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Pharmacological evaluation of ONNO donor quadridentate Schiff bases

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ABSTRACT

Four ONNO donor Schiff bases 2-((*E*)-(2-((*E*)-2-hydroxybenzylideneamino)ethylimino) methyl)phenol (H_2L_1), 2-((*E*)-(2-((*E*)-2-hydroxybenzylideneamino)propylimino)methyl) phenol (H_2L_2), 2-((*E*)-1-(2-((*E*)-1-(2-hydroxyphenyl) ethylideneamino)ethylimino)ethyl) phenol (H_2L_3) and 2-((*E*)-1-(2-((*E*)-1-(2-hydroxyphenyl)ethylideneamino)propylimino) ethyl)phenol (H_2L_4) were synthesized by the reactions of ethylene/propylene diamines with 2-hydroxy benzaldehyde/2-hydroxy acetophenone. The new compounds were characterized by FT-IR and NMR (^{1}H and ^{13}C) spectroscopic techniques accompanied by elemental, GC/MS and single crystal X-ray diffraction analyses. These compounds were screened for various biological studies i.e. brine shrimp cytotoxic, antitumor and antibacterial activities The compound H_2L_3 showed highest cytotoxic and antitumor activities with lowest LD_{50} (14.27) and IC_{50} values (18.90). All the compounds were highly active in protecting DNA against hydroxyl free radicals. Antibacterial studies had shown that these were inactive against Gram positive bacteria (*Staphylococcus aureus* and *Micrococcus luteus)* while active against Gram negative bacteria (*Enterobacter aerogenes*, *Bordetella bronchiseptica* and *Salmonella typhi)* showing variable antibacterial activity.

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1. Introduction

Schiff bases are the class of organic compounds which have azomethine moiety (-C=N). This linkage is a precursor for various biological activities and present in various natural (ancistrocladidine having antimalarial activity) and natural-derived compounds (chitosan derived having antifungal activity). The synthesized Schiff bases possess substantial anticancer, antibacterial, antifungal, antiviral, antioxidant and anti-parasitic activities [1-3]. The azomethine nitrogen may be involved in the formation of hydrogen bonds with the active sites of cell components and affects normal cell processes. The presence of imine group (-C=N) is responsible for elucidating the mechanism of transformation and racemization reaction in biological systems. Schiff bases derived from 2-hydroxy benzaldehyde/2-hydroxy acetophenone and primary amines have recently acquired a considerable importance due to their promising biological properties thus establishing themselves as versatile pharmacophore. These may act as bidentate, tridentate and quadridentate ligands which can be employed for the synthesis of various coordination complexes [4].

Apart from biological activities, Schiff bases have found enormous applications in other fields such as intermediates in organic synthesis, dyes, pigments, polymer stabilizers, corrosion inhibitors, fungicidal, agrochemical, analytical chemistry, electrical conductivity, magnetism, host guest chemistry, ion exchange, nonlinear optics and catalysis [5,6]. They have played an important role in the development of coordination chemistry and inorganic biochemistry as well. These compounds have been used for the synthesis of large number of biologically and industrially active compounds like formazans, 4-thiazolidinines, benzoxazines and so forth via ring closure, cycloaddition and replacement reactions [7,8]. Azomethines and *ortho* hydroxyl groups present in Schiff bases are bioactive moieties which are found to possess immense potential for design and development of various drugs [9]. Such Schiff bases have also been used as drugs as bactericidal, fungicidal, anti-tubercular, antiviral agents and have been extensively studied due to their wide range of applications in medicinal field [10].

Recently our research group has reported the synthesis, characterization and biological activities of novel ON donor bidentate Schiff bases [11]. Exhilarated by these reports, we have synthesised ONNO quadridentate Schiff bases (**H_2L_1-H_2L_4**) derived from 2-hydroxy benzaldehyde/2-hydroxy acetophenone and characterized by various physico-analytical techniques.

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Various biological studies like cytotoxic, antitumor and inhibition of hydroxyl (OH) free radical induced DNA damage assays were carried out to evaluate their future prospects in drug design and discovery.

2. Experimental

2.1. Materials and methods

2-Hydroxy benzaldehyde, 2-hydroxy acetophenone, ethylene diamine and propylene diamine were acquired from Sigma Aldrich Chemie GmbH (Schnelldorf, Germany). Ethanol was procured from Merck (Darmstadt, Germany) and was dried before use following standard drying method [12].

Elemental analysis was carried out on a CHNS 932 (Leco-USA) elemental analyser. Melting points were determined, using a MPD Mitamura Riken Kogyo (Japan) electrothermal melting point apparatus. FT-IR spectra were recorded on a Thermoscientific (USA) Nicolet 6700 spectrometer in the frequency range of 4000-400 cm^{-1}. ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker NMR Spectrometer.

Single crystal of Schiff base (H_2L_4) was obtained by the slow evaporation of ethanol from the mother liquor at room temperature. X-ray data were collected at 173(2) K on a STOE IPDS-II two-circle diffractometer using MoKα radiation (λ = 0.71073 Å). The structure was solved by direct methods and refined on F^2 using all the reflections [13]. All the non-hydrogen atoms were refined using anisotropic atomic displacement parameters and hydrogen atoms were inserted at calculated positions using a riding model. Parameters for data collection and refinement are summarised in Table 1.

Table 1. Crystal data and structure refinement for $\mathbf{H_2L_4}$.

Empirical formula	$C_{19}H_{23}N_2O_{2.50}$
Formula weight	319.39
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	C2/c
Unit cell dimensions	a = 21.8783 (14) Å b = 7.3112 (4) Å c = 22.1486 (14) Å β = 103.612 (5)°
Volume	3443.3 (4) $Å^3$
Z	8
Density (calculated)	1.232 Mg/m^3
Absorption coefficient	0.082 mm^{-1}
F(000)	1368
Crystal size	0.34 × 0.32 × 0.32 mm^3
Crystal description	Yellow
Theta range for data collection	3.38 to 26.41 °
Index ranges	$-27 \le h \le 27$ $-8 \le k \le 9$ $-27 \le l \le 27$
Reflections collected	19557
Independent reflections	3513 [R(int) = 0.0668]
Completeness to theta = 25.00 °	99.8%
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3513 / 0 / 229
Goodness-of-fit on F^2	1.066
Final R indices [I>2sigma (I)]	R_1 = 0.0382, wR_2 = 0.1014
R indices (all data)	R_1 = 0.0492, wR_2 = 0.1062
Extinction coefficient	0.0046(6)
Largest diff. peak and hole	0.275 and -0.255 e.$Å^{-3}$

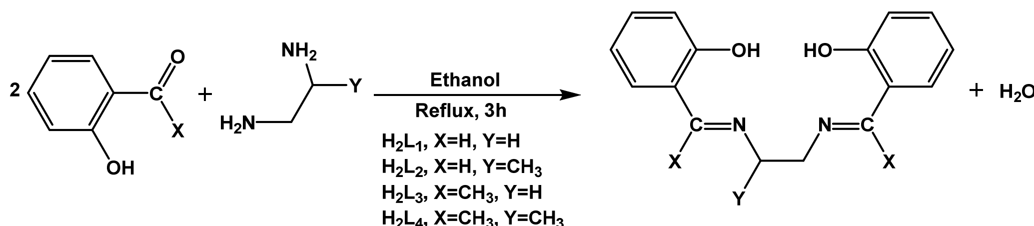
The biological activity of the Schiff bases was assessed by brine shrimp lethality assay [14]. Brine shrimp (*Artemia salina*) eggs were hatched in 48 h using seawater (34 g/L) under constant aeration at 25 °C. After a day phototropic *nauplii* (brine shrimp larvae) were shifted to glass vial by Pasteur pipette and 25 µL of the each stock solution (0.1, 1.0, and 10.0 µg/mL) of the test compound was added. The volume of test compounds from their stock solutions was raised up to 5 mL of artificial seawater with 10, 1, 0.5, 0.25, 0.125 and 0.0625 µg/mL final concentrations. Three replicates were prepared for each concentration. The vials were maintained under illumination at room temperature. After 24 h of incubation survivors were observed and percentage mortality was determined by formula [Percentage mortality = (Number of alive shrimps) control-sample ×100 / (Number of alive shrimps) control]. Then LD_{50} (Lethal Dose that killed 50% of shrimps) was calculated by using Finny (1971) software [15].

Antitumor activity of the synthesized compounds was checked by executing modified potato disc antitumor assay [16]. Inoculum with three different concentrations (1000, 100 and 10 µg/mL) was prepared containing 48 h bacterial culture of *Agrobacterium tumefaciens* (At 10). Red-skinned potatoes were surface sterilized in 0.1% $HgCl_2$ solution and potato discs of size 8 × 4 mm were prepared with the help of sterilized cork borer. Ten discs were placed on the agar plates along with 50 µL of inoculum on the surface of each disc. After 21 days of incubation at 28 °C, discs were stained with Lugol solution (10% KI and 5% I_2) and tumors were counted on each disc. The tumor inhibition was calculated by formula, Percentage inhibition = 100 - average number of tumors of sample/ average number of tumors of negative control × 100). IC_{50} was calculated for describing the results in more compiled form.

Antioxidant or prooxidant activity of the synthesized compounds was assessed by DNA damage assay [17]. Plasmid DNA (pBR322 Fermentas) with a concentration of 0.5 µg/3 µL was treated with three different concentrations of test samples (1000, 100 and 10 ppm). Samples were prepared by dissolving 1 mg of the test compound in 1000 µL of methanol or acetone depending upon their solubility. Concentration of this stock solution was 1000 ppm. From this stock solution further dilutions (100 and 10 ppm) were prepared Fenton reaction was induced by addition of 30% H_2O_2 (4 µL) and 2 mM $FeSO_4$ (3 µL) into the reaction mixture. Three controls, untreated pBR322 DNA as negative, DNA treated with compound (C+P), DNA treated with 2 mM $FeSO_4$ and 30% H_2O_2 as positive control were run simultaneously. Each reaction mixture was incubated at 37 °C for an hour. After incubation, the sample was loaded on a 0.9% agarose gel and was visualized with Doc-IT (VWR). Estimation of antioxidant or pro-oxidant effects on DNA was estimated on the basis of percentage increase or loss of a super-coiled monomer, compared with the control.

Agar well diffusion method was applied to check the antibacterial activity of the newly synthesized Schiff bases [18]. Five bacterial strains including two Gram positive *Staphylococcus aureus*, *Micrococcus luteus* and three Gram negative, *Salmonella typhi*, *Enterobacter aerogenes* and *Bordetella bronchiseptica* were used. To grow the bacterial strains, nutrient broth (Merck) was used as medium. Nutrient broth is composed of peptone from meat (5 g/L) and meat extract (3 g/L). Nutrient broth medium was prepared by dissolving 0.8 g/100 mL in distilled water at neutral pH and then it was autoclaved. The pure glycerol culture of each of the bacterial strain was used. The pure bacterial culture was transferred to nutrient broth medium in LFH to avoid the contamination. The culture was then incubated at 37 °C for 24 h. McFarland standards are used as references to adjust the turbidity of bacterial suspensions. A 0.5 M McFarland standard was prepared by adding 0.5 mL of 0.048 M $BaCl_2$ to 99.5 mL 0.36 N H_2SO_4. All samples (compounds) were assayed by using DMSO (negative control) and Roxithromycin, Cefixime-USP (positive controls) having 1 mg/mL concentration. After 24 h incubation of samples and controls in agar medium, diameter of the clear inhibitory zones was measured with the help of Vernier calliper in three replicates. Then, the MIC for active samples was determined.

MIC is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. Compounds showing good visible growth inhibition at 1 mg/mL concentration were then processsed for MIC determination.



Scheme 1

For MIC determination active samples were diluted to lower concentrations i.e. 0.8, 0.6, 0.4, 0.2, 0.1, 0.08, 0.06 and 0.04 mg/mL and the same agar well diffusion method was repeated. The minimal concentration, above which no inhibition observed, was considered as their MIC.

2.2. Synthesis of ONNO donor Schiff bases (H_2L_1-H_2L_4)

The Schiff bases (**H_2L_1-H_2L_4**) were prepared by condensation reactions of 2-hydroxy benzaldehyde/2-hydroxy acetophenone and ethylene/propylene diamine (Scheme 1). The synthesis of **H_2L_1** is presented as an example to illustrate the general synthetic procedure: a 250 mL two necked round bottom flask equipped with magnetic stirrer and reflux condenser was charged with 5.23 mL (0.05 mol) of 2-hydroxy benzaldehyde and 50 mL of dried ethanol. After complete dissolution of aldehyde, 1.67 mL (0.025 mol) ethylene diamine was added drop wise with constant stirring. The reaction mixture was refluxed for 3 h under inert conditions. The progress of the reaction was monitored by TLC. Ethanol was removed by rotary evaporator to get the solid product.

2-((E)-(2-((E)-2-hydroxybenzylideneamino)ethylimino)methyl)phenol (**H_2L_1**): $H_2L_1$was synthesized by refluxing 5.23 mL (0.05 mol) of salicyaldehyde and 1.67 mL (0.025 mol) ethylenediammine. Color: Yellow. Yield: 85%. M.p: 128-130 °C. FT-IR (KBr, ν, cm^{-1}): 3414 (OH), 1608 (-CH=N), 1371 (-C-N), 1280 (-C-O). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 13.24 (s, 2H, OH), 8.58 (s, 2H, -CH=N), 7.40-7.29 (4H, m, Ar-H), 6.91-6.87 (4H, m, Ar-H), 4.01 (s, 4H, $-CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 163 (C-OH), 160 (CH=N), 133-116 (Ar-C), 69 ($-CH_2-$). MS (EI, *m/z*): 268 (M^+). Anal. calcd. for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.62; H, 5.98; N, 10.43%.

2-((E)-(2-((E)-2-hydroxybenzylideneamino)propylimino) methyl)phenol (**H_2L_2**): H_2L_2 was synthesized by refluxing 5.23 mL (0.05 mol) of 2-hydroxy benzaldehyde and 2.13 mL (0.025 mol) propylenediammine. Color: Yellow. Yield: 72%. M.p: 55-57 °C. FT-IR (KBr, ν, cm^{-1}): 3417 (OH), 1617 (-CH=N), 1376 (-C-N), 1276 (-C-O). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 13.28 (s, 2H, OH), 8.37 (s, 1H, -CH=N), 8.33 (s, 1H, -CH=N), 7.34-7.23 (4H, m, Ar-H), 6.98-6.84 (4H, m, Ar-H), 3.90-3.84 (m, 1H, -CH-), 3.77-3.68 (m, 2H, CH_2), 1.43 (d, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 166 (C-OH), 161, 160 (CH=N), 132-116 (Ar-C), 66 ($-CH_2-$), 65 (-CH-), 21 ($-CH_3$). MS (EI, *m/z*): 282 (M^+). Anal. calcd. for $C_{17}H_{18}N_2O_2$: C, 72.32; H, 6.43; N, 9.92. Found: C, 72.35; H, 6.37; N, 9.95%.

2-((E)-1-(2-((E)-1-(2-hydroxyphenyl) ethylideneamino)ethyl imino)ethyl)phenol (**H_2L_3**): For the synthesis of H_2L_3 6.0 mL (0.05 mol) of 2-hydroxyacetophenone was refluxed with 1.67 mL (0.025 mol) ethylenediammine. Color: Yellow. Yield: 87%. M.p: 196-198 °C. FT-IR (KBr, ν, cm^{-1}): 3407 (OH), 1604 (-CH=N), 1380 (-C-N), 1289 (-C-O). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 15.83 (s, 2H, -OH), 7.69-7.24 (4H, m, Ar-H), 6.91-6.76 (4H, m, Ar-H), 4.04 (s, 4H, $-CH_2$), 1.25 (s, 6H, $-CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 169 (C=N), 162 (C-OH), 133-116 (Ar-C), 65 ($-CH_2-$), 18 ($-CH_3$). MS (EI, *m/z*): 296 (M^+). Anal. calcd. for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45. Found: C, 72.98; H, 6.74; N, 9.22.

2-((E)-1-(2-((E)-1-(2-hydroxyphenyl)ethylideneamino) pro pylimino)ethyl)phenol (**H_2L_4**): H_2L_4 was synthesized by the reaction of 6.0 mL (0.05 mol) 2-hydroxy acetophenone and 2.12 mL (0.025 mol) propylene diamine. Color: Yellow. Yield: 69%. M.p: 101-103 °C. FT-IR (KBr, ν, cm^{-1}): 3410 (OH), 1607 (-CH=N), 1378 (-C-N), 1285 (-C-O). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 16.03 (s, 2H, -OH), 7.54-7.27 (4H, m, Ar-H), 6.94-6.76 (4H, m, Ar-H), 4.36-4.30 (m, 1H, -CH-), 3.82-3.79 (m, 2H, $-CH_2$), 1.43 (d, 6H, $-CH_3$), 0.93 (s, 3H, $-CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 172, 170 (C=N), 163 (C-OH), 133-117 (Ar-C), 57 ($-CH_2-$), 55 (-CH-), 20.0, 14.8, 14.5 ($-CH_3$). MS (EI, *m/z*): 310 (M^+). Anal. calcd. for $C_{19}H_{22}N_2O_2$: C, 73.52; H, 7.14; N, 9.03, Found: C; 73.51; H: 7.13; N, 9.02.

3. Results and discussions

Schiff bases (**H_2L_1-H_2L_4**) have been successfully synthesised in a single step as described in experimental section and were characterised by spectroscopic, elemental and single crystal X-ray diffraction analysis. They are air stable under ambient conditions and soluble in common organic solvents like C_2H_5OH, CH_2Cl_2, $CHCl_3$ and CCl_4 *etc*. The synthesized compounds (**H_2L_1-H_2L_4**) were further assessed for various biological activities.

3.1. Spectral characterizations

The structures of the synthesized compounds were established by means of spectral studies (FT-IR, ^1H NMR, ^{13}C NMR and mass spectrometry), elemental and single crystal X-ray diffraction analyses.

The FT-IR spectral analysis of Schiff bases indicated the presence of all expected functionalities.The presence of an -OH group *ortho* position in the Schiff bases form intramolecular hydrogen bonding which affects ν(OH) vibration by shifting towards lower frequency with broadening. The FT-IR spectra of these compounds exhibited broad absorption bands in the region of 3407-3417 cm^{-1} due to hydroxyl groups (OH). The strong absorption bands in the region of 1604-1617 cm^{-1} were assigned to azomethine bond (-CH=N).

The ^1H and ^{13}C NMR spectra of all the Schiff bases are consistent with the proposed molecular structures. The ^1H NMR spectra showed the characteristic azomethine singlet in the region of δ 8.58-8.37 ppm. Phenyl protons were present in all the compounds which were verified by the appearance of multiplets in the range of δ 7.40-6.87, 7.34-6.84, 7.69-6.76 and 7.54-6.76 ppm for **H_2L_1**, **H_2L_2**, **H_2L_3** and **H_2L_4**, respectively. The resonance signals observed around δ 16.03-13.24 were assigned to phenolic proton.

^{13}C NMR spectra of the synthesized compounds show all the characteristic signals of azomethine and aromatic carbon atoms in their respective ranges of chemical shift values. The resonance signals observed around δ 166-163 ppm were assigned to C-OH carbons. Azomethine carbon resonated around δ 160 ppm. The rest of the peaks from δ 133-116 ppm were due to aromatic carbon atoms.

Table 2. Selected bond lengths and angles for $\mathbf{H_2L_4}$.

Bond length (Å)		Bond angle (°)	
O(1)-C(12)	1.3520(15)	N(2)-C(1)-C(11)	117.19(11)
C(1)-N(2)	1.2908(16)	N(5)-C(6)-C(21)	117.05(15)
N(2)-C(3)	1.4669(15)	N(5)-C(4)-C(3)	109.90(9)
N(5)-C(4)	1.4604(15)	O(1)-C(12)-C(13)	118.24(11)
O(2)-C(22)	1.3235(15)	O(2)-C(22)-C(23)	119.71(11)

Table 3. Hydrogen bonds for $\mathbf{H_2L_4}$ [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...N(2)	1.04(2)	1.55(2)	2.5276(14)	155.6(18)
N(5)-H(2)...O(2)	1.24(2)	1.30(2)	2.4759(13)	155(2)
O(1W)-H(1W)...O(2)	0.94(2)	1.94(2)	2.8806(12)	173.1(18)

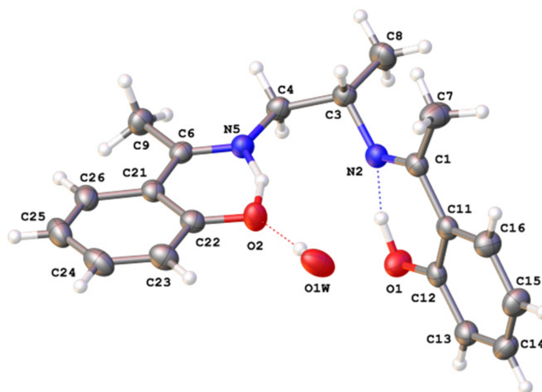


Figure 1. Perspective diagram of $\mathbf{H_2L_4}$.

The mass spectral data has also justified their formation as their molecular ion peaks were obtained at 268, 282, 296 and 310 *m/z* for $\mathbf{H_2L_1}$-$\mathbf{H_2L_4}$, respectively.

3.2. X-ray structure analysis of H_2L_4

The single crystals of Schiff base ($\mathbf{H_2L_4}$) was grown by the slow evaporation of its ethanolic solution at room temperature. A single suitable crystal was selected for X-ray diffraction analysis and was mounted on STOE IPDS-II two-circle diffractometer. The crystal was kept at 173 K during analysis. The perspective diagram of $\mathbf{H_2L_4}$ is shown in Figure 1. Crystal data and refinement details are listed in Table 1. Selected bond lengths and angles for $\mathbf{H_2L_4}$ are summarized in Table 2 and hydrogen bonding parameters in Table 3. The molecular conformation is characterized by two intramolecular hydrogen bonds. In one of them, the H atom is definitely bonded to the O atom, but in the other case the position of the H atom is midway in between O and N. In the crystal, two molecules are connected by a water molecule via O-H...O hydrogen bonds. The water O atom is located on a two-fold rotation axis. It is remarkable that the O atom with the longer O-H bond acts as an acceptor for the hydrogen bond to the water molecule. Furthermore, the bond O2-C22 [1.3235(15) Å] is significantly shorter (and has consequently more double bond character) than the bond O1-C12 [1.33520(15) Å]. Thus, it can be rationalized, that the hybridisation of O2 is between sp^3 and sp^2. The geometric parameters at N2 [N2-C1 1.2908(16) Å; N2-C3 1.4669(15) Å; C1-N2-C3 122.98(10)°] and N5 [N5-C6 1.2973(15) Å; N5-C4 1.4604(15) Å; C4-N5-C6 124.58(10)°] are in agreement with the different kind of H bonds involving O1 and O5.

3.3. Pharmacological studies

3.3.1. Brine shrimp cytotoxic assay

Brine shrimp cytotoxicity assay is a pre-screen test to observe the bioactive nature of compounds. The $\mathbf{H_2L_3}$ showed significant activity against brine shrimp *nauplii* with LD_{50} value of 14.27 μg/mL. The LD_{50} values of $\mathbf{H_2L_1}$-$\mathbf{H_2L_4}$ ranges from 14.27 to 29.85 μg/mL recommending these samples as highly cytotoxic. This cytotoxic action of a compound is simply proved by disturbing the basic mechanisms concerned with mitotic activity, cell growth, function and differentiation (Table 4).

3.3.2. Potato disc antitumor assay

The compounds were screened for possible antitumor activity by using potato disc antitumor assay and results are summarized in Table 4. $\mathbf{H_2L_3}$ has shown significant antitumor activity as its IC_{50} was 18.9 while for remaining compounds it ranged from 21.5 to 28.2. It is thought that these compounds at different doses may be used against different cancer-chemo preventive models and may appear to contrive safer drugs for future.

3.3.3. DNA damage assay

The antioxidant and pro-oxidant behaviour of compounds was checked by free radical induced DNA damage assay. With the attack of •OH produced from the Fenton reaction, super coiled plasmid DNA is broken into two forms, including open circular (OC) and linear form (linear). The intensity of bands formed on 1% agarose gel revealed that all the synthesised compounds were highly protecting at all concentrations (10, 100 and 1000 ppm), showing their antioxidant behaviour as shown in Figure 2 and 3.

3.3.4. Antibacterial assay

According to results, the four compounds $\mathbf{H_2L_1}$-$\mathbf{H_2L_4}$ were inactive against gram positive bacteria (*S. aureus* and *M. luteus)* while active against gram negative bacteria(*E. aerogenes, B. bronchiseptica* and *S. typhi)* showing variable activity (Table 5).

Table 4. Results of cytotoxicity, potato disc antitumor and inhibition of •OH induced DNA damage assays.

Compound	Cytotoxic activity	Antitumor activity	DNA protection activity, ppm		
	LD_{50}	IC_{50}	1000	100	10
H_2L_1	29.85	28.2	+ + +	+ + +	+ +
H_2L_2	24.62	21.5	+ + +	+ + +	+ +
H_2L_3	14.27	18.9	+ + +	+ + +	+ +
H_2L_4	17.65	25.0	+ + +	+ + +	+ +

DNA: Deoxyribonucleic acid; LD_{50}: lethal dose 50 or median lethal dose; IC_{50}: half maximal inhibitory concentration; +++: significant protection; ++: good protection.

Table 5. Antibacterial activity of Schiff bases (**H_2L_1-H_2L_4**).

Compound	Antibacterial zone of inhibition (mm) at 1 mg/mL					Minimum inhibitory conc. (mg/mL)				
	E. aerogenes	*B. bronchiseptica*	*S. typhi*	*S. aureus*	*M. luteus*	*E. aerogenes*	*B. bronchiseptica*	*S. typhi*	*S. aureus*	*M. luteus*
H_2L_1	-	-	-	-	-	-	-	-	-	-
H_2L_2	12.01	-	-	-	-	0.8	-	-	-	-
H_2L_3	18.10	-	12.02	-	-	0.8	-	1.0	-	-
H_2L_4	-	10.10	-	-	-	-	1.0	-	-	-
Roxithromycin	15.1	12.7	20.5	31.5	26.7	0.04	0.07	0.05	0.1	0.6
Cefixime	28.2	31.6	32.5	25.4	21.4	0.0015	0.08	0.004	0.01	0.05

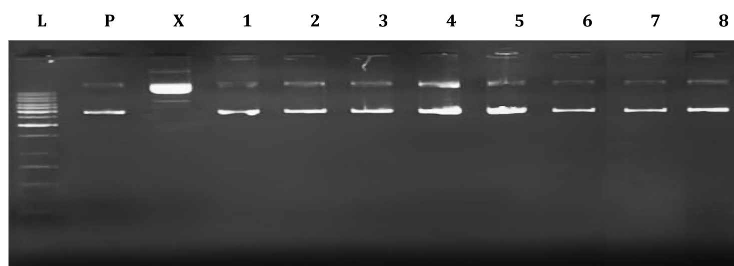


Figure 2. Effect of compounds **H_2L_1**and **H_2L_2** on pBR322 plasmid DNA [L = 1 Kb ladder, P = pBR322 plasmid, X = pBR322 plasmid treated with $FeSO_4$ and H_2O_2 (positive control), 1 (C+P) = pBR322 plasmid + 1000 μg/mL of **H_2L_1** control for the pro-oxidant effect of the compound on DNA, 2 = plasmid + 1000 μg/mL of **H_2L_1**+ $FeSO_4$ + H_2O_2, 3 =plasmid + 100 μg/mL of **H_2L_1**+ $FeSO_4$ + H_2O_2, 4 = plasmid + 10 μg/mL of **H_2L_1** + $FeSO_4$ + H_2O_2, 5 = pBR322 plasmid + 1000 μg/mL of **H_2L_2**; control for the pro-oxidant effect of the compound on DNA, 6 = plasmid + 1000 μg/mL of **H_2L_2**+ $FeSO_4$ + H_2O_2, 7 = plasmid + 100 μg/mL of **H_2L_2**+ $FeSO_4$ + H_2O_2, 8 = plasmid + 10 μg/mL of **H_2L_2**+ $FeSO_4$ + H_2O_2].

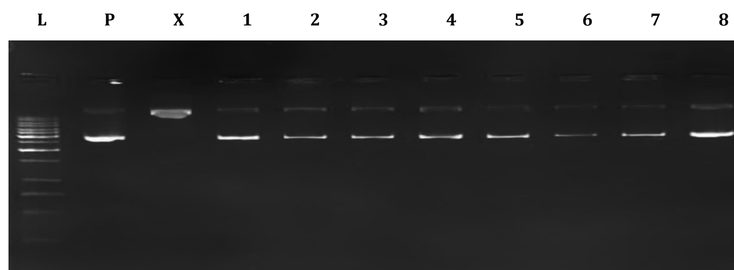


Figure 3. Effect of compounds **H_2L_3** and **H_2L_4** on pBR322 plasmid DNA [L = 1 Kb ladder, P = pBR322 plasmid, X = pBR322 plasmid treated with $FeSO_4$ and H_2O_2 (positive control), 1 (C+P) = pBR322 plasmid + 1000 μg/mL of **H_2L_3** control for the pro-oxidant effect of the compound on DNA, 2 = plasmid + 1000 μg/mL of **H_2L_3**+ $FeSO_4$ + H_2O_2, 3 = plasmid + 100 μg/mL of **H_2L_3**+ $FeSO_4$ + H_2O_2, 4 = plasmid + 10 μg/mL of **H_2L_4** + $FeSO_4$ + H_2O_2, 5 = pBR322 plasmid + 1000 μg/mL of **H_2L_4**; control for the pro-oxidant effect of the compound on DNA, 6 = plasmid + 1000 μg/mL of **H_2L_4**+ $FeSO_4$ + H_2O_2, 7 = plasmid + 100 μg/mL of **H_2L_4**+ $FeSO_4$ + H_2O_2, 8 = plasmid + 10 μg/mL of **H_2L_4**+ $FeSO_4$ + H_2O_2].

The active nature might be due to the presence of hydroxyl, phenyl and azomethine group but variation is due to difference in number of alkyl groups. **H_2L_1** showed no activity against any strain used. The results were compared with standard drugs (Roxithromycin, Cefixime). **H_2L_3** had shown maximum zone of 18.10 mg/mL against *E. aerogenes* which is greater than Roxithromycin (15.1 mg/mL). **H_2L_2** and **H_2L_3** showed lowest MIC with concentration of 0.8 mg/mL against *E. aerogenes*. **H_2L_4** was only active against *B. bronchiseptica.* All the synthesized compounds were less active than Cefixime.

Various biological studies reveal the bioactive nature of these synthons. They were found to be active against gram negative bacterial strains only. The highly cytotoxic and antitumor behavior of these compounds suggests that they have potential to be developed into anticancerous drugs. Furthermore, DNA protection behavior of these compounds signifies their antioxidant potential by scavenging the free radicals produced from Fenton reaction.

4. Conclusion

Four quadridentate ONNO donor Schiff bases (**H_2L_1-H_2L_4**) were synthesised and characterised by various analytical, spectroscopic and single crystal X-ray analysis. The synthesised compounds were further investigated for various biological studies (cytotoxic, antitumor, inhibition of hydroxyl (OH) free radical induced DNA damage and antibacterial) to observe their bioactive nature. The antibacterial studies signified the bioactive nature of the synthesized compounds against Gram negative bacterial strains only. **H_2L_3** was found to

be more active than Roxithromycin against *E. aerogenes* at 1 mg/mL which can further be optimized by further studies. The highly cytotoxic and antitumor nature of these compounds proposes them as anticancer drugs.

Supplementary data

CCDC 1428521 contains the supplementary crystallographic data for the compound $\mathbf{H_2L_4}$. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033.

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