

Synthesis, X-Ray Powder Diffraction Studies and Antimicrobial Activities of Novel Schiff Base Derivatives

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Abstract: Schiff bases are an important class of organic compounds, which play an important role in medicinal and pharmaceutical applications. The synthesized compounds, 1-(4-butoxy-2-hydroxyphenyl) ethylidene- benzohydrazide (I), 1-(4-butoxy-2-hydroxy phenyl) ethylidene-4-chloro benzohydrazide (II) and 1-(4-butoxy-2-hydroxy phenyl) ethylidene-4-methyl benzohydrazide (III) have been characterized by FTIR, ¹H and ¹³C NMR. The structural characterizations of synthesized compounds (I, II and III) have been estimated by analyzing powder X-ray diffraction technique. It is observed that samples I and III have crystallized in triclinic system with P $\bar{1}$ space group and sample II in monoclinic system with P2₁/n space group. The synthesized compounds have been screened for its antimicrobial and antifungal activities against different panel of organisms.

Keywords: Schiff base, Powder x-ray diffraction, Antimicrobial, Antifungal activities

Introduction

Schiff bases are condensation products of primary amines and carbonyl compounds and they discovered by a German chemist, Nobel Prize winner, Hugo Schiff in 1864. Structurally, Schiff base (also known as imine or azomethine) is an analogue of a ketone or aldehyde in which the carbonyl group (C=O) has been replaced by an imine or azomethine group. Schiff base ligands are essential in the field of coordination chemistry, especially in the development of complexes of Schiff bases because these compounds are potentially capable of forming stable complexes with metal ions¹. The individual Schiff bases are considered to be promising antifungal medicines². Isatin Schiff base ligands are marked by antiviral activity, and this fact is very useful in the treatment of HIV³. In addition, it is also found that these compounds have anticonvulsant activity and may be included in the anti-epileptic drugs⁴. Due to the presence of the imine group, the electron cloud of the aromatic ring and electronegative nitrogen, oxygen and sulfur atoms in the Schiff bases molecules, these compounds effectively prevent corrosion of mild steel, copper, aluminum and zinc in acidic

medium⁵. Schiff bases and Mannich bases of isatin are known to possess a wide range of pharmacological properties including antibacterial, anticonvulsant, antifungal and antiviral activities⁶⁻⁹. Schiff's bases are also important compounds owing to their wide range of industrial applications¹⁰. As a part of our ongoing research on syntheses of novel bio-organic molecules and their biological studies¹¹⁻¹³, here we report the synthesis of 1-(2-hydroxy-4-butoxyphenyl) ethanone with benzohydrazide, chloro-benzohydrazide and methyl-benzohydrazide using schiff base method, with the characterizations by IR, ¹H and ¹³C NMR. We also included powder X-ray diffraction study to investigate crystallographic information of the compound to find out the exact position of the different substituents. To understand pharmacological applications on structural basis, we evaluated antimicrobial and antifungal activities of the synthesized products against different panel of organisms.

A large number of different synthetic methods for the preparation of schiff base have been described in the literature¹⁴⁻¹⁵, so considerable attention has been given to the development and synthesis of schiff base for obtaining better biological potent molecules, which have been prepared by the reaction of ketone and different substituted hydrazide in dry benzene. A schiff base has nitrogen analog of an aldehyde or ketone in which the C=O group is replaced by a C=N-R group. It is usually formed by condensation of an aldehyde or ketone with a primary amine.

Experimental

All chemicals and reagents were commercially available from Sigma Aldrich, Merck and were used without further purification. Glassware was oven dried for several hours. General Melting points were determined on an electro thermal melting point apparatus. Completion of reaction and purity of all compounds were checked on aluminum coated TLC plates 60 F245 (E. Merck) using *n*-hexane: ethyl acetate (7.5:2.5, v/v) as mobile phase and visualized under ultraviolet (UV) light, or iodine vapor. Elemental analysis (% C, H, N) was carried out by a Perkin-Elmer 2400 CHN analyzer. IR spectra of all the compounds were recorded on a Perkin-Elmer FT-IR spectrophotometer in KBr. ¹H NMR spectra were recorded on a Varian Gemini 300 MHz and ¹³C NMR spectra on a Varian Mercury-400 (100 MHz) in DMSO-d₆ as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts were reported in parts per million (ppm). Mass spectrum was scanned on a Shimadzu LCMS 2010 spectrophotometer.

Synthesis

Preparation of 1-(4-butoxy-2-hydroxyphenyl) ethylidene-benzohydrazide (I)

1-(2-Hydroxy-4-butoxyphenyl) ethanone (0.208 g, 0.01 mole) and benzohydrazide (0.150 g, 0.01 mole) were dissolved in dry benzene (50 mL). The solution was refluxed for 20-24 h. Completion of reaction was checked by TLC and benzene was distilled out under pressure. The crude mass was poured in water. The resulting solid was filtered and washed with petroleum ether. The final product was crystallized from ethanol as dark yellow needles.

1-(4-Butoxy-2-hydroxyphenyl)ethylidene-4-chlorobenzohydrazide (II)

1-(2-Hydroxy-4-butoxyphenyl) ethanone (0.01 mole) and 4-chlorobenzohydrazide (0.01 mole) were dissolved in dry benzene (50 mL). The solution was refluxed gently for 20-24 h using refluxed apparatus and water has been removed. The completion of reaction was checked by TLC and benzene was distilled out under pressure. The crude mass was poured in water. The resulting solid obtained was filtered and washed with petroleum ether. The final product was crystallized from ethanol as dark yellow needles.

1-(4-Butoxy-2-hydroxyphenyl) ethylidene-4-methylbenzohydrazide (III)

1-(2-Hydroxy-4-butoxyphenyl) ethanone (0.208 g, 0.01 mole) and 4-methylbenzohydrazide (0.150 g, 0.01 mole) were dissolved in dry benzene (50 mL). The solution was refluxed for 20-24 h and water has been removed. The completion of reaction was checked by TLC and benzene was distilled out under pressure. The crude mass was poured in water. The resulting solid obtained was filtered and washed with petroleum ether. The final product was crystallized from ethanol as dark yellow needles.

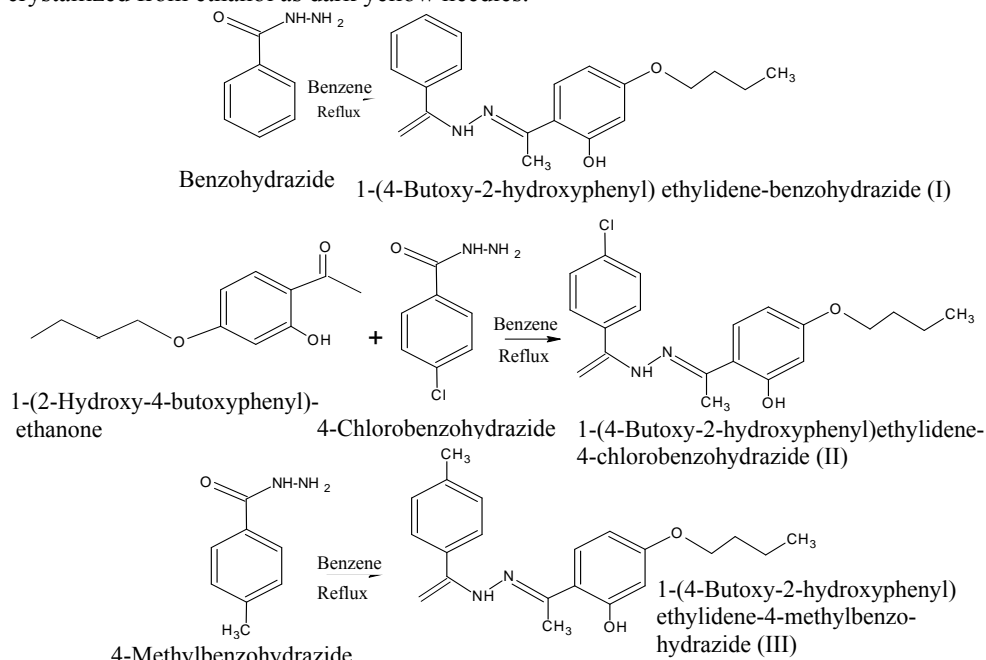


Figure 1. Reaction scheme of the synthesized compounds (I, II and III)

Characterization of compounds*1-(4-Butoxy-2-hydroxyphenyl) ethylidene-benzohydrazide (I)*

IR (cm^{-1}): 3268(Ar -OH, Phenol), 1178(C-O, Phenol), 1274 [C-O-C, (sym) Ether], 1110 [C-O-C (asym.) Ether], 1660 (C=O str. Schiff base), 1559, (N=C str. Schiff base), 3210(N-H str., Schiff base)

¹H NMR (CDCl₃) δ ppm

0.929-0.916, (3H, triplet, -CH₂-CH₃), 1.424-1.528, (2H, sextet, -CH₂-CH₃), 1.68-1.804, (2H, quintet, -CH₂-CH₂-CH₃), 2.482, (3H, singlet, N=C-CH₃), 3.985-3.993, (2H, triplet, -OCH₂), 6.442-6.458, (2H, dd, Ar-H, J = 1.2 Hz.) 7.531-7.911, (6H, complex, Ar -H), 11.180, (1H, singlet, -NH), 13.61, (1H, singlet, -OH)

¹³C NMR (CDCl₃) δ ppm

161.484(C-1), 14.05(C-2), 167.668 (C-3), 66.809 (C-4), 160.944 (C-5), 106.402(C-6), 129.932 (C-7), 105.124 (C-8), 155.855(C-9), 102.424 (C-10), 67.653(C-11), 31.113(C-12), 19.154 (C-13), 14.132(C-14), 128.457(C-15), 112.301 (C-17,19), 121.606 (C-18), 151.925 (C-16,20). Yield: 69%, m.p.: 175°C, R_f Value: 0.37, [Solvent system- Toluene: Ethyl acetate (10 : 1)], Analysis: C₁₉H₂₂N₂O₃, Found: C-69.94%, H-6.80%, N-8.56%

1-(4-Butoxy-2-hydroxyphenyl)ethylidene-4-chlorobenzohydrazide (II)

IR(cm^{-1}): 3310(Ar -OH, Phenol), 1182(C-O, Phenol), 1265[C-O-C, (sym)Ether], 1134[C-O-C (asym.) Ether], 1670(C=O str. Schiff base), 1590(N=C str. Schiff base), 3354(N-H str., Schiff base).

 $^1\text{H NMR}$ (CDCl_3) δ ppm

0.901-0.937, (3H, triplet, -CH₂-CH₃), 1.390-1.444, (2H, sextet, -CH₂-CH₃), 1.812-1.829, (2H, quintet, -CH₂-CH₂-CH₃), 2.426, (3H, singlet, N=C-CH₃), 3.960-3.990, (2H, triplet, -OCH₂), 6.435-6.471, (2H, dd, Ar-H, J= 1.2 Hz.), 7.511-7.533, (1H, doublet, Ar-H) 7.593-7.957, (4H, complex, Ar-H), 8.142, (1H, singlet, -NH), 13.558, (1H, singlet, -OH)

 $^{13}\text{C NMR}$ (CDCl_3) δ ppm

161.441(C-1), 14.15(C-2), 166.781 (C-3), 66.912 (C-4), 161.865 (C-5), 106.454(C-6), 128.971 (C-7), 105.453 (C-8), 155.768(C-9), 102.532 (C-10), 68.124(C-11), 31.091(C-12), 19.232 (C-13), 14.438(C-14), 127.897(C-15), 112.342 (C-17,19), 120.789 (C-18), 153.224 (C-16,20). Yield: 67%, m.p.:218 °C, R_f Value: 0.36, [Solvent system- Toluene: Ethyl acetate (10:1)], Analysis: C₁₉H₂₁ClN₂O₃, Found: C-63.25%, H-5.90%, N-7.73%, X-9.85%.

1-(4-Butoxy-2-hydroxyphenyl) ethylidene-4-methylbenzohydrazide (III)

IR (cm^{-1}): 3271 (Ar -OH, Phenol), 1180(C-O, Phenol), 1270 [C-O-C, (sym)Ether], 1121 [C-O-C (asym.) Ether], 1681(C=O str. Schiff base), 1567 (N=C str. Schiff base), 3242(N-H str., Schiff base)

 $^1\text{H NMR}$ (CDCl_3) δ ppm

0.901-0.938, (3H, triplet, -CH₂-CH₃), 1.392-1.559, (2H, sextet, -CH₂-CH₃), 1.658-1.827, (2H, quintet, -CH₂-CH₂-CH₃), 2.335, (3H, singlet, -CH₃), 2.418 (3H, singlet, N=C-CH₃), 3.957-3.987, (2H, triplet, -OCH₂), 6.432-6.444, (2H, dd, Ar-H, J= 1.2 Hz.), 6.467, (1H, doublet, Ar-H) 7.322-7.340, (2H, doublet, Ar -H), 7.823-7.841, (2H, doublet, Ar -H) 8.413, (1H, singlet, -NH), 13.662, (1H, singlet, -OH)

 $^{13}\text{C NMR}$ (CDCl_3) δ ppm

161.264(C-1), 14.134(C-2), 164.415 (C-3), 67.687 (C-4), 161.678 (C-5), 106.458(C-6), 128.483 (C-7), 106.458 (C-8), 158.934(C-9), 102.463 (C-10), 68.321(C-11), 31.122(C-12), 19.166 (C-13), 14.430(C-14), 129.370(C-15), 113.068 (C-17,19), 128.064 (C-18), 130.651 (C-16,20). Yield: 54%, m.p.:171°C, R_f Value: 0.35, [Solvent system: Toluene: Ethyl acetate (10: 1)], Analysis: C₂₀H₂₄N₂O₃, Found: C-70.54%, H-7.12%, O-14.10%, N-8.24%

Results and Discussion*Spectroscopic analysis*

The infrared spectrum has been recorded using the KBr pellets in the spectral range 4000-400 cm^{-1} . The IR spectra of title compounds I, II and III have been displayed absorption band at 1559, 1590 and 1567 cm^{-1} respectively, which shown characteristics of the -N=C str. band. -NH stretching band at 3210 cm^{-1} is observed and carbonyl group of Schiff base showed characteristic absorption band at 1660 cm^{-1} . The strong broad band observed at 3268 cm^{-1} recognized the Ar -OH and the band due to C-O stretching appeared at 1178 cm^{-1} . Due to ether linkage (C-O-C) two bands appeared in the range of 1275-1200 cm^{-1} (symmetric) and 1019-1020 cm^{-1} (asymmetric) as stretching band. The aromatic in plane bending was observed at 1117 cm^{-1} . The aromatic out of plane bending was observed at 848 cm^{-1} . Multi substituted benzene ring gives out of plane deformation at 900-860 cm^{-1} range. At 682 cm^{-1} band has been observed in compound II which shown that the halogen (Cl) present in the structure.

$^1\text{H NMR}$ Spectrum of the title compounds (I, II and III) exhibited as singlet of N=C-CH₃ group at 2.482 δ ppm and the singlet appeared at 13.61 δ ppm indicated the presence of OH group. The proton of NH shows as singlet at 7.911 δ ppm. In butoxy group, the Sextet

of $-\text{OCH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_3$, appeared at 1.42-1.52 δ ppm, the triplet of $-\text{CH}_2\text{-CH}_3$ observed at 0.929-0.916 δ ppm, the quintet of $-\text{OCH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_3$ and the triplet of $-\text{OCH}_2\text{-CH}_2\text{-}$ observed at 1.684 δ ppm and 3.985 δ ppm respectively. The aromatic region of the spectrum exhibited two protons of the ketonic nucleus as double doublet in the range of 6.442-6.458 ($J = 1.2$ Hz.) δ ppm, while protons of the benzohydrazide nucleus showed as complex pattern integrating five protons in the range of 7.531-7.723 δ ppm.

In ^{13}C NMR spectra of the title compounds (I, II and III), carbons of the Schiff group were resonated at 161.484 δ ppm (C-1) and 14.045 δ ppm (C-2). The carbon of carbonyl group (N-C=O) resonated at 167.668 δ ppm (C-3). The carbons of $-\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ group showed signals at 14.132 δ ppm (C-14), 19.154 δ ppm (C-13), 31.113 δ ppm (C-12), and 67.653 δ ppm (C-11). The carbon of $-\text{CO-CH}_2$ group resonated at 66.809 δ ppm (C-4). The aromatic carbon showed signals at 102.424 (C-10), 105.124 (C-8), 106.402 (C-6), 112.301 (C-17), 113.533 (C-20), 121.606 (C-19), 128.457 (C-15), 129.932 (C-7), 148.118 (C-18), 155.849 (C-9), 160.944 (C-5) and 151.925 (C-16, C-18) δ ppm.

Powder x-ray diffraction study

The crystalline phase of all the compounds are identified at room temperature using Powder x-ray diffraction which routinely has been used as a non-destructive fingerprinting technique in laboratory and industry for several decades. Every solid crystalline compound gives its own unique x-ray diffraction pattern consisting of a set of Bragg peaks. The diffraction pattern for a compound can be considered analogous to a fingerprint, or barcode, with the peak positions determined by the unit cell symmetry and lattice parameters. When we collect XRD data, we can use these fingerprints to identify not only what phases are present in our sample but also index the pattern to obtain information about the unit cell size and shape. The experimental 2θ range is $2\text{--}120^\circ$ with a step size of 0.01° and a counting time of 60s per step. The program of graphic tool for powder diffraction named Win-PLOTTR package¹⁶ was used to determine the observed diffraction peak positions of the title compound. Analytical indexing of the powder pattern and determination of the space group were performed using EXPO 2009¹⁷, which is designed to launch the most common indexing programs. The powder diffraction patterns for all the title compounds (I, II and III) are presented in the Figure 2. The lattice parameters of the all the compounds are calculated and summarized in Table 1.

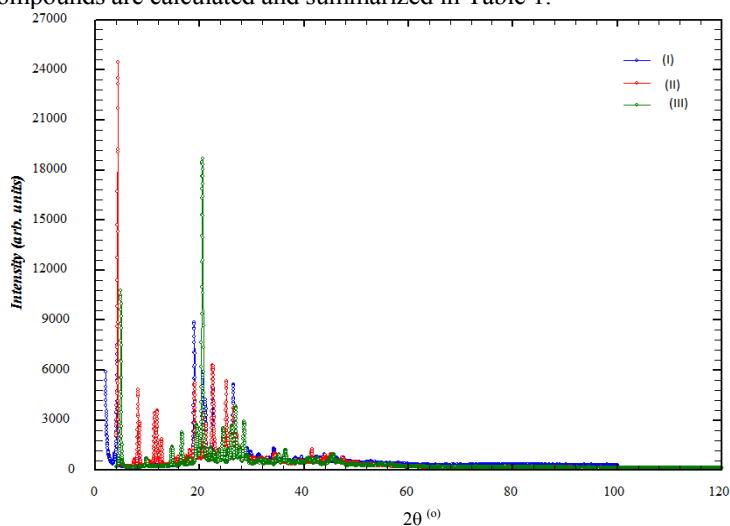


Figure 2. The powder diffraction patterns for the title compounds (I, II and III)

Table 1. The lattice parameters of the compounds

R-group	4-H (I)	4-H (II)	4-H (III)
Molecular Weight	C ₁₉ H ₂₂ N ₂ O ₃	C ₁₉ H ₂₁ N ₂ O ₃ Cl	C ₂₀ H ₂₄ N ₂ O ₃
Lattice Parameters			
System	Triclinic	Triclinic	Monoclinic
a(Å)	18.2954	21.7945	29.1853
b(Å)	5.4862	10.9915	10.2414
c(Å)	5.1188	5.2139	22.2292
α (°)	100.1732	103.5663	90.00
β (°)	92.0484	95.9758	107.2078
γ (°)	97.7921	97.1843	90.00
Space Group	Pī	Pī	P2 ₁ /n

Antimicrobial activities

Synthesized title compound has been screened for their antimicrobial activity against different panel of organisms, *i.e.*, *E.coli*, *P.aeruginosa*, *S.aureus*, *S.pyogenes* and antifungal strains *C. albicans*., using *Gentamycin* and *K. Nystatin* as reference standards respectively. The Serial dilution technique was followed by micro method as per NCCLS-1992 manual 21. The observed Minimum Inhibitory Concentrations (MIC) values, for bacterial and fungal strains of the title compound are presented in Table 2. The results revealed that they showed varying degree of inhibition against the tested microorganisms. The MIC values suggest that the tested compound exhibited moderate antibacterial activity against gram negative bacterial strain *E.coli* and gram positive bacterial strain *S. aureus* comparable to reference agent *Gentamycin*.

Table 2. Minimal Inhibition Concentrations of Bacterial Strains (MIC) in µg/mL

S. No.	-R	Bacterial activity				Fungal activity	
		Minimal Inhibition Concentrations (MIC) in µg/mL				Minimal Inhibition Concentration (MIC) in µg/mL	
		<i>S.aureus</i> MTCC	<i>S.pyogenus</i> MTCC	<i>E. coli</i> MTCC	<i>P.aeruginosa</i> MTCC	<i>C.albicans</i> MTCC	<i>A.niger</i> MTCC
		96	443	442	441	227	282
1.	4-H (I)	250	500	250	500	1000	200
2.	4-Cl (II)	12.5	500	12.5	500	100	>1000
3.	4-CH ₃ (III)	100	250	100	150	1000	500
Std.	<i>Gentamycin</i>	0.25	0.50	0.05	1.0	-	-
Drug	<i>K.Nystatin</i>	-	-	-	-	100	100

Conclusion

A series of novel derivatives, 1-(4-butoxy-2-hydroxyphenyl) ethylidene- benzohydrazide (I), 1-(4-butoxy-2-hydroxy phenyl) ethylidene-4-chloro benzohydrazide (II) and 1-(4-butoxy-2-hydroxy phenyl) ethylidene-4-methyl benzohydrazide (III) have been synthesized by Schiff base method. The spectroscopic characterizations have been confirmed by the chemical analysis, IR, 1H NMR and 13C NMR. Powder X-ray diffraction graph shows that the synthesized compounds have been crystalline in nature and it belongs to triclinic and monoclinic systems. From the results of biological study, it is clear that the chlorine containing schiff base derivative exhibit more degree of inhibition against specific bacteria.

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