

# Synthesis, Characterization and Antioxidant Activity Studies of New Azo-Compounds

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## Abstract

Four new azo-azomethine ligands (b2, b3, c2 and c3) have been prepared from the reaction of 4, 4' (diamino diphenyl) methane with 5-(4-X-phenyl)-azo-salicylaldehyde (X = H, CH<sub>3</sub> and Br). Furthermore, derivatives of Schiff bases (c1-3) have been prepared by the reduction of imine function (b1-3) with Sodium borohydride. The dyes have been characterized by IR, UV-Vis and <sup>1</sup>H NMR spectroscopic techniques as well as elemental analysis. In addition, the assessment of the antioxidant activity of the compounds (c1-3) by DPPH essay gave positive results comparing to ascorbic acid (VC) as a reference compound.

**Keywords:** Azo-azomethine, Salicylaldehyde, Schiff base, antioxidant activity, DPPH.

## Introduction

The azo compounds are very important molecules and have attracted much attention in both academic and applied research (Zollinger, 1961; Zollinger, 1987; Kamellia et al, 2009). These compounds are key chromophores in the chemical industry as dyes and pigments, food additives, indicators, radical reaction initiators and therapeutic agents (Hamid et al, 2013; Koh and Greaves, 2001; Bhatti and Seshadri, 2004), and have been used as cosmetics (Bouchoul et al, 2004), in dyeing wool and wood (Desai and Desai, 2004), medicine industry as drugs to prevent the growth of germs (Bondock et al, 2006). The name of Schiff bases was given to the organic compounds containing the group of imine or (C=N) which is known also as the group of azomethine (Kurahashi and Bull, 1976). The spectral properties of azo dyes depend on the nature of both the azo and the coupling

components. For example, azomethine compounds which have some interesting structural properties and uses can be prepared by coupling of azo and methine groups (Ispir, 2009; Alghool et al, 2010; Zhou et al, 2000; Ghosh et al, 2002). They are important structures in the medical and pharmaceutical fields (Jarraphour et al, 2004). Furthermore, considerable attentions have been paid to the study of azo-azomethine dyes containing hydroxyl groups in recent years as their metabolites are toxic, carcinogenic, and mutagenic (Odabas et al, 2007; Refat et al, 2006; Dincaple et al, 2007; Myslak and Bolt 1988). These compounds have the ability to form different type of intra- and intermolecular hydrogen bonds and intra-molecular proton transfer between their nitrogen atoms. This tautomerization can be induced either by light, heat or the solvent (Basu Baul et al, 2009; Hamid and Abdollahi 2012; Kakaneja difard et al, 2013; Kakaneja difard et al, 2013; Ghasemian et al, 2014).

The new azo-azomethine compounds (b2, b3, c2 and c3) were synthesized through the following steps (figure-1-)

- The preparation of azo compounds (a1-3)
- The preparation of azo-azomethine compounds (b1-3)
- Reducing the imine group for the azo-azomethine compounds (b1-3)

These new compounds were characterized by IR, UV-Vis, and <sup>1</sup>H NMR as well as elemental analysis; finally, antioxidant activity for the (c1-3) compounds were studied using DPPH essay

## Experimental:

### Materials:

All reaction chemicals were used as received without further purification. Azo-coupled precursors (a1-3) were prepared according to the well-known procedure (Salem et al, 2015; Hamid and Khatereh, 2012).

### Instrumentation

NMR spectra were measured using Bruker-Avance 400 MHz spectrometer and IR spectra were measured by Bruker vertex 70.

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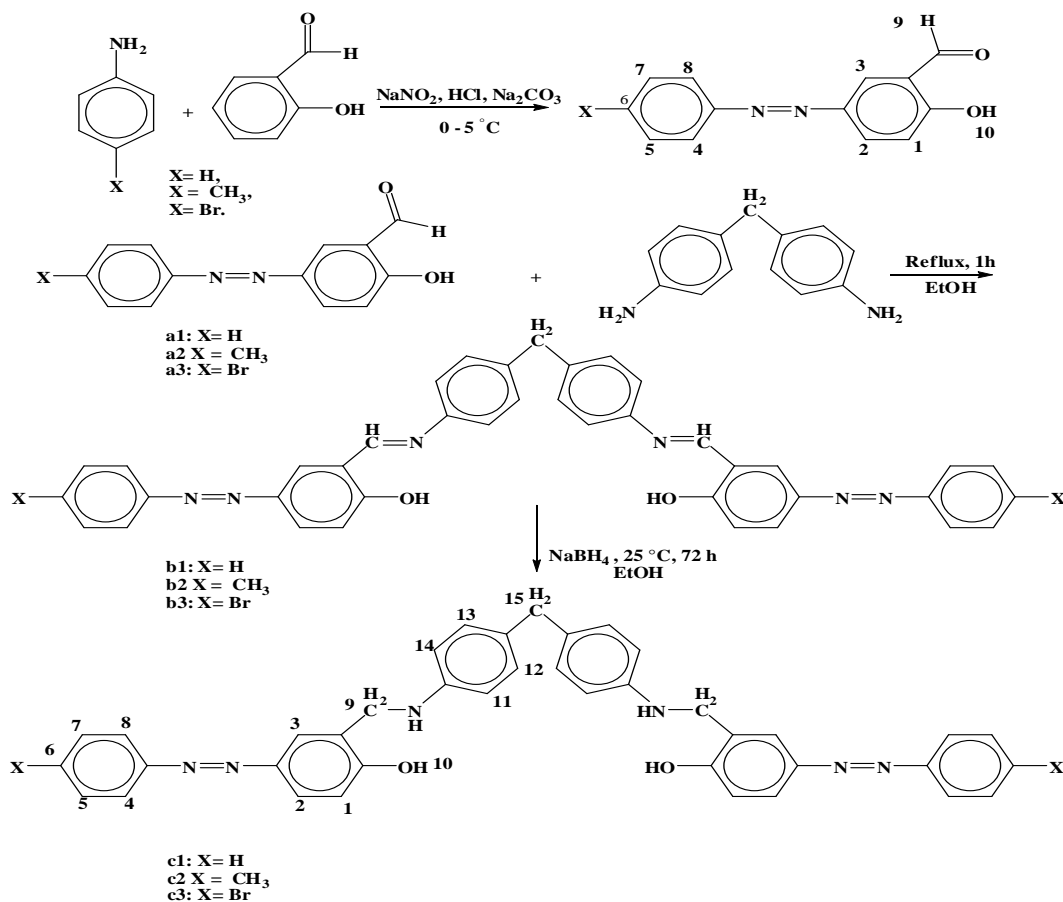
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Elemental analysis was carried out using a Euro vector E.A.3000 instrument using copper sample-tubes. Melting points were measured by a Stuart Scientific melting apparatus (uncorrected  $\pm$

0.1°C). The UV/Vis spectra were recorded in acetonitrile by using TIDAS fiber optic diode array spectrometer.



**Figure 1.** Synthesized azo-azomethine dyes

*General procedure for the synthesis of azo-coupled precursors a1-3*

Ice-cold  $\text{NaNO}_2$  (0.62 g, 9 mmol) solution was added dropwise to the ice cold aryl amine solution. The resulting solution was kept in ice-bath; it was then added to an ice-cold solution of Salicylaldehyde (10 mmol), dissolved in water (20 mL) containing 10 mmol of sodium hydroxide and 40 mmol of sodium carbonate at  $0^\circ\text{C}$ . The resulting solution was added slowly to a solution of diazonium chloride (10 mmol) in water at  $0-5^\circ\text{C}$ . The reaction mixture was stirred for 1 h at  $0^\circ\text{C}$  and then allowed to warm slowly to room temperature. The product was collected by filtration and washed with 100 mL of NaCl. The obtained solid was dried overnight under vacuum at  $80^\circ\text{C}$ .

#### 5-phenylazo-2-hydroxybenzaldehyde (a1)

Brown solid, yield 63.09 %, mp =  $128^\circ\text{C}$ . Elemental analysis calcd (%) For ( $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_2$ ): C 69.02, H 4.46, N 12.38, found: C 68.76, H 4.63 and N 12.29.

IR (KBr,  $\text{cm}^{-1}$ ): 1664 (C=O), 1570 (C=C, aromatic), 1478 (N=N), 1284 (C-O), 1155, 682.

#### 5-(4-methyl phenylazo)-2-hydroxybenzaldehyde (a2)

Yellow solid, Yield: 95%, mp =  $155-156^\circ\text{C}$ . Elemental analysis calcd (%) For ( $\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_2$ ): C 69.99, H 5.03, N 11.66, found: C 70.13, H 5.23 and N 11.82. IR (KBr,  $\text{cm}^{-1}$ ): 1666 (C=O), 1579 (C=C, aromatic), 1479 (N=N), 1286 (C-O), 1154, 820, 738.

#### 5-(4-bromo phenylazo)-2-hydroxybenzaldehyde (a3)

Light green solid, Yield: 91%, mp =  $206-207^\circ\text{C}$ . Elemental analysis calcd (%) For ( $\text{C}_{13}\text{H}_9\text{N}_2\text{O}_2\text{Br}$ ): C 51.17, H 2.97, N 9.18, found: C 51.30, H 3.10 and N 9.34, IR (KBr,  $\text{cm}^{-1}$ ): 1666 (C=O), 1477 (N=N), 1579 (C=C, aromatic), 1288 (C-O), 1152, 825 and 714.

*General procedure for the synthesis of azo-azomethine dyes: b1-3.*

A solution of diamine (1 mmol) in absolute ethanol (10 ml) was added to a stirring solution of a1-3 (2 mmol) in absolute ethanol during a period of 30 min at 50 °C (Hamid and Maryam 2009). The solution was heated in water bath for 2 h at 80 °C with stirring, cooled to ambient temperature. The resulted product was collected by filtration, washed successively with diethyl ether and dried in air.

**Bis[5-(phenylazo)-2-hydroxybenzaldehyde]-4,4'-diiminophenyl methane (b1)**

Yellow solid, yield 94.27 %, mp >260 °C, Elemental analysis calcd (%) For (C<sub>39</sub>H<sub>30</sub>N<sub>6</sub>O<sub>2</sub>): C 76.20, H 4.92, N 13.67, found: C 76.06, H 4.84 and N 13.38, IR (KBr, cm<sup>-1</sup>): 1617 (C=N), 1569 (C=C, aromatic), 1495(N=N), 1353, 1280 (C-O), 1188 (C-N), 832, 683.

**Bis [5-(4-methylphenylazo)-2-hydroxybenzaldehyde]-4, 4'-diiminophenyl methane (b2)**

Orange solid, yield 88.13%, mp >290 °C, Elemental analysis calcd (%) For (C<sub>41</sub>H<sub>34</sub>N<sub>6</sub>O<sub>2</sub>): C 76.61, H 5.33, N 13.08, found: C 76.48, H 5.56 and N 13.82. IR (KBr, cm<sup>-1</sup>): 1618 (C=N), 1576(C=C, aromatic), 1492(N=N), 1277 (C-O), 817, 658.

**Bis [5-(4-bromophenylazo)-2-hydroxybenzaldehyde]-4, 4'-diiminophenyl methane (b3)**

Orange solid, yield 78.2 %, mp >290°C, Elemental analysis calcd (%) For (C<sub>39</sub>H<sub>28</sub>N<sub>6</sub>O<sub>2</sub>Br<sub>2</sub>): C 60.64, H 3.65, N 10.88, found: C 61.18, H 4.02 and N 11.80, IR (KBr, cm<sup>-1</sup>): 1621 (C=N), 15687(C=C, aromatic), 1485(N=N), 1280 (C-O), 833, 658.

*General procedure for synthesis of the reduce form c1-3*

These compounds were synthesized as follows: (0.001 mol) of the azo-imine (c1-3) were dissolved in 100 mL of absolute ethanol and then 0.075 g (0.002 mol) of sodium borohydride, was added [25]. The mixture was stirred at room temperature for 72 h and the solvent was removed by rotary evaporation. The remaining solid was dissolved in 150 mL of distilled water, and extracted with CHCl<sub>3</sub> three times (3×50 mL). The CHCl<sub>3</sub> layer was dried by 40 g of anhydrous sodium sulfate powder, filtered and the CHCl<sub>3</sub> was removed by rotary evaporation. The isolated brown-yellow powder was stored in desiccator.

**Bis[5-(phenylazo)-2-hydroxybenzaldehyde]-4,4'-diaminophenyl methane (c1)**

Yellow solid, yield 88.44 %, mp 156°C. Elemental analysis calcd (%) For (C<sub>39</sub>H<sub>34</sub>O<sub>2</sub>N<sub>6</sub>): C 75.71, H 5.54, N 13.58, found: C 75.45, H 5.83 and N 13.35. IR (KBr, cm<sup>-1</sup>): 3268 (N-H), 3034 (C-H, aromatic), 2910 (C-H, aliphatic), 2857, 1591 (C=C, aromatic), 1508 (N=N), 1258(C-O), 769, 691. <sup>1</sup>H NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ=10.51 (s, H-10), 7.82 (s, H-3), 7.76 (d, J = 7.2 Hz, H-4, H-8), 7.67 (dd, J = 2, 8.5 Hz, H-2), 7.50 (m, 3H, H-5, H-6 (X), H-7), 6.99 (d, J = 8.6 Hz, H-1), 6.85 (d, J = 8.3 Hz, H-12, H-13), 6.50 (d, J = 8.1 Hz, H-11, H-14), 5.98 (m, N-H), 4.22 (s, 2H, H-9), 3.57 (s, H-15). <sup>13</sup>C NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ= 158.58, 151.88, 146.48, 144.88, 130.10, 129.20, 129.06, 128.82, 127.04, 123.07, 122.55, 121.81, 115.14, 111.96.

**Bis [5-(4-methylphenylazo)-2-hydroxybenzaldehyde]-4, 4'-diaminophenyl methane(c2)**

Light brown solid, yield 75.89 %, mp 169°C. Elemental analysis calcd (%) For (C<sub>41</sub>H<sub>38</sub>O<sub>2</sub>N<sub>6</sub>): C 76.14, H 5.92, N 12.99, found: C 75.76, H 5.95 and N 13.13. IR (KBr, cm<sup>-1</sup>): 3294(N-H) 1587(C=C, aromatic), 1501(N=N), 1240(C-O), 833, 658. <sup>1</sup>H NMR( 400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ=9.42(br, s.; H-10), 7.84 (br s, H-3), 7.77(br, s; 2H, H-4, H-8), 7.26(s; H-2), 7.31(br.s.;2H, H-5, H-7), 7,26 (s; H-2), 7.04 (br.s;2H, H-12, H-13), 6.97 (s; H-1), 6.82 (d, J = 7.9,2H, H-11, H-14), 5.98 (br s, N-H), 4.51 (s; 2H, H-9), 3.83 (s, H-15), 2.43 (s; 3H, H-6 (X)). <sup>13</sup>C NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ= 159.89, 150.82, 146.48, 144.85, 134.59, 132.27, 129.75, 129.72, 124.80, 123.02, 122.84, 122.52, 117.21, 116.58.

**Bis [5-(4-bromophenylazo)-2-hydroxybenzaldehyde]-4, 4'-diaminophenyl methane (c3)**

Sepia solid, yield 69.20%, mp 188°C. Elemental analysis calcd (%) For (C<sub>39</sub>H<sub>32</sub>O<sub>2</sub>N<sub>6</sub>Br<sub>2</sub>): C 60.32, H 4.15, N 10.82, found: C 59.83, H 4.04 and N 10.97. IR (KBr, cm<sup>-1</sup>): 3286(N-H) 1595 (C=C, aromatic), 1507 (N=N), 1232(C-O), 832, 652. <sup>1</sup>H NMR( 400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ=9.57(br, s.; H-10), 7.85 (br s, H-3), 7.74 (br, s.; 2H, H-4, H-8), 7.64 (br, s.; 2H, H-5, H-7), 7.26(s; H-2), 7.04 (s; 2H, H-12, H-13), 6.98 (d, j=7.8, H-1), 6.83 (s; 2H, H-11, H-14), 5.98 (br s, N-H), 4.52(s; 2H, H-9), 3.83 (s, H-15). <sup>13</sup>C NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ= 160.51, 151.47, 146.25, 144.76, 134.68, 132.27, 129.77, 125.06, 124.58, 124.04, 123.18, 123.12, 117.35, 116.61.

*Antioxidant activity*

Antioxidant activity of compounds (Motaleb et al, 2014) was investigated by using DPPH method. Briefly, 200 µl of test compounds in DMSO at concentrations of 50, 150, 250, 350 and 450 Mm were added to 4 ml of DPPH (6 × 10<sup>-5</sup> M) in methanol. The mixtures were kept in dark at room temperature. After 30 min, the absorbance at 517 nm against blank samples lacking scavenger was measured. Decreasing DPPH solution absorbance indicates increasing DPPH radical scavenging activity.

The inhibition percentage was calculated using the following formula:

% Inhibition = [(A<sub>Control</sub> - A<sub>Sample</sub>) / A<sub>Control</sub>] × 100 where A<sub>Control</sub> is the absorbance of DPPH without sample and A<sub>Sample</sub> is the absorbance of DPPH in the presence of the sample.

**Results and Discussion**

Compounds azo-azomethine (b1-3) were prepared from the reaction of 4, 4' (diaminodiphenyl) methane with 5-(4-X-phenyl)-azo-salicylaldehyde (X = H, CH<sub>3</sub> and Br) in ethanol (scheme 1). Azo ligand (c1-3) was prepared from the reaction of the imine reducing found in the compound (b1-3) by sodium borohydride. The structures of all compounds were then characterized by elemental analysis, FT-IR, UV-vis, <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy.

Table 1: Melting point, Elemental analysis of (a1-3), (b1-3) and (c1-3).

compounds	Yield (%)	Mp (°C)	%C		%H		%N	
			Calculated	found	Calculated	found	Calculated	found
<b>a1</b>	63.09	122	69.02	68.76	4.46	4.63	12.38	12.29
<b>a2</b>	75.12	155-156	69.99	70.13	5.03	5.23	11.66	11.82
<b>a3</b>	70.13	205-206	51.17	51.30	2.97	3.10	9.18	9.34
<b>b1</b>	94.27	>260	76.20	76.06	4.92	4.84	13.67	13.8
<b>b2</b>	88.04	>290	76.61	76.48	5.33	5.56	13.08	13.82
<b>b3</b>	78.2	>290	60.64	61.02	3.65	3.95	10.88	11.20
<b>c1</b>	82.44	156	75.71	75.45	5.54	5.83	13.56	13.35
<b>c2</b>	75.28	169	76.14	75.85	5.92	5.95	12.99	13.13
<b>c3</b>	69.2	188	60.32	59.90	4.15	4.04	10.82	10.97

### Infrared analysis

The IR analysis data of the synthesized compounds are shown in table1. The presence of a band at 1568-1595 is due to the vibration of C=C group. The two bands at 1477-1508 and 1258-1290 are due to N=N and C-O, respectively. The bands in the

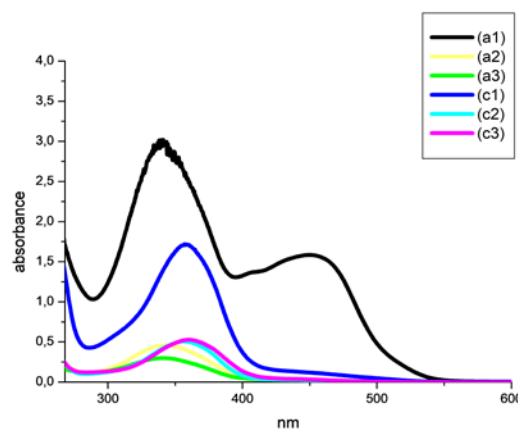
range of 1664-1666 are due to C=O of the three primary compounds (a1-3). The imine group C=N related to Schiff bases (b1-3) are appeared in the range 1617-1621. However, the NH functional group for (c1-3) are appeared in the range 3268-3286 (Hamid and Khatereh, 2012; Hamid and Malihe, 2012; Hamid et al, 2014).

Table 2: Tentative assignments of some selected IR (KBr, cm<sup>-1</sup>) and UV-Vis data of the prepared azo-azomethine dyes and their azo precursors.

compounds	$\nu(\text{C-O})$	$\nu(\text{C=O})$	$\nu(\text{C=C})$	$\nu(\text{C=N})$	$\nu(\text{N-H})$	$\nu(\text{N=N})$	$\lambda_{\text{max}}(\text{nm})$ in DMF
<b>a1</b>	1284	1664	1570	-	-	1478	341,453
<b>a2</b>	1286	1666	1579	-	-	1477	347
<b>a3</b>	1290	1666	1578	-	-	1479	346
<b>b1</b>	1280	-	1574	1617	-	1495	-
<b>b2</b>	1277	-	1576	1618	-	1492	-
<b>b3</b>	1272	-	1568	1621	-	1476	-
<b>c1</b>	1258	-	1591	-	3268	1508	358
<b>c2</b>	1280	-	1586	-	3283	1490	360
<b>c3</b>	1290	-	1595	-	3286	1484	363

### The UV-Visanalysis

The UV absorption data for the synthesized compounds (a1-a3 and c1-c3) were measured in the range of 200–900 nm using in DMF at room temperature (figure 2). The results are given in Table 2. The UV spectra of compounds (a-c) show two bands at 341–363 and 453 nm which are assigned to  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$ , respectively (Saeid et al, 2013; Motaleb et al, 2014).



**Figure 2:** Absorption spectra of (a1-3) and azo ligands dyes (c1-3) in DMF

### The <sup>1</sup>H NMR spectra

NMR analysis Spectra for synthesized compounds using CH<sub>2</sub>CL<sub>2</sub>, and solvents were mainly distinguished by signals of the proton OH at the range of 9.42-10.51 ppm and phenyl protons in the range of 6.50-7.85 ppm. The CH<sub>2</sub> protons were observed as signals in the range of 4.22-4.52ppm. Moreover, the signal peak at 2.43 ppm was assigned to the methyl group (CH<sub>3</sub>) in the compound c2.

### Antioxidant activity:

DPPH is a relatively free stable radical. It accepts hydrogen or an electron to be more stable. The DPPH percentage inhibition of the compounds (c1–3) and the standard compound (VC) are shown in the table 3.

Table 3: Antioxidant activity of synthesized compounds (c1- 3)

Concentration ( $\mu$ M)	DPPH Scavenging Effect (%)			
	C1	C2	C3	Ascorbic acid
50	10.05	13.60	7.82	27.03
150	25.00	28.46	12.39	40.02
250	27.11	45.08	13.04	65.11
350	32.65	52.39	13.69	90.00
450	55.20	60.20	18.69	91.03

The compounds (c1 and c2) proved a good antioxidant activity but the compounds c2 showed a better activity. However, the compound c3 displayed a less significant activity may be due to the substituent of Br on the phenyl ring (Motaleb et al, 2014; Motaleb et al, 2015)

### Conclusion

In the present study, new azo-azomethine compounds (b2, b3, c2 and c3) were synthesized from the reaction of reducing the imine group. These compounds were characterized by IR, UV-Vis, <sup>1</sup>H NMR and elemental analysis. The antioxidant activity of the compounds from (c1-3) was appraised and gave average results according to the following order c2> c1> c3.

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