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Research Article

SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION, ANTIOXIDANT AND LIPOXYGENASE INHIBITION ACTIVITY OF N'-[(Z)-(1,5-DIMETHYL-3-OXO-2-PHENYL-2,3-DIHYDRO-1H-PYRAZOL-4-YL)METHYLIDENE]-2-HYDROXYBENZOHYDRAZIDE

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ABSTRACT

A new Schiff base, $C_{19}H_{18}N_4O_3$, **(3)** was prepared by condensation of *1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carbaldehyde* **(1)** and *2-hydroxybenzohydrazide* **(2)** in the presence of conc. H_2SO_4. Single crystal X-ray crystallographic and spectroscopic techniques including ^{1}H-NMR, IR, EI-MS along with elemental analysis were employed for characterization of synthesized Schiff base **(3)**. The said Schiff base **(3)** showed moderate antioxidant and lipoxygenase activities.

Keywords: Schiff base, Azomethine linkage, Single crystal X-ray crystallographic, Antioxidant activity, Lipoxygenase activity.

INTRODUCTION

The researchers are involved in developing new and novel structures which can be employed against pathogenic fungi and bacteria and such type of synthesis has got prime significance with the passage of time. As a number of pathogenic strains have become resistant to many of the drugs used in routine which has increased the need for the development of new templates to combat with the resistant strains[1]. Schiff bases are thought as promising compounds in this regard and Hugo Schiff was the first researcher to report these gleaming compounds[2]. Azomethine (-C=N-) functionality is the characteristic feature of Schiff base compounds. Literature is full of reports regarding application of such compounds as anti-bacterial, anti-fungal[3], anti-tubercular[4], analgesic[5], CNS depressant[6], anti-inflammatory[7], anti-convulsant[8], insecticidal[9], plant growth inhibitors[10], anti-viral and anti-cancer[11] agents. The prime significance of Schiff bases with respect to medicinal value has attracted many researchers towards the synthesis and evaluation of simpler Schiff bases or larger frame works with azomethine functionality for various diseases[12]. The biochemical and medicinal significance of Schiff bases has drawn our attention towards the synthesis of such compounds[13].

Herein we account the synthesis, characterization and biological evaluation of Schiff base **(3)**, synthesized from the condensation of *1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carbaldehyde* **(1)** and *2-hydroxybenzohydrazide* **(2)**, and also the synthesized Schiff base was investigated against different biological activities, such as antioxidant and lipoxygenase.

MATERIALS AND METHODS

Reagent grade solvents and chemicals were employed for all reactions which were used without any change upon receipt commercially from Merck. Washing of all the glassware during the reaction was accomplished by using distilled water and drying was carried out at 110 ^{o}C.

Physical measurements

The balance used for the weighing of compounds is known as electric Mettler Toledo balance and the model of balance was AL 204. The synthesized compound was monitored *via* Gallenkamp melting point apparatus for the measurement of melting point and uncorrected melting point was reported. For finding out elemental composition, Perkin-Elmer 2400 Series II elemental analyzer was used. In order to take IR spectra, Thermo Nicolet Avatar 320 FT-IR spectrometer within 400-4000 cm^{-1} range were used by employing KBr disc method. Pre-coated silica gel G-25-UV$_{254}$ plates (E-Merck) were utilized for checking the purity of Schiff base by TLC method. The solubility of compound was checked and dissolved in DMSO-d_6 for the measurement of ^{1}H-NMR spectra of compound on Bruker AMX-400 spectrometer. The values for chemical shift (δ) are given in ppm, while employing TMS as internal standard and the data of scalar coupling constants (*J*) is presented in Hertz.

Synthesis of Schiff base (3)

The Schiff base was synthesized by adding *1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carbaldehyde* **(1)** (0.01 mol in 50 ml ethanol) to *2-hydroxybenzohydrazide* **(2)** (0.01 mol in 50 ml ethanol) along with 3-4 drops of conc. H_2SO_4. The mixture was refluxed with stirring for 5 h at 70 °C on water bath. The volume of the resulting solution was reduced to one-third using a rotary evaporator and cooled on ice water bath after adding acetone. The reaction mixture was kept at room temperature overnight and white needle like crystals were obtained. These were filtered, washed with cooled methanol, dried and recrystallized from absolute methanol. The recrystallized product was dried over anhydrous calcium hydroxide under the reduced pressure. The reaction was examined by TLC with time to time till completion (Scheme 1)[13](c).

N'-[(Z)-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)methylidene]-2-hydroxybenzohydrazide (3)

The compound was obtained as white needle like crystals and 83 % yield. m.p: 219 ºC; IR (KBr, ν_{max} cm^{-1}): 3238 (-OH), 1637 (C=N), 1584 (C=O), 1531 (C=C), 1489 (-CH_3), 1334 (C-N), 856 (C-H); ^{1}H-NMR (DMSO-$_6$*d*, 400 MHz) δ: 14.32 (OH, s), 12.77 (NH, s), 7.85 (1H, dd, *J* = 7.2, 1.6 Hz, H-3''), 7.56-7.63 (3H, m, H-3', -4', -5'), 7.47 (2H, d, *J* = 7.2, H-2', -6'), 7.38 (H, br. t, *J* = 8.0, H-5''), 7.34 (1H, s, H-1'''), 6.89 (1H, d, *J* = 8.0, H-6''), 6.82 (1H, br. t, *J* = 8.0, H-4''), 3.34 (s, Me(1)), 2.11 (s, Me(5)); EI-MS *m/z* (%): 350.39 [M]$^+$; Elemental analysis for $C_{19}H_{18}N_4O_3$: Found (Calcd %); C: 65.17 (65.13), H: 5.20 (5.18), N: 15.89 (15.99).

Biological assays

Antioxidant: DPPH radical scavenging assay

The solution of DPPH (0.3 mM) was prepared in ethanol. 5 µL methanol solution of each sample of different concentration (5-500 µg) was mixed with 95 µL of DPPH solution in ethanol. The mixture was then dispersed in 96 well plate and incubated at 37 °C for 30 min, then absorbance was

measured at 515 nm by microtitre plate reader (Spectramax plus 384 Molecular Device, U.S.A.). BHA is used as standard. The percent radical scavenging activity was determined in comparison to the methanol treated control with the following formula:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Absorbance of control (DMSO treated) - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The IC_{50} value of the compounds was determined by monitoring the effect of different concentrations (1-1000 µM). The IC_{50} of the compounds were calculated using EZ-fit enzyme kinetic program (Pellera Scientific Inc. Amherst, U.S.A)[14].

Lipoxygenase inhibition assay

All the chemicals including linoleic acid and lipoxygenase (EC 1.13.11.12) purchased from Sigma (St. Louis, Missouri, USA). 160 µL of 100 mM sodium phosphate buffer (pH 8.0) and 10 µL of test compound solution in methanol (of concentrations 5-500 µM) was added in each well. 20 µL of lipoxygenase (LOX) solution (enzyme 130 units per well) was added, mixed and incubated for 10 min at 25 °C. The reaction was then initiated by the addition of 10 µL substrate solution (linoleic acid, 0.5 mM, 0.12 % w/v tween-20 in ratio of 1:2) in each well. The absorption changed with the formation of (9*Z*,11*E*)-13*S*)-13-hydroperoxyo-ctadeca-9,11-dienoate and was measured after 15 min at 234 nm. Baicalein was used as standard and IC_{50} values were determined by EZ-fit enzyme kinetic program (Pellera Scientific Inc. Amherst, U.S.A)[15].

RESULTS AND DISCUSSION

Chemistry and spectroscopic properties

1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carbaldehyde **(1)** has undergone a condensation reaction upon treatment with *2-hydroxybenzohydrazide* **(2)** as represented by scheme 1 which lead to the Schiff base (**3**). The sharp m.p. and stability of the compound in air reflected that it is almost pure.

Schiff base upon recrystallization yielded white coloured, needle shaped crystals. TLC was used for direct comparison of spots of reactants with that of reaction mixture in order to monitor the progress and success of reaction. The data obtained from elemental analysis of the compound reflected that there is a complete agreement between the data and structure of compound. IR, ^{1}H-NMR and single crystal x-ray crystallographic data[13](c) (Fig. 1 showing ORTEP of Schiff base) were used for further confirmation of the structure.

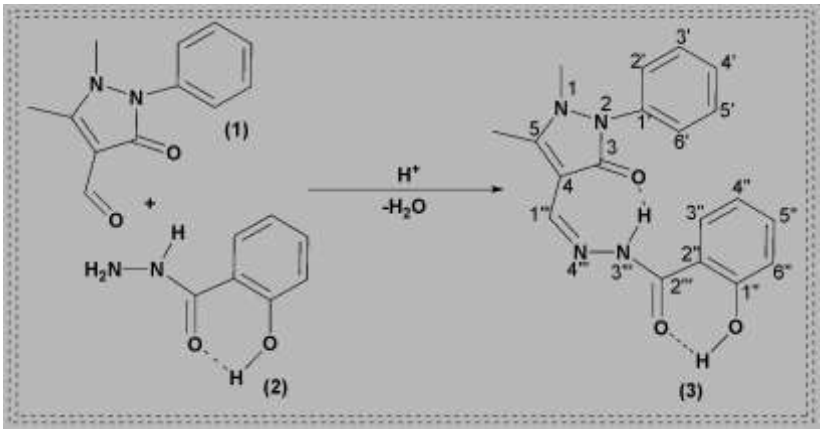
A band at 1637 cm^{-1} observed in IR spectrum of the Schiff base was assigned to the azomethine (C=N) functionality which was further strengthened by the disappearance of a band at 2800-2700 cm^{-1} due to aldehydic moiety of *1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carbaldehyde* **(1)**[16].

A clear evidence of azomethine linkage present in the synthesized compound was the appearance of a singlet at 7.34 ppm due to C-H of azomethine group in ^{1}H-NMR spectrum of Schiff base. The signals observed at 7.85-7.38 ppm and also at 7.38 and 6.82 ppm were attributed to aromatic protons. The relatively broader signal at 14.32 ppm was attributed to the OH group. The signals observed as singlets at 3.34 and 2.11 ppm were assigned to Me (1), Me (5) and due the presence of N-H a signal at 12.77 ppm was also observed.

EI-MS spectra showed molecular ion peak $[M]^+$ at *m/z* 350.39 for the Schiff base and was found consistent to the theoretical molecular weight *i.e.* 350.13. This fact was also supported by the data of elemental analysis.

Biological studies

Screening of the synthesized Schiff base **(3)** was conducted for antioxidant and lipoxygenase inhibition activities. Table-1 shows the data for these activities. The Schiff base **(3)** was found fairly active in both cases.



Scheme 1: Synthesis of Schiff base (3).

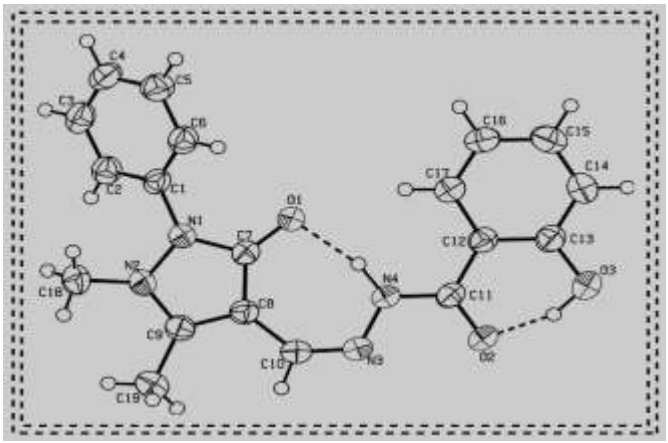


Fig. 1: ORTEP of Schiff base (3).

Table 1: IC_{50} (µM) values of the antioxidant and lipoxygenase inhibition assay.

Compound	DPPH scavenging activity IC_{50} (µM)	Lipoxygenase inhibition activity IC_{50} (µM)
3	91.3	60.7
BHA	44.2	-
Baicalein	-	22.6

Butylated hydroxyanisole (BHA, for DPPH scavenging activity) and Baicalein (for lipoxygenase inhibition activity) were used as positive controls and each bioassay was performed in triplicate.

CONCLUSION

The Schiff base **(3)** was found to have moderate antioxidant and lipoxygenase activities.

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