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由蒽醌衍生的二价和三价过渡金属偶氮配合物及其致突变–畸变作用

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摘要: 制备了 4-(5′-氨基萘-3′-磺酸)偶氮-(2″-*N*-(4‴,6‴-二氯-*S*-三嗪)苯-5″-(β-羟乙基砜硫酸酯)-2-甲基蒽醌(**2a**),4-(2′-氨基苯磺酸)偶氮-(2″-*N*-(4‴,6‴-二氯-*S*-三嗪)-3″-苯磺酸)-2-甲基蒽醌,4-(5′-氨基萘-3′-磺酸)偶氮-(2″-*N*-(4‴,6‴-二氯-*S*-三嗪)苯-5″-(β-羟乙基砜硫酸酯)-2-甲基蒽醌和 4-(*p*-(β-羟乙基砜硫酸酯)偶氮-(2″-*N*-(4‴,6‴-二氯-*S*-三嗪)萘-5″-磺酸)-2-甲基蒽醌的 Fe^{III},Co^{II} 金属配合物,并在安姆/沙门氏菌/微粒体致突变试验(Ames/Salmonella/Microsome Test)和大鼠胚胎中测定其致突变–畸变作用。不管是否使用代谢赋活剂 S9mix,**2a**-Fe 和 **2a**-Co 对 TA98 and TA100 菌株均无致突变–畸变作用。

关键词: 蒽醌;偶氮;金属配合物;致突变作用;致畸变作用
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Bivalent and Trivalent Transition Metal Complexes of Azo Compounds Derived from Anthraquinone and Their Mutagenic-Teratogenic Effects

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Abstract: 4-(5′-aminonaphtalene-3′-sulfonic acid)azo-(2″-*N*-(4‴, 6‴-dichloro-*S*-triazine) benzene-5″-(β-sulfatoethyl sulfonyl)-2-methyl anthraquinone (**2a**), 4-(2′-aminobenzenesulfonic acid)azo-(2″-*N*-(4‴, 6‴-dichloro-*S*-triazine)-3″-benzensulfonic acid)-2-methyl anthraquinone, 4-(5′-aminonaphtalene-3′-sulfonic acid)azo-(2″-*N*-(4‴, 6‴-dichloro-*S*-triazine)benzen-5″-(β-sulfatoethyl sulfonyl)-2-methyl anthraquinone and 4-(*p*-(β-sulfatoethyl sulfonyl) azo-(2″-*N*-(4‴, 6‴-dichloro-*S*-triazine) naphtalene-5″sulfonic acid)-2-methyl anthraquinone with Fe^{III}, Co^{II} metal complexes were prepared and used for determination of mutagenic and teratogenic effects in Ames/Salmonella/Microsome Test and in embryos of rats. **2a**-Fe and **2a**-Co were not mutagenic for both TA98 and TA100 strains with and without metabolic activator S9mix.

Key words: anthraquinone; azo; metal complex; mutagenicity; teratogenicity

0 Introduction

Metal^{II}-azo complexes are studied widely because of their excellent optical and thermal properties which are very important for the practical application as next generation of high-density optical recording media high technology areas and textile industry[1-4].

The linear azo dyes based on 2-anilinoethanol have been synthesized using *o*-, *m*-, *p*-position of phenylazoanilinoethanoles for this aim[5]. Although all

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their technological benefits, some of chemicals cause metabolic diseases and health problems[6-7]. Astrazon Yellow, Red, Blue, and Black commercial textile dyes also cause embryotoxicity and teratogenicity in Xenopus leavis embryos[8].

In this paper, we report the synthesis of a series of compounds including azo and anthraquinone moieties, their metal complexes with cobalt^{II} and iron^{III} chlorides.

The second aim of this study was to investigate the mutagenic and teratogenic effect of new synthesized compounds using Ames/Salmonella/Microsome Test in TA98 and TA100 strains of Salmonella typhimurium and teratogenicity test in pregnant rats.

1 Experimental

1.1 Materials

All chemicals were obtained from commercial sources and some of them purified prior to use. In the synthesis process, the following reagents were used without further purification: 1-amino-4-bromo-2-methyl anthraquinone, 98.8%; 2, 4, 6-trichloro-1,3,5-triazine, 99% (Sigma-Aldrich). Intermediate chemicals such as 2, 2′-Bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) were weighed under the nitrogen atmosphere in glove box.

1.2 Instruments

FTIR spectra were recorded on a Perkin Elmer RX I spectrometer as KBr disc, electronic spectra on Model Shimadzu UVG-54 spectrophotometer in the range 200 ~400 nm. ^{1}H-NMR spectra were recorded on a Bruker/XWIN-NMR (400 MHz). UV-Vis spectra were measured on Perkin Elmer Lampda 25 Spectrometer. The metal content was calculated according to the results of AAS (Perkin Elmer Analyst-400). The magnetic measurements were carried out by the Gouy method, Sherwood Scientific Cambridge MS No: MK 1 using $CuSO_4 \cdot 5H_2O$ as the calibrant.

1.3 Preparation of the ligands

1-amino-4-(2′ -amino benzene sulfonicacid)-2-methyl anthraquinone (**1**), 1-amino-4-(amino-*p*-(*β*-sulfatoetyl sulfonicacid)-2-methyl anthraquinone (**2**) and 1-amino-4-(5′-amino naphtalene-3′-sulfonic acid)-2-methyl anthraquinone (**3**) were prepared with a stirred mixture of $Pd_2(dbap)_3$, BINAP and Cs_2CO_3 in previous work[9].

1.3.1 Preparation of the diazonium salts and coupling component

Diazonium salts were synthesized according to previous work[10].

1.3.2 Preparation of the metal complexes

1.3.2.1 General Co(Ⅱ) complexes

The ligands were dissolved in ethyl alcohol (20 mL) and added to $CoCl_2 \cdot 6H_2O$ (0.270 mmol, 64.0 mg) and refluxed for 5 h. The crude product was dissolved in dichloromethane and filtrated through silica to remove polar impurities and evaporated in vacuum.

1.3.2.2 Fe(Ⅲ) complex

2a (0.136 mmol, 11.5 mg) was dissolved in Ethanol (10 mL) and added to $FeCl_3 \cdot 6H_2O$ (0.136 mmol, 22.0 mg) in Ethanol (10 mL). The mixture was stirred for 2 h at room temperature and cooled. Diethyl ether was used for precipitation. The solution was filtered and dried.

4-(2′ -amino benzene sulfonic acid)azo-(2″ -*N*-(4‴ , 6‴ -dichloro-*S*-triazine)-3″ -benzene sulfonic acid)-2-methyl anthraquinone (1a)

Brown powder. M.P. 299.4 ℃ Yield 72%. FTIR (KBr, cm^{-1}): 3 317, 1 590, 1 550, 1 673, 1 437, 1 303,1 261, 1 068. UV-Vis (Ethanol, nm): 251, 330, 450; *ε* (max): 1.29×10^3. ^{1}H NMR (DMSO-d_6, ppm): *δ*: 2.0 (SO_3H, -OH), 2.4 (-CH_3), 6.7~7.3 (m, 20H, 4Ph), 9.1 (NH, Ph-NH-Ph), 10.9 (NH, triazine). ^{13}C NMR (DMSO-d_6, ppm): *δ*: 17.0 (-CH_3), *δ* 121 ~140.5 (C, aromatic), *δ* 143 (C-N =N-Ph), *δ* 154 (NH-C-N-(triazine)), *δ* 163 (N-C(Cl)-N- (triazine)), *δ* 182 (C=O).

1a-Co: Brown powder, M.P.: >300 ℃. Yield 55%. FTIR (KBr, cm^{-1}): 3 412, 1 673, 1 588, 1 581, 1 442, 1 339, 1 163, 1 110, 559. UV-Vis (Ethanol, nm): 203, 254, 333, 452; ε_{max}: 1.0×10^3. ^{1}H-NMR(DMSO-d_6, ppm): 13.0 (O-H), 9.0, AAS: Calculated (%):10.80, Found(%): 10.25

4-(5′ -amino naphthalin-3′ -sulfonic acid)azo-(2″ -*N*-(4‴ , 6‴ -dichloro-*S*-triazine) benzene-5″ -(*β*-sulfatoethyl sulfonyl)-2-methyl anthraquinone (2a)

Red powder. M.P: 232 ℃ 71%. FTIR (KBr,cm^{-1}):

3 418, 1 673, 1 590, 1 549, 1 398, 1 267, 1 177, 1 036. UV-Vis (Ethanol, nm): 248, 318, 481; ε_{max}: 6.96×10^3. ^{1}H-NMR (DMSO-d_6, ppm): δ: 2.0 (SO_3H, -OH), 2.5 (-CH_3), 3.0~4.5 (-CH_2), 6.8~8.4 (m, 9H, Ph), 9.8(s, NH, Ph-NH-Ph), 11.1 (NH, triazine). ^{13}C-NMR (DMSO-d_6, ppm): δ: 19.0 (-CH_3), 121.0~146.0 (C, aromatic), δ 146 (C-N=N-Ph), δ 150 (Ph-N=N-C), δ 152 (NH-C-N- (triazine)), δ 170 (N-C(Cl)-N-(triazine)), δ 184 (C=O).

2a-Co: Light red powder, M.P.: >300 ℃. Yield 51%. FTIR (KBr, cm^{-1}): 3 330, 1 673, 1 590, 1 548, 1 437, 1 292, 1 162, 1 068, 577. UV-Vis (Ethanol, nm): 248, 284, 328, 480; ε_{max}: 7.98×10^3. AAS: Calculated(%): 9.43, Found(%): 9.67

2a-Fe: Purple powder, M.P.: >300 ℃. Yield 67%. FTIR (KBr, cm^{-1}): 3 317, 1 673, 1 590, 1 552, 1 436, 1 263, 1 207, 1 067, 584. UV-Vis (Ethanol, nm): 202, 247, 318, 480; ε_{max}: 5.57×10^3. AAS: Calculated(%): 5.02, Found(%):5.46. μ_{eff}: 5.17 B.M.

4-(*p*-(*β*-sulfatoethyl sulfonil aniline)azo-(2″-*N*-(4‴, 6‴-dichloro-*S*-triazine) naphthalin-5″-sulfonic acid)-2-methyl anthraquinone (3a)

Khaki powder, M. P.: 255.5 ℃ 79%. FTIR (KBr, cm^{-1}): 3 301, 1 675, 1 589, 1 558, 1 424, 1 230, 1 141, 1 033. UV-Vis (Ethanol, nm): 201, 260, 276, 342, 458; ε_{max}: 4.31×10^3. ^{1}H NMR (DMSO-d_6, ppm): δ: 2.2 (s, SO_3H, -OH), 2.5 (s, -CH_3), 3.6-3.8 (d, -CH_2), 6.9~8.5 (m, 9H, Ph), 11.0 (s, NH, triazine), 10.7 (s, NH, Ph-NH-Ph). ^{13}C NMR: δ: 21.0 (-CH_3), δ 55.5~58.0 (-CH_2-CH_2-), δ 122.0~136.0 (C, aromatic), δ 142 (NH-C(Ph), δ 146.0 (C-N=N-Ph), δ 151 (Ph-N=N-C), 152.0 (NH-C-N-(triazine)), δ 162.0 (N-C (Cl)-N-(triazine)), δ 182.0 (C=O).

3a-Co: Dark brown powder, M.P.: >300 ℃. Yield 47%. FTIR (KBr, cm^{-1}): 3 371, 1 723, 1 676, 1 591, 1 399, 1 136, 1 094, 535. UV-Vis (Ethanol, nm): 220, 254, 334, 489 ε_{max}: 3.12×10^3. AAS: Calculated (%): 9.43, Found(%): 9.84.

1.3.3 Mutagenicity Assay

The mutagenic effects of synthesized compounds were investigated using Ames/Salmonella/Microsome Test. The three different nontoxic concentrations of test compounds were tested using TA98 and TA100 strains of *S. typhimurium* in the presence and the absence of microsomal fraction (S9)[11].

1.3.3.1 Chemicals

Nicotinamide adenine dinucleotide phosphate (NADP) (N5755), glucose-6-phosphate (G7879), DMSO (D8418), l-histidin (H8125), d-biotin (B4501), ampicillin (A6140), sodium azid (SA) (S2002) and 2-aminofluorene (2-AF) (A9031) were purchased from Sigma -Aldrich. 4-nitro-*o*-phenylenediamine (NPD) was purchased from Sigma -Aldrich. The other chemicals like agar (aquamedia, 7178), nutrient broth (NB) (oxoid B241116) and 3-methylcolanthrene (oekanal, 200-276-4) were also purchased. 2-AF (dissolved in DMSO), NPD (dissolved in DMSO) and SA (dissolved in distilled water) were used as positive controls.

1.3.3.2 Preparation of S9

The S9 mix was prepared as follows before use (for totally 20 mL). 0.5 mL of S9 mix was used for each plate.

Rat liver microsome fraction (S9) (3-methylcolanthrene induced)	2 mL
$MgCl_2$ (8 mmol·L^{-1})-KCl (33 mmol·L^{-1}) salts	0.25 mL
Glucose-6-phosphate	0.04 g
NADP	0.102 g
0.2 mol·L^{-1} phosphate buffer (pH value of 7.4)	6.25 mL
Sterile double distilled water	11.50 mL

1.3.3.3 Bacterial Strains

For the test mutagenicity, all the test substances were dissolved in acetone and used at different concentrations per plate (Table 1).

1.3.3.4 Statistical Significance

The significance between control revertants and revertants of treated groups were determined using *t*-test. Dose-response relationships were determined using regression and correlation (*r*) test systems.

1.3.3.5 Teratogenicity Assay

The teratogenicity assay was performed according to OECD guideline [12-13]. The embryos of the rats (female, 10-12 weeks old) were used for teratogenicity studies as shown in Table 2. The rats were maintained

Table 1 Concentrations of test compounds (μg/plate) used in mutagenicity assay

1a	**1a**-Co	**2a**	**2a**-Fe	**2a**-Co	**3a**	**3a**-Co
4.44	15.00	2.70	30.00	32.25	2.52	33.00
8.88	30.00	5.40	60.00	64.50	5.04	66.00
17.76	60.00	10.80	120.00	129.00	10.08	132.00

Table 2 Concentrations of test compounds ($\mu g \cdot kg^{-1}$(bw)) used in teratogenicity assay

1a	**1a**-Co	**2a**	**2a**-Fe	**2a**-Co	**3a**	**3a**-Co
0.08	1.0	0.045	1.0	1.29	0.04	1.1
0.16	2.0	0.090	2.0	2.58	0.08	2.2

during 5 days for adaptation before teratogenicity studies under controlled temperature (22±1 ℃) with 12 hour light/12 hour dark cycle. Test compounds were dissolved in distilled water and the skeletal segments were characterized[14].

At the present study, it was reported that one substance (**1a**) was mutagenic at TA98 strain with and without metabolic activation while it was also mutagenic at TA100 strain with metabolic activation. The other two substances (**2a**-Fe and **2a**-Co) were not mutagenic for both TA98 and TA100 strains with and without metabolic activator S9mix. The three substances (**2a**, **3a** and **3a**-Co) were mutagenic in TA98 strain especially in the absence of S9mix. All the test compounds decreased the fetus body weight and caused embryotoxicity that three of them (**3a**, **2a**-Co and **1a**-Co) caused to incomplete ossify because of growth retardation. However **1a**-Co was teratogenic and **3a** and **2a**-Co could be concluded as questionable teratogenic substances.

It could be concluded that **1a**-Co, **2a**-Fe and **2a**-Co substances showed a weak mutagenic effects or they could be determined as non mutagenic in Ames Test. **2a**, **3a** and **3a**-Co substances were mutagenic because of increasing the number of revertants in TA98 strain without S9mix. However these substances did not cause mutation in tester strains with S9mix. So, it could be concluded that **2a**, **3a** and **3a**-Co substances would be converted to non-mutagenic metabolites *in vivo* systems.

All the test substances decreased the body weight of fetuses and increased the number of dead fetuses in rats. Incomplete ossify was observed in fetuses with growth retardation. **1a**-Co showed teratogenic effect because of causing skeletal abnormality such as wave ribs in all concentrations. **3a** and **2a**-Co had a teratogenic effect only in one concentration (0.08 and 1.20 $mg \cdot kg^{-1}$ body weight, respectively). According to this result **2a**-Co and **3a** could be concluded as questionable teratogenic substances.

2 Results and discussion

The mixed ligands including azo, anthraquinone and s-triazine moieties substituted 2-amino benzene sulfonic acid, 1-amino-*p*-(β-sulfatoetyl sulfonic acid and 5-amino naphthalene-3-sulfonic acid (in Table 3), were synthesized according to previous work as shown in Figure 1[9-10]. The products were obtained in 47%~79% yield after purification. Analytical data indicate that there are correlations among the results.

Table 3 Synthesized compounds and binding functional groups

Compound	Functional Groups	
	R1	R2
1a	$PhSO_3H$	$PhSO_3H$
2a	$PhSO_2CH_2CH_2OSO_3H$	$NaphtSO_3H$
3a	$NaphtSO_3H$	$PhSO_2CH_2CH_2OSO_3H$

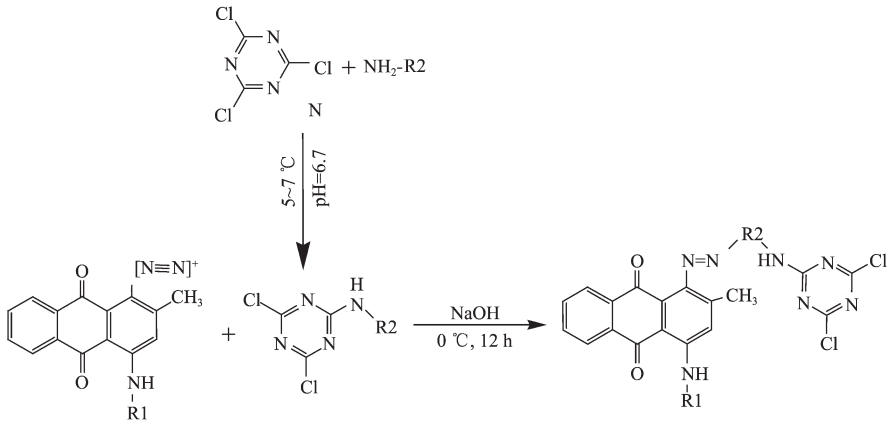


Fig.1　General coupling reaction for the synthesized azo compounds (R1 and R2 are shown in Table 3)

2.3　FTIR Spectra

The primer amine asymmetric-symmetric stretching vibration bands at 3 400 ~3 200 cm^{-1} assigned to ν(N-H) are belong to the starting material vanished after diazotization and the sec-amine peaks were observed near 3 400 cm^{-1} in the FTIR spectra of the all ligands. Typically, **1a**, **2a** and **3a** showed the peaks assigned to ν(S=O) band at 1 136~1 261 cm^{-1}, ν(C=N) band at 1 548~1 591 cm^{-1} and ν(Ar-Cl) band between 1 033 ~1 110 cm^{-1} in *S*-triazine group in Table 4. ν (N =N) stretching bonds belong to the ligands shifted from 1 398 ~1 437 cm^{-1} to 1 399 ~ 1 442 cm^{-1} in their metal complexes [10]. This situation indicates coordination of azo group to the metal ions and as expected adjacent carbonyl acts as complementary dentate for the coordination number [15-16]. Moreover intensities of ν(C=O) bands decrease without any shifts. The new observing peaks between 535~584 cm^{-1} could be attributed to metal-oxygen bonds ν (M-O) [17-18] as shown in Table 4.

Table 4　Characteristic bands in the FTIR spectra (cm^{-1}) of the synthesized compounds

Compound	N=N	N-H	C=N	Ar-Cl	C=C	C=O	CH_3	S=O	M-O	M-N
1a	1 437	3 317	1 550	1 068	1 590	1 673	1 303	1261	–	
1a-Co	1 442	3 412	1 581	1 110	1 588	1 673	1 339	1 163	559	432
2a	1 407	3418	1 549	1 036	1 590	1 673	1 267	1 177	–	
2a-Co	1 437	3 330	1 548	1 068	1 590	1 673	1 292	1 162	577	435
2a-Fe	1 436	3 317	1 552	1 067	1 590	1 673	1 263	1 207	584	437
3a	1 424	3 301	1 558	1 033	1 589	1 675	1 230	1 141	–	
3a-Co	1 486	3 371	1591	1 094	1 676	1 676	1 399	1 136	535	broad band

2.4　UV-Vis Spectra

Electronic absorption spectra of the synthesized compounds were recorded at room temperature in EtOH as shown in table 5.

The three different absorption bands were observed belong to synthesized compounds. The absorption band of the starting material, 1-amino 4-bromo 2-methyl anthraquinone, is attributed to $n\rightarrow\pi^*$ transition (λ_{max}=452 nm). The same attributed peaks were observed at 450, 481 and 458 nm for **1a**, **2a** and **3a**, respectively. The characteristic $n\rightarrow\pi^*$ transition peaks were observed between at 318~342 nm for the azo compounds. In UV spectra, the shoulder is assigned to $n\rightarrow\pi^*$ transition at 323~347 nm for -N=N- group[19].

When the metal attaches to the **3a**, its absorption intensity decreases and $n\rightarrow\pi^*$ transition band shifts batochromically for **3a**-Co[18].

2.5　^{1}H and ^{13}C NMR Spectra

The NMR spectra of all compounds are simple

Table 5 UV Spectra of the synthesized compounds, EtOH

Compounds	λ / nm π →π*	λ / nm (azo) n→π*	λ / nm n→π*	ε_{max} / (L·mol^{-1}·cm^{-1})
1a	251	330	450	1.29×10^3
1a-Co	254	333	452	1.00×10^3
2a	248	318	481	6.96×10^3
2a-Co	284	328	480	7.98×10^3
2a-Fe	247	318	480	5.57×10^3
3a	276	342	458	4.31×10^3
3a-Co	254	334	489	3.12×10^3

and confirm the expected structures. The samples were measured in DMSO-d_6 solvent at 25 ℃.

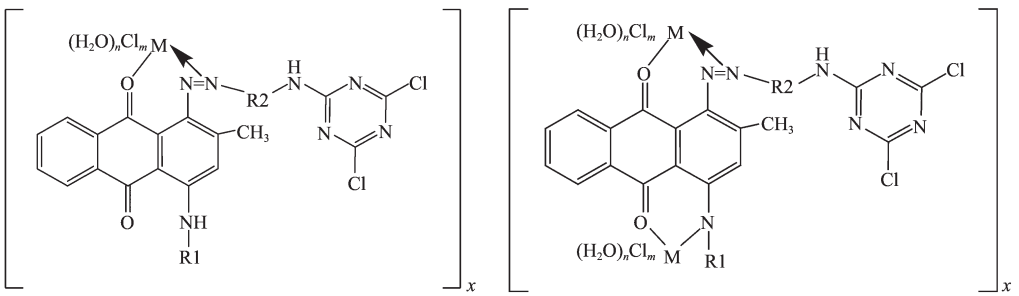
The singlet signal at δ 2.4 ppm is assigned to 3 protons (-CH_3), at δ 7.6~7.7 ppm multiple peaks of 2 protons in quinone ring. The multiple signals belong to (C-H) neighbour of carbonyl group in quinone appear at δ 7.9~8 ppm. The protons over the carbon which is neighbour of methyl group appear at δ 7.1~7.2. The value of chemical shift for methyl group which is electron donor moiety indicates shielding effect. In the ^{1}H-NMR spectra of ligands, the peak belong to the starting material, (NH_2) at δ 3.3 ppm disappears after diazotization process. The singlet peak for the (N-H) binding *S*-triazine ring showed around δ 10 ppm but another contribution was not observed by the heterocyclic structure which has not any protons[16,19].

The carbon peaks belong to (CH_3) group bonding anthraquinone was measured in the range δ 17~21 ppm as singlet in all the ligands. The aromatic carbons belong to anthraquinone ring gave multiple signals near δ 121~146 ppm. Furthermore, the peaks between δ 143~146 are assigned to (C-N=N-Ph). The carbon peaks of s-triazine were observed at 152~163 (NH-C-N- (triazine)). Typically, the signals of carbonyl units (C=O) which is common in all the ligands on ketone group give in the range δ 182~184 ppm as singlet[20-22].

2.6 Atomic Absorption Spectra and analytical data

The calculations of metal percentages show that the molar ratios are estimated as 2∶1 for all cobalt complexes and 1∶1 for **1a**-Fe as given in experimental section. These values are supported by the obtained results of Job′ s method. The synthesized metal complexes include two chloride atoms in their second valance shells, the resultant of argentometric titration data are shown in Figure 2[23].

The Bohr Magnetone of **2a**-Fe, which has free valance electrons, was measured as 5.17 B.M. The mononuclear complex exhibits the weak field ligand effects [24-25]. It is high spin complex for d^5 electron configuration. The magnetic interaction between ligand and metal causes deviation in magnetic susceptibility value [26].



a. M:$Fe^{Ⅲ}$ (n=3, m=1) b. M:$Co^{Ⅱ}$ (n=3~4, m=1~0)

Fig.2 Plausible structures for the synthesized metal complexes (n_L:n_M), a. 1:1 b. 1:2 ratios (coordination number 6), x=Cl

2.6 Biological Results

The test compounds were pre-treated for their toxic effects on *S. typhimurium* tester strains before application of the mutagenicity test system. The tester strains were treated with three concentrations of the test compounds in the presence and absence of microsomal fractions. The results of mutagenicity of test compounds are shown in Table 6.

All substances decrease the fetuses born weight. All the substances also increase the rate of dead fetuses except **2a**-Fe. The **3a**, **2a**-Co and **1a**-Co also moderately increase the skeletal abnormalities in the

Table 6　Mutagenic effects of synthesized compounds inTA98 and TA100 strains of *S. typhimurium* in the presence and absence of microsomal fraction (S9)

Test compd.	Concentrat. μg / plate	TA98		TA100	
		-S9	+S9	-S9	+S9
Control	-	25.66±2.33	24.00±2.72	136.33±5.58	149.00±8.32
Acetone	100 μL/plate	28.50±2.26	28.16±5.05	140.66±5.15	142.16±11.63
NPD	100	6139.00±138.40	-	-	-
2-AF	20	-	2715.33±287.92	-	1591.00±146.84
SA	2	-	-	1170.67±101.39	-
1a	4.44	137.16±4.29 a3b3	49.33±7.72 a1b1	180.00±7.47 a3b2	144.16±5.69
	8.88	266.34±17.11 a3b3	80.83±8.61 a3b2	207.83±10.81 a3b2	130.83±2.73
	17.76	665.17±13.18 a3b3	323.50±36.29 a3b3	298.33±14.82 a3b3	154.66±3.87 b1
1a-Co	15.00	26.16±1.66	29.83±4.11	171.00±9.49 a1	140.83±12.93
	30.00	35.17±1.30 a3b2	27.50±4.03	160.83±6.33 a1	142.66±8.57
	60.00	33.66±0.84 a3b2	26.83±1.72	183.50±0.85 a3	144.66±9.64
2a	2.70	34.33±1.02 a3b2	29.83±3.34	128.00±8.62	134.50±4.29
	5.40	44.00±2.72 a3b2	30.66±4.46	157.66±6.92 a1b1	170.50±11.37
	10.80	51.00±2.28 a3b2	43.66±4.82 a2b1	120.00±9.39 b2	151.33±07.00
2a-Fe	30.00	33.00±3.07	25.33±2.60	167.50±3.01 a3	137.16±6.09
	60.00	32.00±1.75 a2	20.00±2.15	176.50±6.41 a2	135.66±9.29
	120.00	36.83±2.12 a2b1	22.66±2.71	165.00±8.95 a1	136.66±8.74
2a-Co	32.25	36.16±1.60 a3b2	23.83±4.52	158.66±9.22	156.66±6.13
	64.50	30.50±1.47 a2	29.33±2.18	172.33±7.76 a2	142.66±10.60
	129.00	29.83±0.60 a3	22.50±3.62	171.00±6.60 a2	145.33±08.57
3a	2.52	58.50±4.91 a3b2	27.16±4.01	126.00±8.71	158.16±5.94
	5.04	82.50±4.80 a3b3	39.00±6.13 a1	155.50±8.97	153.16±9.62
	10.08	128.00±3.33 a3b3	59.33±11.26 a1b1	154.00±5.26 a1b1	155.66±8.86
3a-Co	33.00	37.16±1.57 a3b2	26.66±3.63	168.50±6.16 a2	132.16±12.17
	66.00	43.16±2.18 a3b3	24.00±2.58	166.83±6.16 a2	158.16±7.84
	132.00	45.50±1.23 a3b3	22.16±3.04	177.50±5.50 a3	149.84±4.82

Significant when compared with control (a) and with solvent control acetone (b)

a1b1: $P<0.05$; a2b2: $P<0.01$; a3b3: $P<0.001$

NPD: 4-nitro-*o*-phenylenediamine, 2AF: 2-aminoflourene, SA: sodium azid

Table 7 Effects of synthesized compounds on caesarean section parameters and on skeletal systems in rats

Test compd.	Conc./ (mg.kg^{-1} bw)	Dam weightaGD0/ GD19	Corpora lutea± SD[d]	Total implants± SD[d]	Dead fetuses± SD[c]	Live fetuses± SD[d]	Fetus weight± SD[d]	Skeletal abnormality	
								Dams with abnormal fetuses(%)[e]	Number of abnormal fetuses(%)[f]
Control	–	195.4±5.30/ 252.2±7.2***	11.6±1.69	11.0±0.70	0.0	10.4±0.40	2.63±0.09	0(0)	0(0)
Acetone	0.5 mL·k·g^{-1}	197.2±10.2/ 251.2±9.3**	9.4±1.24	9.0±0.31	0.0	9.0±0.70	2.60±0.03	1(20)	1(2.32)
EMS	100 g·k·g^{-1}	231.0±9.4/ 244.4±10.4	10.4±1.16	8.6±1.43	12.9±7.19	1.6±2.07	1.63±0.05	2(40)	2(40)
1a	0.08	180.6±18.6/ 206.8/14.4	10.2±0.96	12.6±0.97	20.6±4.03**	9.8±0.80	2.12±0.05***	0(0)	0(0)
	0.16	160.2±9.9/ 211.0±7.5**	9.6±0.24	9.6±0.24	12.4±3.69*	8.4±0.40	2.32±0.04***	0(0))	0(0)
1a-Co	1.0	195.2±8.9/ 234.0±10.1*	9.2±0.91	6.8±1.95	0.0	6.8±1.95	1.83±0.06***	3(60)	5(14.70)
	2.0	180.0±3.4/ 221.0±6.3**	10.0±0.70	7.0±0.70*	14.2±0.31***	6.0±0.30***	1.40±0.04***	4(80)	5(16.66)
2a	0.045	206.6±7.8/ 224.2±0.3***	9.0±0.31	8.4±0.24	16.9±3.29**	5.8±0.73*	1.87±0.12***	0(0)	0(0)
	0.090	182.8±7.6/ 222.8±7.4**	7.4±0.60*	7.8±0.73	6.9±2.81*	7.2±0.58*	2.25±0.04***	0(0)	0(0)
2a-Fe	1.0	217.6±11.0/ 239.4±8.7	9.0±0.29	5.0±1.22*	0.0	4.4±1.46*	1.89±0.09***	0(0)	0(0)
	2.0	177.2±0.4/ 209.2±14.7	8.0±0.54	8.4±0.40	0.0	8.4±0.40	2.12±0.02***	0(0)	0(0)
2a-Co	1.29	138.0±2.5/ 171.0±4.6	11.0±0.70	11.0±0.73	62.0±6.63***	2.0±0.35***	0.92±0.01***	5(100)	10(100)
	2.58	199.6±12.5/ 222.2±12.3	10.0±0.44	8.0±0.70	0.0	8.0±0.32*	1.85±0.04***	0(0)	0(0)
3a	0.04	190.0±3.1/ 230.0±3.1***	11.0±0.63	8.0±0.31*	25.0±1.58***	6.0±0.89*	2.04±0.01***	0(0)	0(0)
	0.08	186.0±9.2/ 215.8±13.9	4.8±0.48***	7.2±0.70*	8.8±5.44	5.2±0.49***	1.83±0.13***	2(40)	4(16.66)
3a-Co	1.1	190.0±3.1/ 221.0±2.8***	10.0±0.92	7.4±1.16	0.0	6.0±0.70*	1.79±0.01***	0(0)	0(0)
	2.2	188.0±2.5/ 205.0±3.1**	11.0±0.94	10.0±0.63	10.0±0.63***	9.0±0.44	2.31±0.02***	0(0)	0(0)

[a]:Totally 5 dams were used for each group and concentrations. GD0: Starting of gestation, 0.day, GD19: end of gestation, 19.day, the weight at GD0 and GD19 were statistically compared; [b]: No fetuses obtained; [c]: The percentage of died fetuses in total number of implants; [d]: Mean±SD (standard deviation); [e]: The percentage of Dams with abnormal fetuses in total number of dams; [f]: The percentage of number of abnormal fetuses in examined fetuses; *: Significant when compared with control; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$.

fetuses such as incomplete ossify. Two test compounds (**3a** and **2a**) only decrease the number of *C.lutea* at the highest concentration while the others do not decrease the number of *C.lutea*. **3a**, **2a**-Fe and **1a**-Co decrease the number of implantations.

According to the results of Ames Test, **2a**-Fe and **2a**-Co substances are not mutagenic for both TA98 and TA100 strains with and without metabolic activator S9 mix. However, **2a**, **3a** and **3a**-Co substances are mutagenic in TA98 strain especially in the absence of S9mix. **2a** and **3a** show a mutagenic effect in TA100 strain in the absence of S9mix at the high concentration only. In the presence S9mix, **2a** and **3a** show a mutagenic effect in TA98 strain at the highest doses only. It can be concluded that, **2a** and **3a** would not be mutagenic whether they are used at low concentrations. Also, there are no any dose-dependent effects in all substances used except **3a** substance which causes dose-dependent increase in number of revertants in TA98 strain without S9mix (r= 1, P<0.009). On the other hand, **1a** is mutagenic at TA98 strain with and without metabolic activator while it is mutagenic at TA100 strain without metabolic activator, however it is not mutagenic in TA100 strain with S9mix (Table 7).

3 Conclusions

In this paper, we report the synthesis and characterization of 1-amino,4-bromo,2-methyl anthraquinone with anilin-2-sulfonic acid, 5-Amino-2 naphthalene-sulfonic acid and *p*-β-sulfatoetyl sulfonic acid using *S*-triazine ring as a bridge. Their Co^{II}, and Fe^{III} complexes have been discussed. The estimated structures of the synthesized compounds were characterized with UV-Vis, FTIR, ^{13}C and ^{1}H NMR, AAS, magnetic susceptibility and argentometric titration techniques. Single crystals of the synthesized compounds could not be isolated from any solution; thus no definite structure may be described. However, our proposed structural formula is given in Figure 1. The octahedral structures are supported by using characterization techniques. The divalent Co complexes and trivalent iron complex exhibit as 2:1 and 1:1 molar ratio, respectively. They have two chloride atoms in their second valance according to argentometric methods.

As a result, it is determined that **2a**-Fe substance is not mutagenic and teratogenic. **2a**-Co is not mutagenic however is questionable for its teratogenic effect. **3a**-Co also is not mutagenic in the presence of S9mix and it is not teratogenic. Consequently **2a**-Fe, **2a**-Co and **3a** could be carefully used as a dyeing substance in textile industry.

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Abbreviations

BINAP: 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl

DCM: Dichloromethane

Ph: Phenyl

Napht: Naphthalene

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