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Synthesis, crystal structure, spectral and antimicrobial studies of 4-(2,5-dimethoxybenzaldehydene)-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one

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A novel Schiff base, 4-(*N*-2,5-dimethoxybenzaldehydene)-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (DMBDPP) was prepared by the reaction between 4-aminoantipyrine and 2,5-dimethoxybenzaldehyde in dry methanol at room temperature. The synthesized compound was characterized by elemental analysis, FT-IR, UV-Vis and NMR spectral studies. Single crystal X-ray diffraction studies showed that the title compound belongs to monoclinic system, with space group Cc having four molecules per unit cell (Z = 4). The parameters of the unit cell are *a* = 19.587(2) Å, *b* = 6.8406(5) Å, *c* = 13.4652(11) Å at 296 K. The molecular structure of the compound shows that the phenyl-pyrazole and dimethoxy phenyl group on alternate sides of the azomethine moiety. The *in vitro* anti-microbial activity of compound was tested against three Gram-negative (*Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumoniae,)* and two Gram-positive (*Staphylococcus aureus, Streptococcus mutans*) bacterial strains, and two fungal strains (*Candida albicans* and *Aspergillus niger*) by the agar well diffusion method.

Keywords: 4-Aminoantipyrine, Schiff base, crystal structure, antimicrobial studies.

Introduction

4-Aminoantipyrine and its derivatives are an interesting class of heterocyclic compounds because of their medicinal properties and participation in chemical processes. They have a five membered ring which contains two nitrogen atoms and a carbonyl group in the same molecule, and exhibit a wide range of biological activities[1–5]. Pyrazoles based on 4-aminoantipyrine have also applications outside the pharmaceutical field, such as in the solvent extraction of metal ions and act as ligands of metal complexes with catalytic activity.

The compound 4-aminoantipyrine (4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) (4-AAP) is a metabolite of aminophenazone and an aromatic substance with antipyretic, analgesic and anti-inflammatory and antimicrobial activity[6–13]. 4-AAP has a pyrazole-phenyl ring and a methyl group on either side of a polar carbonyl group, thus similar to *N*-substituted amides[14,15]. 4-Aminophenazone is used in colorimetric determination of phenols and also identified as a reagent in the biochemical reactions producing peroxides or phenols[16]. 4-AAP has pyrazol-5-one fragment which are also found in several biologically active molecules that have important roles in plant as well as animal kingdoms[17].

Mostly, the electron releasing-withdrawing nature and the position of substituents present in the phenyl ring affect the antimicrobial activities. Inhibition is enhanced with the introduction of an electron withdrawing nitro group in the phenyl ring[18]. 4-Aminoantipyrine that forms a variety of Schiff bases with aldehydes and a remarkable number of compounds have been reported with a wide range of biological activities and applications[19,20]. However, various inter- or intra-molecular hydrogen bonds are present in Schiff bases derived from 4-aminoantipyrine and these bonds are often used to evaluate the physicochemical and chemical properties of the compounds[21,22]. Recently, there are many reports on the antimicrobial activities of Schiff bases derived from 4-aminoantipyrine derivatives and their metal complexes. The study showed that the complexes showed more antimicrobial activity than the Schiff bases[23,24].

The present manuscript reports the synthesis, crystal structure and spectral studies of a novel Schiff base derived from 4-aminoantipyrine and 2,5-dimethoxybenzaldehyde and its antimicrobial activity.

Experimental

Materials and methods:

4-Aminoantipyrine (1-phenyl-2,3-dimethyl-4-amino-3-pyrazolin-5-one) and 2,5-dimethoxybenzaldehyde were of A.R. grade purchased from Merck. A.R. grade methanol was purchased from Merck and used as received.

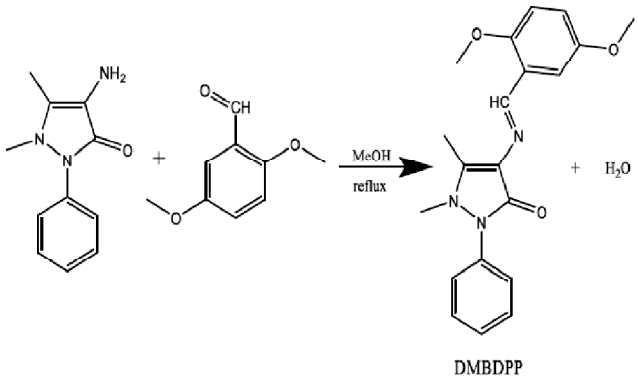
Elemental analysis (C, H and N) was performed with a Vario EL-III CHN Elemental Analyzer. IR spectra were recorded on a Perkin-Elmer Infrared Spectrometer using KBr pellets in the range 4000–400 cm^{-1}. Electronic absorption spectra were recorded in DMSO solution on a Shimadzu UV 2450 UV-Visible spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded using Bruker DRX-MHz NMR spectrometer with $CDCl_3$ as solvent and TMS as the internal standard. XRD data were collected using a Bruker AXS Kappa Appex2 CCD diffractometer, with graphite monochromated Mo Kα radiation (λ =0.71073 Å) at 296 K. The structure was solved using SIR 92 and refinement was carried out by full-matrix least squares methods on F^2 using SHELXL-97. The molecular and packing diagrams were created by the DIAMOND Program. The C-H hydrogen atoms were fixed with idealized geometry and refined using a riding model. Non-hydrogen atoms were refined using anisotropic displacement parameters.

Biological studies:

The *in vitro* antibacterial and antifungal activity of the compound was evaluated by the agar-well diffusion method using Streptomycin and Clotrimazole as standard drug for bacteria and fungi respectively. The five bacteria selected were *Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumoniae, Streptococcus mutans* and *Staphylococcus aureus* and the two fungi were *Candida albicans* and *Aspergillus niger.*

Synthesis of DMBDPP:

To a solution of 2,5-dimethoxybenzaldehyde (0.166 g, 1 mmol) in methanol (10 ml) was added 1-phenyl-2,3-dimethyl-4-amino-3-pyrazolin-5-one (0.203 g, 1 mmol) in methanol (15 ml). The mixture was refluxed for 4 h on water bath. On slow evaporation, shiny needle-like yellow crystals were formed. The crystals were washed with methanol and diethyl ether and dried over P_4O_{10} in vacuo (m.p. 160°C). The elemental analysis data were in good agreement with the formula of the compound. The synthesized compound was also characterized by FT-IR, UV-Vis. and NMR spectral techniques.



Scheme 1. Synthesis of 4-(*N*-2,5-dimethoxybenzaldehydene)-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (DMBDPP).

Results and discussion

The DMBDPP is a non-hygroscopic solid, stable in air and soluble in common organic solvents. The purity of the compound is ascertained by elemental analysis. The elemental analysis data of the compound is consistent with the stoichiometry. Anal. Calcd. for $C_{20}H_{21}N_3O_3$: C, 68.36; H, 6.02; N, 11.96. Found: C, 67.65; H, 6.09; N, 12.08%.

Crystallographic results:

Crystal data of DMBDPP is listed in Table 1. The selected molecular structure parameters (bond lengths and bond angles) are listed in Table 2. The hydrogen bonds are listed in Table 3.

The compound, DMBDPP crystallizes in monoclinic of Cc space group. The unit cell of DMBDPP was found to be composed two pairs of two juxtaposed molecules (Z = 4). The compound possesses two different planes, i.e. the pyrazolone and benzylidene groups are almost coplanar and the phenyl group connected to N1 atom of pyrazolone ring creates the other plane. Fig. 1 represents the molecular structure with atom numbering of DMBDPP. In this the *ortho* and *para* positions of the phenyl ring, are substituted by C2 and

Table 1. Crystal data and structure refinement parameters for DMBDPP

Empirical formula	$C_{20}H_{21}N_3O_3$
Formula weight	351.40
Temperature (K)	296(2)
Wave length (Å)	0.71073
Crystal system	Monoclinic
Space group	Cc
Unit cell dimensions:	
a, *b*, *c* (Å)	19.587(2), 6.8406(5), 13.4652(11)
α, β, γ (°)	90, 98.997(6), 90
Volume V (A^3)	1782(3)
Z	4
D_{calc} (ρ) (mg/m^3)	1.310
Absorption coefficient (mm^{-1})	0.090
F (0 0 0)	744
Crystal size (mm)	0.35×0.35×0.30
Colour, nature	Yellow, needle
Θ range for data collection (°)	3.06–28.34
Limiting indices	$-26 \le h \le 26$, $-8 \le k \le 8$, $-17 \le l \le 17$
Reflections collected	10447
Independent reflections R (int)	358 (0.0250)
Completeness to Θ	28.34 (99.5%)
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9736 and 0.9693
Refinement method	Full- matrix least square on F^2
Data/restraints/parameters	4358/2/240
Goodness-of-fit on F^2	R1 = 0.0262, wR2 = 0.0665
Final R indices [I > 2σ (I)]	R1 = 0.0483, wR2 = 0.0979
Largest diff. peak and hole (e A^{-3})	0.156 and –0.153

Table 2. Selected bond lengths (Å) and bond angles (°) of DMBDPP

Bond lengths		Bond angles	
C(14)-N(1)	1.392 (3)	C(9)-N(2)-C(10)	119.80 (2)
C(15)-N(1)	1.433 (3)	C(11)-N(3)-N(1)	106.78 (16)
N(1)-N(3)	1.402 (3)	C(14)-N(1)-N(3)	110.13 (19)
N(2)-C(9)	1.282 (3)	N(3)-N(1)-C(15)	120.66 (17)
C(10)-N(2)	1.395 (3)	N(2)-C(9)-C(6)	120.70 (2)
C(11)-N(3)	1.362 (3)	C(11)-N(3)-C(13)	123.80 (2)
C(13)-N(3)	1.452 (3)		
C(2)-O(1)	1.366 (3)		
C(5)-O(2)	1.377 (3)		
C(14)-O(3)	1 .231 (3)		
C(10)-C(11)	1.370 (3)		

C5 respectively. The dimethoxy substituted phenyl ring (C2-C6) is in the same plane, while the phenyl ring (C15-C20) of the antipyrine moiety is tilted by 36.64° with respect to the central five-membered ring. The tilt of the phenyl group C15-C20 with respect to central five-membered ring can be attributed to various molecular interactions involved in the packing pattern.

DMBDPP was found to adopt an *E*-configuration about its azomethine group, -N2=C9- double bond. The bond length of its azomethine group, N2-C9 is 1.282(3) Å which is in conformity with a formal C=N (1.29 Å). The C14-O3 bond distances of 1.231(3) Å, is very close to exocyclic C=O bond length (1.23 Å). It proves the existence of pyrazolone in the keto form in the solid state. The distance between C10 and C11 in the pyrazolone ring is 1.370(3) Å, indicating a double bond character. In the crystal of DMBDPP, the five mem-

Table 3. Hydrogen- bonding geometry (Å, °)

D-H···A	D-H	H···A	D···A	D-H···A	Symmetry codes
Inter C1-H1C···O3	0.96	2.54	3.4311(4)	154	X-1/2, –Y+1/2, Z-1/2
Intra C9-H9···O2	0.93	2.44	2.7523(3)	100	
Intra C9-H9···O3	0.93	2.33	3.0174(3)	131	X, Y-1, Z
X-H(I)-Cg(J)	H···Cg (Å)	X-H···Cg (Å)			
CH···π Interactions:					
C1-H1A-Cg(3)	2.74	133			
C1-H1B-Cg(1)	2.93	128			

Cg(1) = N(1), N(3), C(11), C(10), C(14)

Cg(3) = C(15), C(16), C(17), C(18), C(19), C(20)

D = donor, A = acceptor, Cg = centroid.

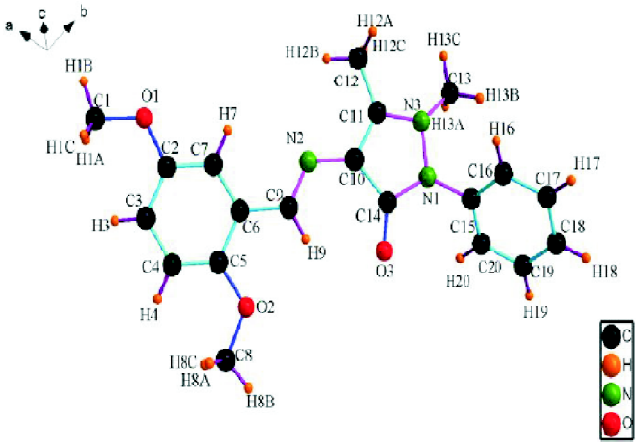


Fig. 1. The molecular structure with the atom numbering for DMBDPP.

bered ring N3-C11-C10-C14-N1 is almost planar.

In the crystal structure, two types of hydrogen bonds co-exist. Intermolecular interactions are established between the ligand molecule O3, the exocyclic carbonyl oxygen atom of pyrazolone ring and hydrogen atom (H1B) of the 2,5-dimethoxybenzaldehyde ring. Intramolecular interactions are established between the O2 and azomethine nitrogen atoms O2-H9-C9 and another interaction is established between the O3 and azomethine nitrogen atoms O3-H9-C9.

The packing of the compound in a unit cell along the b-axis is given in Fig. 2. The group of molecules in the respective manner in the unit cell result in C-H···π interactions as illustrated in Table 4. The C-H···π interactions are established between the methyl hydrogen of antipyrine ring from one molecule and the centre of phenyl ring from another molecule. The C-H···π interactions C1-H1A→Cg3 and C1-H1B→Cg1 are at a distance of 2.74 and 2.93 Å contribute stability to the unit cell packing (atom numbering according to Fig. 1).

FT-IR, UV-Vis. and NMR spectral studies:

In the IR spectrum, the broad peak ~3450 cm^{-1} is assigned to hydrogen bonding. A very sharp band occurring at 1642 cm^{-1} is due to C=O stretching. The sharp peak at 1567 cm^{-1} which is assigned to the C=N stretching vibration shows the formation of the Schiff base. The N-N stretching mode of pyrazole ring is observed at 1176 cm^{-1}. The strong peak appeared at 1495 cm^{-1} is assigned to the stretching vibration of C=C group. The peaks at 2955 and 2836 cm^{-1} are

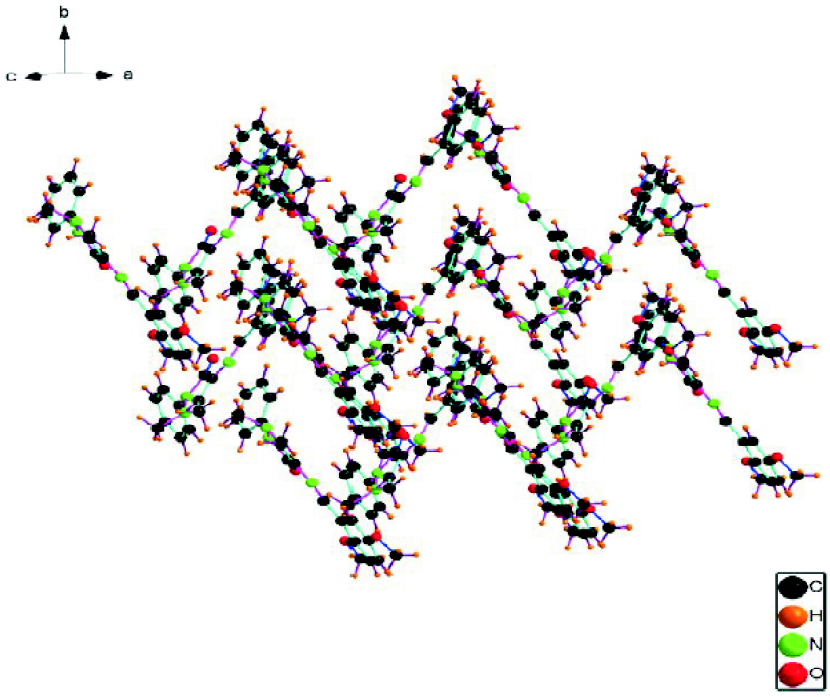


Fig. 2. Packing diagram of DMBDPP viewed along b-axis.

Table 4. Antibacterial screening data of DMBDPP

Bacterial strains	Inhibition zone (mm)		
	Streptomycin	Conc. (μg/mL)	DMBDPP
Escherichia coli	32	25	–
		50	10
		100	13
Pseudomonas aeroginosa	29	25	–
		50	12
		100	15
Klebsiella pneumoniae	28	25	–
		50	10
		100	10
Streptococus mutans	30	25	–
		50	11
		100	10
Staphylococcus aureus	28	25	10
		50	11
		100	16

assigned to the C-H stretching vibration of the O-CH_3 group. The C-O stretching frequencies of methoxy group appeared at 1352 cm^{-1} as a strong band.

The UV-Vis. spectrum of the DMBDPP is shown in Fig. 5. The absorption spectrum of the compound consists of intense bands centred at 336 and 370 nm which are assignable to n $\rightarrow \pi^*$ transitions of carbonyl and azomethine groups respectively (C=O and C=N). Another intense band at 270 nm in higher energy region of the spectrum of the compound is assigned to $\pi \rightarrow \pi^*$ transitions of aromatic rings.

The NMR spectrum of the compound is shown in Fig. 6. The ^1H NMR spectrum shows a signal at δ 10.06 ppm which is assigned to azomethine proton, -CH=N. The signal for benzene rings appears in the region δ 6.83–7.68 ppm. The signal at δ 3.80–3.84 ppm is assigned to methyl proton of two methoxy groups attached to benzylidenephenyl ring. The signals for C-CH_3 and N-CH_3 appear at δ 2.48 ppm and δ 3.13 ppm respectively. All the protons are found to be in their expected region.

There are 18 unique carbon atoms in the molecule, which give a total of 18 different peaks in the ^{13}C NMR spectrum. The peak at 160.92 ppm is assigned to C=O carbon C14. The peak at 154.05 ppm is assigned to azomethine >C=N carbon C9. The phenyl ring carbons are: C16 and C20, 124.26 ppm; C17 and C19, 129.11 ppm; C18, 124.24 ppm; C15, 134.99 ppm. The pyrazolone ring resonate at 35.97 ppm and 10.17 ppm represent N-CH_3 and C-CH_3 carbons. The signal at 56.38–55.81 ppm corresponds to methoxyl protons of the

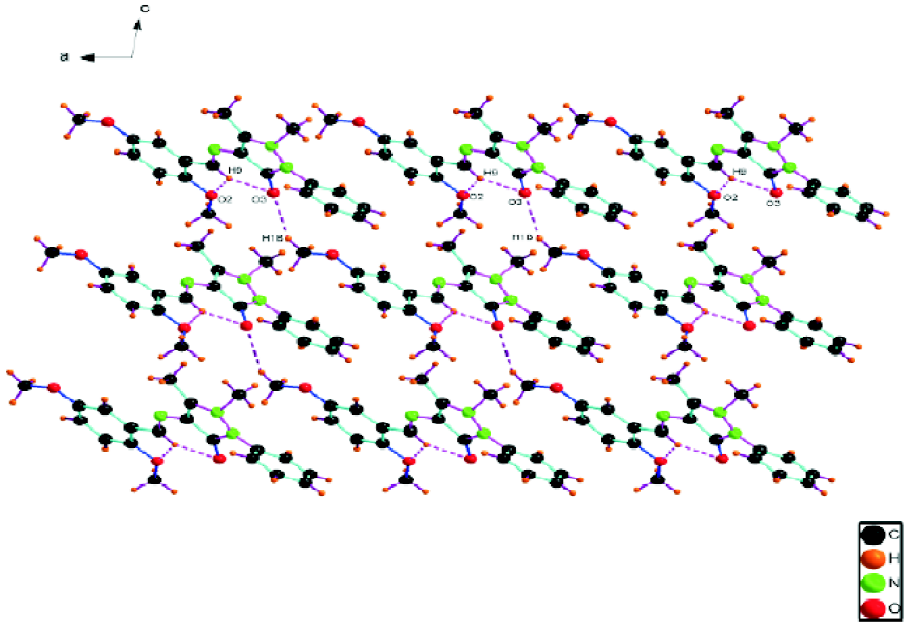


Fig. 3. Hydrogen bonding interactions of DMBDPP viewed along b-axis.

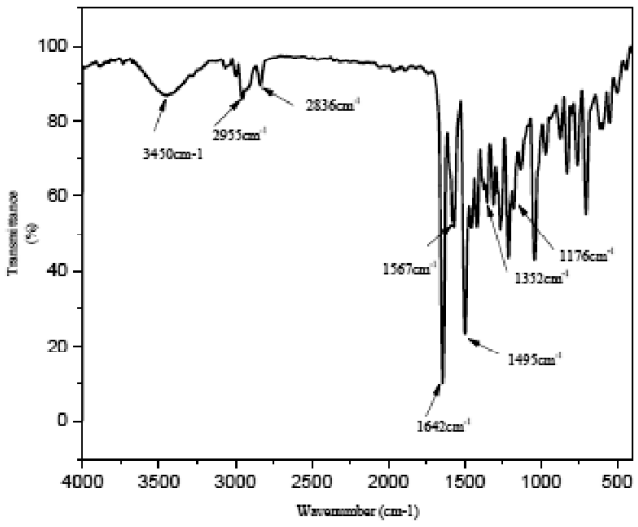


Fig. 4. FT-IR spectrum of DMBDPP.

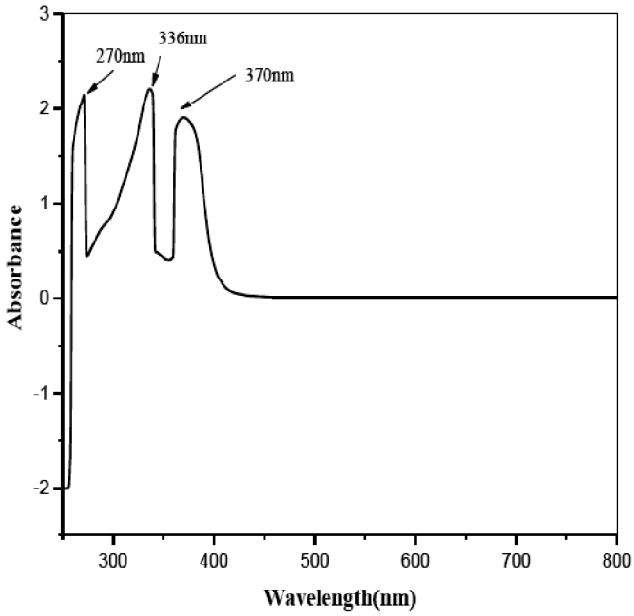


Fig. 5. UV-Visible spectrum of DMBDPP.

ligand. The ^{13}C spectra of the DMBDPP supports the molecular structure obtained from X-ray crystallography.

Biological evaluations

In vitro antibacterial study:

The *in vitro* antibacterial assay of the synthesized DMBDPP was investigated using Muller Hinton Agar plates through agar-well diffusion method[25]. The compound was screened against Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeroginosa*, and *Klebsiella pneumoniae* and Gram-positive bacteria *Streptococcus mutans* and *Staphylococcus aureus*. Streptomycin was used as a standard for the comparison of activity. The title compound in different concentrations was allowed to distribute out into the medium and interact in plate lately seeded with the test organisms. The antibacterial activity was examined by measuring the diameter of the inhibition zone formed around the wells were calculated for different concentrations; 25, 50 and 100 µg/

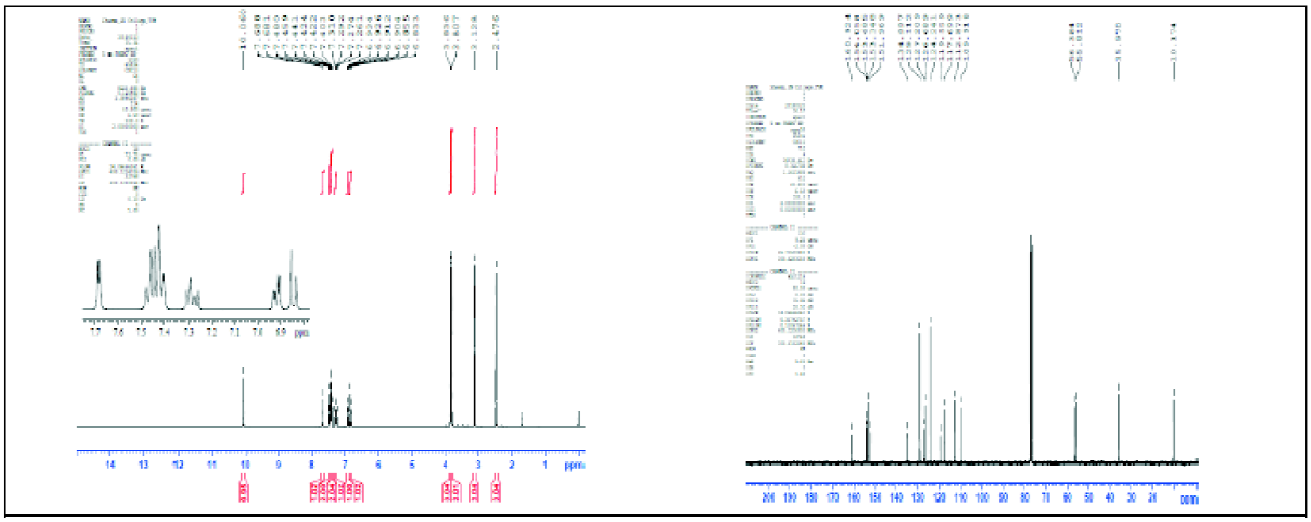


Fig. 6. ^{1}H and ^{13}C NMR spectrum of DMBDPP.

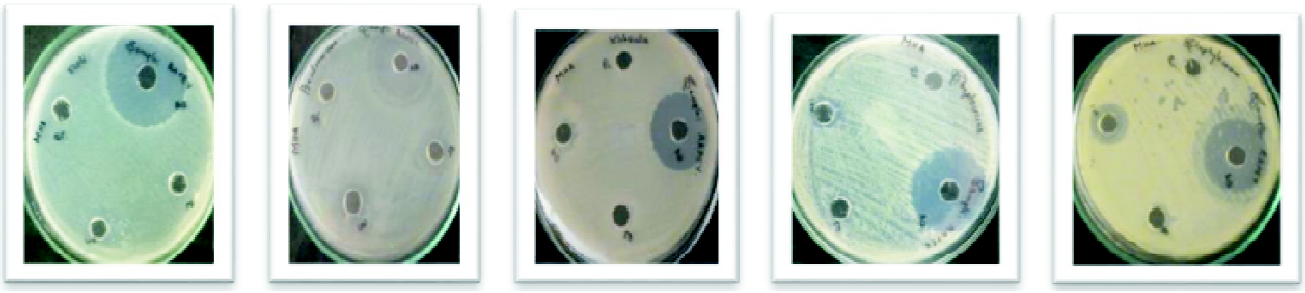


Fig. 7. Showing zone of inhibition against *E. coli*, *P. aeroginosa*, *K. pneumoniae*, *S. mutans* and *S. aureus*.

mL (Fig. 7). The diameter of zone of inhibition in mm is given in Table 4. Compared to the given concentration or 10 μg of Streptomycin, DMBDPP has no activity at lower concentrations of 25 μg/mL against Gram-negative bacteria. But at higher concentration of 100 μg/mL, DMBDPP shows good activity against *E. coli, P. aeroginosa* and *K. pneumonia*. DMBDPP shows moderate activity against Gram-positive bacteria at medium and higher concentration.

In vitro antifungal study:

The antifungal activity of DMBDPP was assessed against two fungal cultures *Candida albicans* and *Aspergillus niger* by Potato Dextrose agar plates using agar well diffusion method for three concentrations (25, 50, 100 μg/mL) using a standard Clotrimazole. Against both *Aspergillus niger* and *Candida albicans*, DMBDPP shows no activity at lower concentration of 25 μg/mL. But at higher concentration of 100 μg/mL, DMBDPP shows comparable activity against both the organisms (Table 5 and Fig. 7.1).

Table 5. Fungicidal screening data of DMBDPP

Fungal strains	Inhibition zone (mm)		
	Clotrimazole	Conc. (μg/mL)	DMBDPP
Aspergillus niger	14	25	–
		50	9
		100	10
Candida albicans	15	25	–
		50	11
		100	13

Conclusion

A Schiff base derivative of 4-aminoantipyrine, 4-(*N*-2,5-dimethoxybenzaldehydene)-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (DMBDPP) was synthesized and characterized it by elemental analysis, FT-IR, UV-Vis., ^{1}H and ^{13}C NMR spectral studies. The structure of DMBDPP was unambiguously determined by single crystal X-ray diffraction. The newly synthesized DMBDPP exhibits moderate antibacterial activity against *Escherichia coli*, *Pseudomonas aeroginosa*, *Klebsiella pneumoniae*, *Streptococcus mutans* and *Staphylococcus aureus* and moderate antifungal activity against *Aspergillus niger* and *Candida albicans*.

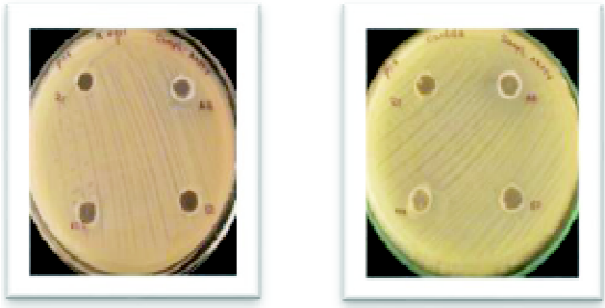


Fig. 7.1. Showing zone of inhibition against *A. niger* and *C. albicans*.

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Appendix A: Supplimentary material

CCDC-1900210 contains the supplementary crystallographic data for DMBDPP. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data-request/cif.

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