2021;26(10):1-14. DOI: https://doi.org/10.3390/molecules26102992