Articles

Development of Cobalt(3,4-diarylsalen) Complexes as Tumor Therapeutics

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[1,6-Bis(2-hydroxyphenyl)-3,4-diaryl-2,5-diazahexa-1,5-diene]cobalt(II) complexes (cobalt(3,4-diarylsalen)) with 2-, 3-, or 4-OCH_3/OH substituents in the 3,4-standing aryl rings were synthesized and tested for antitumor activity in vitro on the MCF-7, MDA-MB 231, and LNCaP/FGC cell lines. The cytotoxicity depended on both the configuration of the diene ligand and the kind of substituents in the 3,4-standing aromatic rings. **d,l-7** (2-OCH_3), **d,l-8** (3-OCH_3), and **d,l-9** (4-OCH_3) were equipotent to cisplatin, while the respective hydroxy-substituted complexes (**d,l-10** (2-OH), **d,l-11** (3-OH), and **d,l-12** (2-OH)) as well as all of the meso-configured compounds (**m-7** to **m-12**) did not influence the cell growth. Interestingly, a high catalytic potency and a rapid and high accumulation in MCF-7 cells (15- to 25-fold compared to the cell culture medium (5 μM)) were demonstrated for **m-7** (2-OCH_3), **m-8** (3-OCH_3), and **m-9** (4-OCH_3). Therefore, a mode of action based on a cobalt-catalyzed oxidative damage of the DNA is not very likely.

Introduction

The pioneering work of Barnett Rosenberg on the antitumor activity of *cis*-diamminedichloroplatinum(II) (cisplatin) is an important milestone in cancer chemotherapy.[1,2] Cisplatin found its first clinical application in the early 1970s and is currently one of the most successful cancer chemotherapeutics. This great success of an "old" drug (cisplatin was first synthesized by Peyrone in 1844) led to the search for well-known compounds that were rarely discovered as potential active agents.

Recently, we identified acetylenehexacarbonyldicobalt complexes as a new class of antitumor agents.[3,4] We demonstrated a strong dependence of the antitumor activity as well as the tumor selectivity on the kind of alkyne ligand. The most active derivative [(2-propyn-1-yl)acetylsalicylate]hexacarbonyldicobalt showed a high selectivity for human breast cancer cells and was even more active than cisplatin.[3]

A further very interesting class of potential cytotoxic drugs represent cobalt(salen) complexes (salen = 1,6-bis(2-hydroxyphenyl)-2,5-diazahexa-1,5-diene) mainly developed as reversible oxygen carriers and catalysts for oxidation reactions.[5] However, there are several indications that these compounds can also catalyze the building of hydroxy radicals under physiological conditions.[6] Singlet oxygen and superoxides described as reactive species may inflict damages to the DNA.[7] Therefore, we tried to design cobalt(salen) complexes with high cytotoxicity and selectivity against tumor cells.

While designing these complexes, it was possible to consider the results of structure−activity relationship studies in the class of [1,2-diamino-1,2-diarylethane]-platinum(II) complexes. It was demonstrated that the 1,2-diarylethane moiety has to be OCH_3- or OH-substituted to get complexes with high tumor selectivity.[8−12] Because this pharmacophor can be realized in a similar spatial arrangement in [1,6-bis(2-hydroxyphenyl)-3,4-diaryl-2,5-diazahexa-1,5-diene]cobalt(II), we synthesized OH-phenyl and OCH_3-phenyl substituted cobalt(3,4-diarylsalen) complexes and studied their antiproliferative effects on the MCF-7 and MDA-MB 231 breast cancer as well as the LnCaP/FGC prostate cancer cell lines.

Synthesis and Structural Characterization

The 1,6-bis(2-hydroxyphenyl)-3,4-diaryl-2,5-diazahexa-1,5-dienes were synthesized by reaction of the corresponding diastereomerically pure 1,2-diamino-1,2-diarylethanes[8,9,12] with salicylaldehyde (Scheme 1). The subsequent coordination to cobalt was performed according to Berkelew and Calvin[13] using cobalt(II) acetate.

IR spectroscopy, mass spectrometry, and elemental analysis allowed the characterization of the new complexes. NMR spectroscopy could not be used because of the marked paramagnetism of the complexes.

The coordination of 3,4-diarylsalenes and the building of a square-planar chelate complex led to characteristic changes in the fingerprint region of the IR spectra. The C=N stretching vibration determined for the ligands at 1623−1627 cm^{-1} was shifted to lower wavenumbers between 1601 and 1608 cm^{-1}. Furthermore, the bending vibration disappeared in the area at about 1278 cm^{-1}.

A characteristic fragmentation of the cobalt(3,4-diarylsalen) complexes could be observed in the mass spectra. Two fragments resulting from the consecutive

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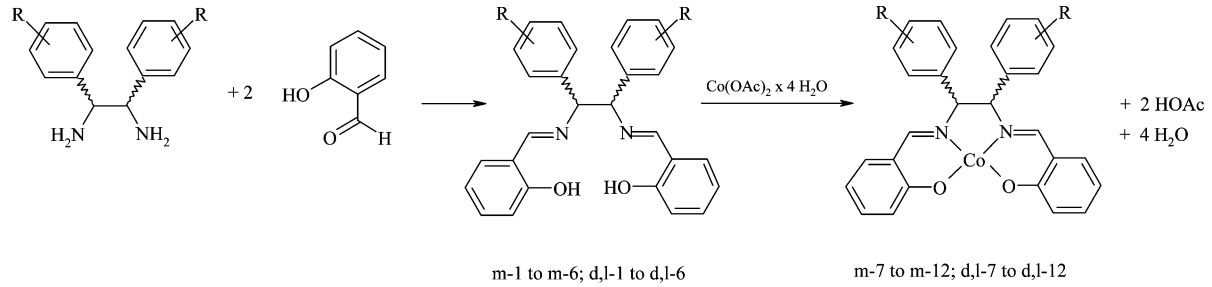
Scheme 1

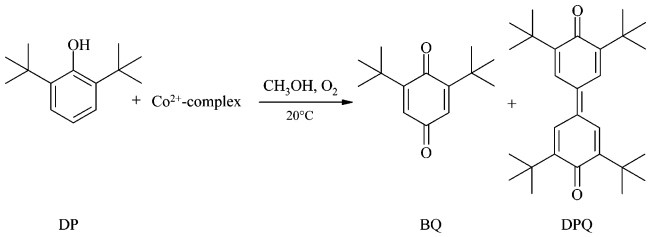


m-1 to m-6; d,l-1 to d,l-6

m-7 to m-12; d,l-7 to d,l-12

R	compound		R	compound	
2-OCH_3	m-1	m-7	**2-OH**	m-4	m-10
	d,l-1	d,l-7		d,l-4	d,l-10
3-OCH_3	m-2	m-8	**3-OH**	m-5	m-11
	d,l-2	d,l-8		d,l-5	d,l-11
4-OCH_3	m-3	m-9	**4-OH**	m-6	m-12
	d,l-3	d,l-9		d,l-6	d,l-12

Scheme 2



DP BQ DPQ

elimination of a salicylimine fragment and a benzyl fragment were found besides the molecular ion peak.

Investigations of the Catalytic Activity

The catalytic activity was evaluated using the cobalt-catalyzed oxidation of 2,6-di-*tert*-butylphenol (DP) first described by van Dort and Geursen.[14] This reaction yielded a mixture of benzoquinone (BQ) and diphenoquinone (DPQ) (Scheme 2) whereby the formation of BQ served as a measure of the catalytic potency of the complexes. The higher the relation between BQ and DPQ, the stronger the catalytic effect of the cobalt complex.

Nishinaga[15] and Drago[16] already elucidated the steps involved in this reaction, which starts with the building of a cobalt/O_2 complex. The reaction of this superoxo compound with DP yields a phenoxy and an H_2O radical. While the latter disproportionates into H_2O_2 and O_2, the phenoxy radical reacts with the cobalt/O_2 complex to give a cobalt peroxocyclohexa-2,5-dienone, which decomposes into BQ. If the reaction with the activated cobalt complex is disturbed, the concentration of the phenoxy radical correspondingly increases and the coupling to DPQ is observed.

The quantification of the reaction was performed by ^{1}H NMR spectroscopy using the integrals of the *tert*-butyl groups as a parameter (DP, $\delta = 1.28$; DPQ, $\delta = 1.37$). The reaction conditions were chosen (see Materials and Methods) to get a quantitative conversion (100% BQ) with cobalt(3,4-diarylsalen).

The results of the diastereomeric complexes **m-7** and **d,l-7** to **m-9** and **d,l-9** are listed in Table 1. Complexes **m-7** to **m-9** oxidized DP nearly quantitatively (> 90%) into BQ (see Table 1), while the *d*,*l*-configured compounds **d,l-7** to **d,l-9** caused only an approximately 50%

Table 1. Catalytic Effect of [1,6-Bis(2-hydroxyphenyl)-3,4-diphenyl-2,5-diazahexa-1,5-diene]cobalt Complexes, Showing the Conversion of 2,6-di-*tert*-butylphenol (DP) to Benzochinone (BQ) and Diphenochinone (DPQ) According to Scheme 2

	m-7, %	**m-8**, %	**m-9**, %	**d,l-7**, %	**d,l-8**, %	**d,l-9**, %
DP	5	8	1	56	42	45
DPQ	1	1	2	38	54	51
BQ	94	91	97	6	4	4

conversion of DP mainly into DPQ. Interestingly, the catalytic behavior of the complexes was independent of the position and the kind of substituents in the 3,4-standing aromatic rings. The hydroxy-substituted compounds **m-10** and **d,l-10** to **m-12** and **d,l-12** showed effects simliar to those of their methoxy derivatives (data not shown).

Biological Activity

The antitumor activity was determined in vitro using the hormone-dependent MCF-7 and the hormone-independent MDA-MB 231 breast cancer and the LNCaP/FGC prostate cancer cell lines.

In a preliminary study, the influence of Co(salen) (Figure 1, right) and $CoCl_2$ (Figure 1, left) was evaluated against MCF-7 cells. Both compounds showed a concentration-dependent reduction of cell proliferation. Co(salen) was more active than $CoCl_2$ and caused

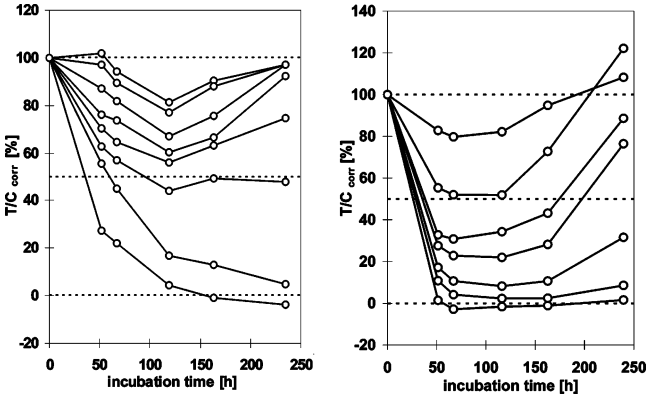


Figure 1. Antiproliferative effects of $CoCl_2$ and Co(salen) on the MCF-7 breast cancer cell line: (left) $CoCl_2$ concentrations of 20, 40, 60, 80, 100, 120, 150, 200 μM; (right) Co(salen) concentrations of 10, 20, 30, 40, 50, 60, 70 μM.

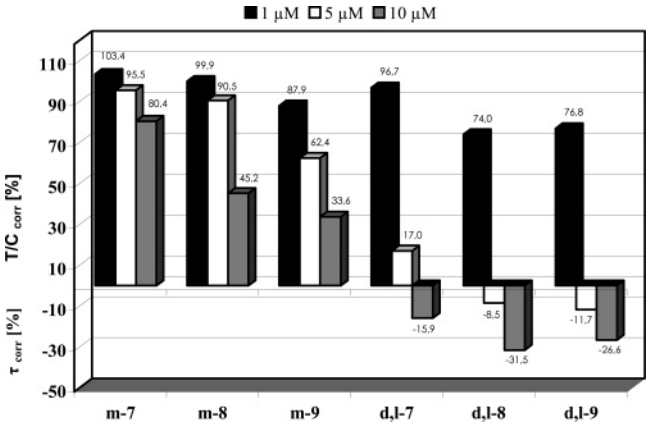


Figure 2. Cytotoxicity (maximal effects) of cobalt(3,4-diarylsalen) complexes against MCF-7 cells.

antiproliferative effects in concentrations higher than 10 μM. Cytocidal effects were achieved in concentrations of about 60−70 μM. Cisplatin possessed a considerably higher activity and completely blocked the growth of the MCF-7 cells at a concentration of 5 μM (Figure 7).

Introduction of aryl rings into the 3,4-positions of the salen ligand enhanced the antitumor activity of Co(salen), which is dependent on the configuration at the asymmetric C atoms and the substitution pattern of the aromatic rings. The growth inhibition increased in the following series (Figure 2): **m-7** (2-OCH_3) < **m-8** (3-OCH_3) < **m-9** (4-OCH_3) < **d,l-7** (2-OCH_3) < **d,l-8** (3-OCH_3) = **d,l-9** (4-OCH_3).

All complexes reached their maximum effects at the beginning of the test. While **m-7** showed only marginal effects (T/C = 80.4% at 10 μM), **m-8** and **m-9** reduced the cell proliferation by more than 50% (T/C = 45.2% (**m-8**) and T/C = 33.6% (**m-9**) at 10 μM). Even cytocidal effects were achieved with **d,l-7** (τ = −15.9% (10 μM)), **d,l-8** (τ = −8.5% (5 μM) and −31.5% (10 μM)), and **d,l-9** (τ = −17.7% (5 μM) and −26.6% (10 μM)).

The time−activity curves of **d,l-7** to **d,l-9** presented in Figure 3 show a recuperation of the tumor cells after a prolonged exposition. Because exponential cell growth is guaranteed for at least 250 h of incubation, the rise of the growth curve can be explained by the development of drug resistance,[17] which depends on the position of the OCH_3 group in the aromatic ring. While the cells treated with **d,l-7** showed at 5 and 10 μM a strong development of resistance, the shift of the methoxy group into the 3- (**d,l-8**) and the 4-positions (**d,l-9**) reduced this effect significantly. **d,l-8** and **d,l-9** were comparably active to cisplatin and even caused cytocidal effects ($\tau \approx -20\%$ to -40%) at 10 mM concentration during the entire incubation period (244 h).

In contrast to cisplatin, which reached its maximum effect toward the end of the test (see Figure 7), the onset of antiproliferative effects was observed much earlier.

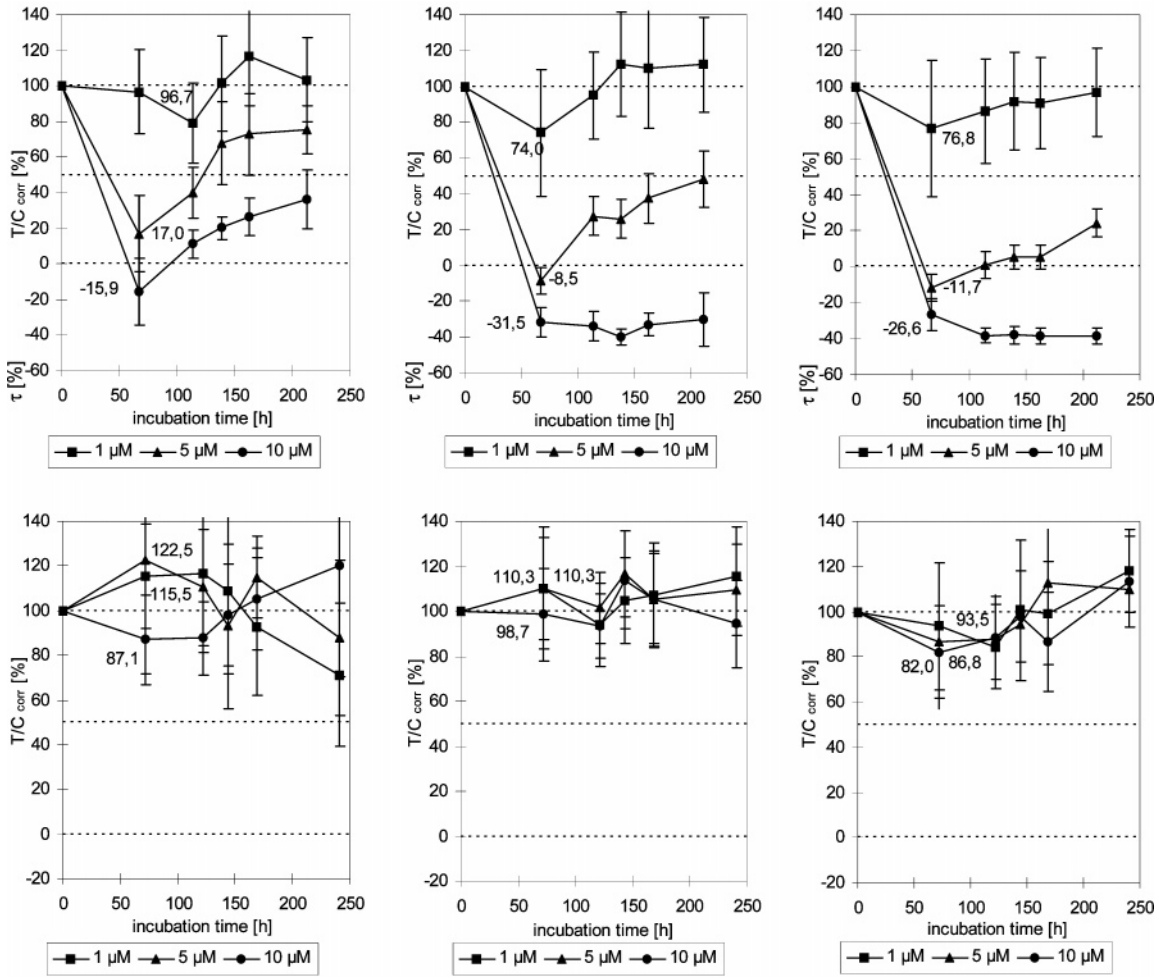


Figure 3. Antiproliferative effects of the cobalt(3,4-bis(methoxyphenyl)salen) complexes **d,l-7** (up, left), **d,l-8** (up, middle), and **d,l-9** (up, right) as well as the respective hydroxyl derivative **d,l-10** (down, left), **d,l-11** (down, middle), and **d,l-12** (down, right).

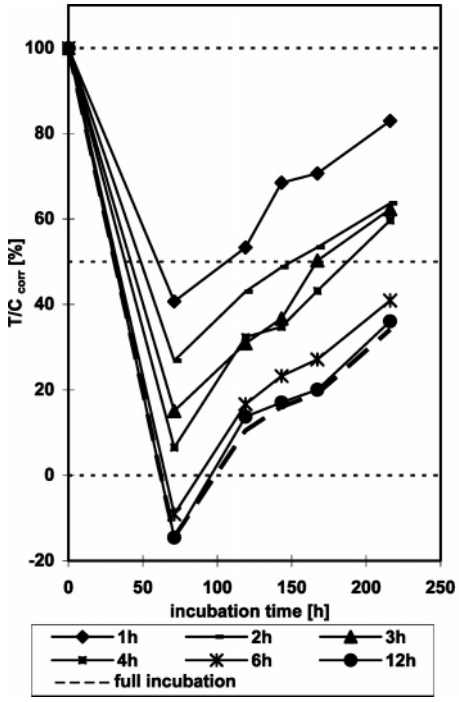


Figure 4. Cytotoxicity of **d,l-9** on the MCF-7 cell line, dependent on the time of drug exposure.

Therefore, we investigated the minimal time of drug exposure to achieve cytotoxic effects on the example of **d,l-9**.

The MCF-7 cells were treated with a drug concentration of 5 μM for 1, 2, 3, 4, 6, or 12 h. The medium was subsequently replaced by a drug-free one, and the test was continued for about 200 h.

As shown in Figure 4, a 1 h drug exposure already led to a cell growth inhibition of about 60%. This effect was continuously enhanced with the elongation of the incubation time. The maximal effect was achieved after 6 h, and the time−activity curve showed only marginal differences compared to the one obtained in the regular experiment (see Figure 3). Therefore, it can be concluded that the complexes were taken up in MCF-7 cells during the incubation time of 6 h in cytotoxic amounts.

To quantify this process, we used electrothermic atomic absorption spectroscopy according to the method already described for cellular studies on [1,2-diamino-1,2-diarylethane]platinum(II) complexes.[18]

The intracellular cobalt concentration increased in a fast rate (see Figure 5) and already reached a saturation point after 100 min. Figure 5 demonstrates that the meso-configured complexes **m-7** to **m-9** were taken up in MCF-7 cells to a much higher level than their diastereomers. The highest intracellular level was achieved with **m-9** (128 μM), which means a 25-fold enrichment compared to the cell culture medium (5 μM). The shift of the *O*-methyl group into the 3- or the 2-position distinctly reduced the accumulation. MCF-7 cells treated with **m-7** and **m-8** showed a cobalt content of 83.5 and 73.0 μM, respectively. The uptake of their *d*,*l*-configured congeners was comparable to that of the lead compound Co(salen) (26.8 μM) and nearly independent of the position of the *O*-methyl substituent (**d,l-7** (23.8 μM), **d,l-8** (22.7 μM), and **d,l-9** (30.7 μM)).

Hydroxy groups in the aromatic rings prevented the uptake of both the *d*,*l*- and *meso*-configured cobalt(3,4-diarylsalen) complexes. Only the incubation with **m-11** (6.1 μM) and **m-12** (7.4 μM) led to a slightly higher cobalt concentration in the tumor cells compared to the medium (5 μM). This finding correlates very well with the missing cytotoxicity of the OH-substituted complexes (see Figure 3).

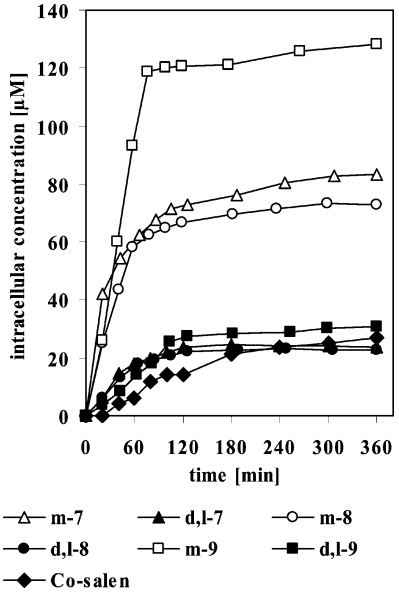


Figure 5. Accumulation of cobalt(3,4-diarylsalen) complexes into MCF-7 cells.

Furthermore, the antiproliferative properties of *d*,*l*-configured cobalt(3,4-diarylsalen) complexes depended on an intact metal complex. The free ligands **d,l-1**, **d,l-2**, and **d,l-3** reduced the cell growth at 5 μM only minimally to *T*/*C* = 83%, 75%, and 71%, respectively.

The same dependence on the salen ligand was determined on the MDA-MB 231 cell line. The OH-substituted and *meso*-configured complexes were completely inactive, while the *O*-methyl derivatives showed a concentration-dependent reduction of the cell growth (Figure 6). They reduced the cell growth at a concentration of 5 μM by 70−90% after an incubation time of 48 h. At higher concentration, the complexes caused cytocidal effects. A comparison of the time−activity curves indicates that the antiproliferative properties decreased slightly with the shift of the *O*-methyl group from the 4-position to the 2-position. Generally, the effect on the MDA-MB 231 cell line was somewhat lower than on the MCF-7 cell line.

The complexes **d,l-7**, **d,l-8**, and **d,l-9** were also tested against LNCaP/FGC cells (Figure 6). They were highly active and reduced the cell growth, comparable to cisplatin (Figure 7). Interestingly, the complexes showed nearly identical cell growth characteristics on the three cell lines. A very fast onset of antitumor activity during the first 48 h of incubation was followed by a development of resistance.

Chemoresistance is a multifunctional phenomenon; however, reduced uptake and increased efflux are the first evidence of resistance for metal complexes. Therefore, we incubated MDA-MB 231 cells with **m-9** and **d,l-9** at a concentration of 5 μM, respectively, and quantified the cobalt content over a period of 120 h. On one hand, **m-9** showed a 21-fold accumulation in the tumor cells after 24 h, which decreased to about 13-fold

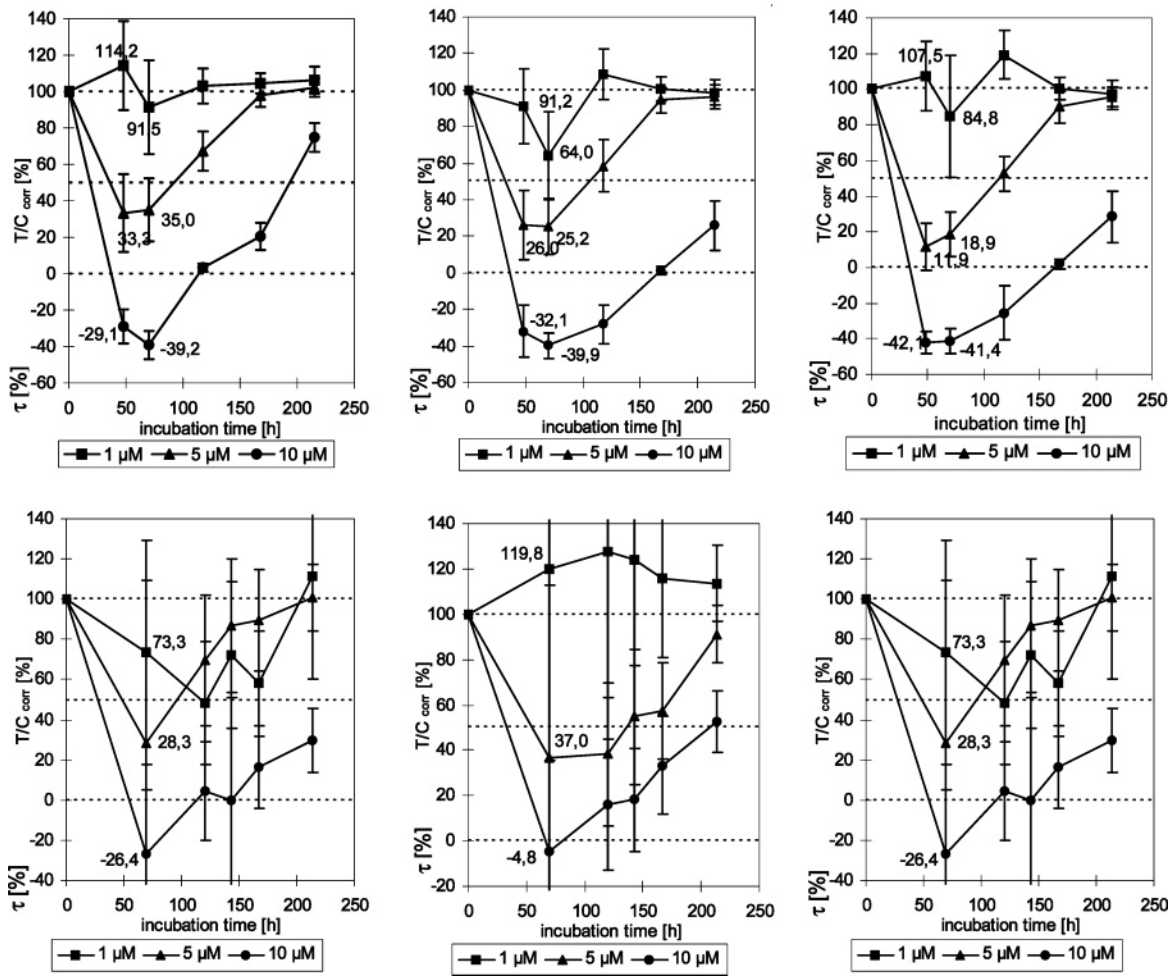


Figure 6. Cytotoxic properties of cobalt(3,4-diarylsalen) complexes **d,l-7** (left), **d,l-8** (middle), and **d,l-9** (right) on the MDA-MB 231 (up) and the LNCaP/FGC cell lines (down).

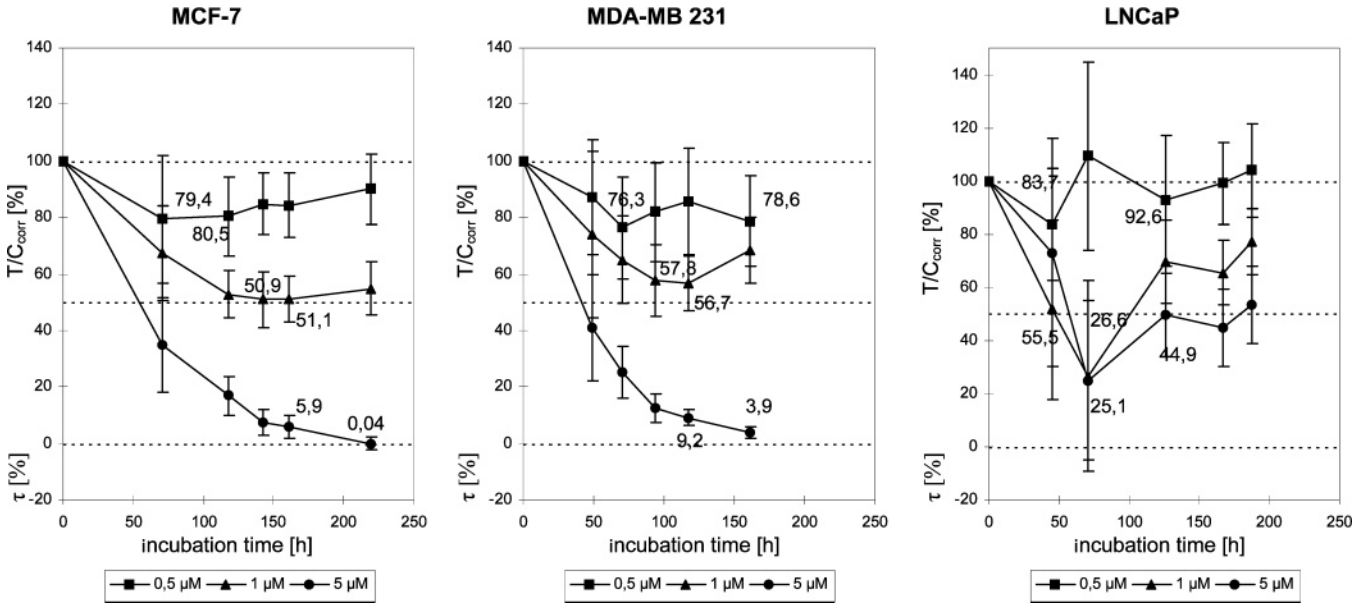


Figure 7. Cytotoxicity of cisplatin on the MCF-7, MDA-MB 231, and LNCaP/FGC cell lines.

during the following 96 h. On the other hand, **d,l-9** holds its 16-fold accumulation during the incubation time of 120 h.

These values did not correlate with the growth curves depicted in Figure 6 and contradict the above-mentioned resistance mechanism. Therefore, other mechanisms have to be considered for the observed phenomenon. Metal complexes such as cisplatin enter the tumor cells by passive diffusion, facilitated diffusion, or active transport. The loss of activity and development of resistance are often associated with elevated glutathione (GSH) levels, enhanced repair of drug-induced DNA

lesions, or overexpression of the P-170 glycoprotein.[19] On the basis of our accumulation studies, the decrease of activity observed for cobalt(salen) complexes after a longer drug exposure may be attributed to these phenomena. However, other cellular defense mechanisms become more likely because cisplatin caused its antitumor effect on the breast cancer cell lines slowly and reached its maximal effect at the end of the test (Figure 7).

Discussion

[1,6-Bis(2-hydroxyphenyl)-3,4-diaryl-2,5-diazahexa-1,5-diene]cobalt(II) complexes represent a new class of antitumor active metal complexes. They showed high antiproliferative activity against hormone-sensitive MCF-7 and hormone-insensitive MDA-MB 231 breast cancer cells as well as LNCaP/FGC prostate cancer cells. The cytotoxicity of the complexes depended on an intact metal complex. The free ligands (data not shown) and Co^{2+} ions did not influence significantly the cell growth at concentrations less than 50 μM.

The aromatic rings at the 3,4-position in the cobalt(salen) complexes played an essential role (see Figures 2 and 3). Both the substituents in the aromatic rings and the configuration of the asymmetric C atoms determined the cytotoxic potency. On one hand, hydroxylation prevented the uptake into the tumor cells and led to completely inactive compounds. On the other hand, *O*-methylation resulted in compounds with cytotoxic potency comparable to that of cisplatin. In this case, however, the ligand had to be *d*,*l*-configured. The respective *meso*-configured diastereomers were enriched in the tumor cells in higher amounts than the corresponding *d*,*l*-configured compounds and showed lower antiproliferative effects despite their higher catalytic activity. It indicates the involvement of more specific processes or interactions inside the tumor cells. Differences in the intracellular distribution and the uptake into the nuclei as well as distinct covalent or intercalative DNA interactions caused by the different stereochemistry of *meso*- and *d*,*l*-configured derivatives must be taken into account in future studies on cobalt(salen) complexes.

The observed discrimination between diastereomers supports the evidence that the uptake of the complexes into tumor cells is carrier-mediated, as already confirmed for the aqua[1,2-diamino-1,2-bis(4-fluorophenyl)-ethane]sulfatoplatinum(II) complexes *d*,*l*-4F-$PtSO_4$ and *meso*-4F-$PtSO_4$.[20] The accumulation of *d*,*l*-4F-$PtSO_4$ was very rapid, with a 14-fold intracellular platinum concentration compared to that of the cell culture medium (5 μM) that was reached within 4 h. With *meso*-4F-$PtSO_4$, only a 3-fold enrichment was achieved.

The 1,2-diarylethane pharmacophor is also realized in the 1,6-bis(2-hydroxyphenyl)-3,4-diaryl-2,5-diazahexa-1,5-diene. These ligands can be considered to be salicylimine derivatives of the 1,2-diamino-1,2-diarylethane carrier ligands.

The spatial structure of [1,6-bis(2-hydroxyphenyl)-3,4-diaryl-2,5-diazahexa-1,5-diene]cobalt(II) complexes could not be evaluated by ^{1}H NMR spectroscopy because of the paramagnetism of the complexes. Therefore, we propose a three-dimensional structure based on already published bond length and angles.[21−23]

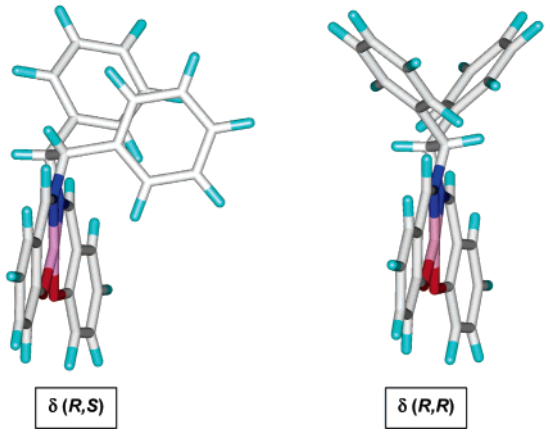


Figure 8. Proposed spatial structure of diastereomeric cobalt-(3,4-diarylsalen) complexes.

Cobalt(3,4-diarylsalen) complexes are square-planar with either bisequatorially (*d*,*l*-configured compounds) or axially/equatorially standing (*meso*-configured compounds) aromatic rings at the ethane bridge (Figure 8). The differing arrangements of the 1,2-diarylethane pharmacophor are of great importance for the pharmacological properties of the complexes. In contrast to the platinum complexes, the orientation towards the cobalt central atom in *meso*-configured compounds forced the transport into the tumor cells and increased the oxidative potency compared to the *d*,*l*-configured diastereomers. Nevertheless, the complexes **d,l-7** to **d,l-9** showed much higher cytotoxicity. Therefore, we assume that DNA damage inflicted by superoxide radical mediated active species only plays a subordinate role.

A mode of action postulated by Liu et al.[24] seems to be more likely. They investigated the interactive effects of co(salen) complexes on the DNA and found that the bonding to DNA decreased the fluorescence intensity accompanied by a bathochromic shift of the excitation and emission peaks. A hypochromism in the UV absorption spectra was also observed. KI quenching and competitive binding to DNA between Co(salen) and ethidium bromide showed that the interactive model between Co(salen) and DNA was an intercalative one.

These studies are quite difficult for cobalt(3,4-diarylsalen) complexes because of their relatively low water solubility. Therefore, we used in an initial study the [*meso*-1,6-bis(2-hydroxy-4-trimethylaminophenyl)-3,4-bis(4-methoxyphenyl)-2,5-diazahexa-1,5-dien]cobalt(II) complex **m-9**-NMe_3^+ as a model. **m-9**-NMe_3^+ induced a significant change of the melting point (ΔT_m) of the DNA. ΔT_m amounted to 2.7 °C at a DNA base pair/ligand ratio of 20 and amounted to 7.2 °C at a DNA base pair/ligand ratio of 40. Compared to other metal(salen) complexes, however, these values were low.[25] This seems to be the consequence of the aryl substituents at the salen ligand, which hinder the approach to the DNA.

This effect was already discussed for [1,2-diamino-1,2-diarylethane]platinum(II) complexes. *meso*-4F-$PtSO_4$ shows a dynamic interconversion of the five-membered chelate ring. The orientation of the aryl substituents during the interconversion of the chelate ring towards the platinum central atom reduced the binding tendency to guanine bases of the DNA, resulting in low cytotoxic effects.

The *d,l*-4F-$PtSO_4$ and related hydroxy- or methoxy-substituted complexes exist in a stable conformation, with exclusively equatorial arranged aromatic rings at the puckered five-membered chelate ring. A conversion into the conformation with axial standing phenyl rings is not observed. A better approach of *d,l*-4F-$PtSO_4$ to the DNA is therefore very likely, resulting in higher cytotoxicity.

This might also be true for **d,l-9** and **m-9**. Unfortunately, the introduction of trimethylamonium groups into the 1,6-standing phenol rings did not increase the solubility of **d,l-9** to determine the DNA intercalation significantly. Nevertheless, these findings demonstrated clearly that the DNA intercalation of cobalt(3,4-diarylsalen) complexes must be taken into consideration as a possible mode of action.

To confirm this assumption, it is necessary to study the interaction of the cobalt(3,4-diarylsalen) complexes with the DNA in detail. Therefore, we intend to optimize the physical properties of cobalt(3,4-diarylsalen) complexes to perform DNA binding studies.

Materials and Methods

General Procedures. The following instrumentation was used: IR spectra (KBr pellets), Perkin-Elmer model 580 A; ^{1}H NMR, Bruker ADX 400 spectrometer at 400 MHz (internal standard, TMS); EI-MS spectra, CH-7A-Varian MAT (70 eV) and Kratos MS 25 RF (80 eV). Elemental analyses were carried out at the Microlaboratory of the Free University of Berlin. Computational graphics were built using SYBYL 6.7 (Tripos Inc. 1699 South Hanley Road, St. Louis, MO, 63144). Geometry optimization was carried out using the MM3 force field within the program, running on an INDY work station.

Syntheses. The 1,2-diamino-1,2-diarylethanes were synthesized as described earlier.[8,9,12]

General Procedure for the Synthesis of 1,6-Bis(2-hydroxyphenyl)-3,4-diaryl-2,5-diazahexa-1,5-dienes. An amount of 1 mmol of the respective 1,2-diamino-1,2-diarylethane was suspended in 50 mL of acetonitrile and reacted with 2.1 mmol of salicylaldehyde. The reaction mixture was heated to reflux for 6 h. The solvent was reduced by half, and the diimine was subsequently allowed to crystallize. The crystals were filtered off, washed with diethyl ether, and dried over $CaSO_4$.

***meso*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(2-methoxyphenyl)-2,5-diazahexa-1,5-diene (m-1). m-1** was obtained from *meso*-1,2-diamino-1,2-bis(2-methoxyphenyl)ethane (1.00 mmol, 272.0 mg) and salicylaldehyde (2.10 mmol, 256.1 mg). Yield: 1.97 mmol (947.5 mg), 94%, pale-yellow crystals, mp 208 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2837 m (OCH_3), 2600 m, br (OH), 1627 s (C=N), 1583 s, 1493 s, 1461 s, 1402 m, 1278 s (OH), 1246 s, 1153 m, 1111 m, 1048 m, 1026 m, 849 m, 754 s. ^{1}H NMR (DMSO-d_6): δ = 3.67 (s, 6H, OCH_3), 5.42 (s, 2H, C*H*), 6.77 (m, 4H, Ar*H*-3, Ar′*H*-3), 6.91 (m, 4H, Ar*H*-5, Ar′*H*-5), 7.26 (m, 8H, Ar*H*-4, Ar*H*-6, Ar′*H*-4, Ar′*H*-6), 8.40 (s, 2H, *NCH*), 13.36 (s, 2H, O*H*).

***d,l*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(2-methoxyphenyl)-2,5-diazahexa-1,5-diene (d,l-1). d,l-1** was obtained from *d,l*-1,2-diamino-1,2-bis(2-methoxyphenyl)ethane (0.491 mmol, 133.8 mg) and salicylaldehyde (1.13 mmol, 126.0 mg). Yield: 0.34 mmol (145.9 mg), 62%, yellow crystals, mp 199 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2937 m (OCH_3), 2600 m, br (OH), 1627 s (C=N), 1600 m, 1594 m, 1492 s, 1460 m, 1277 s (OH), 1246 s, 1150 m, 1050 m, 1028 m, 849 m, 751 s, 664 w. ^{1}H NMR (DMSO-d_6): δ = 3.79 (s, 6H, OCH_3), 5.44 (s, 2H, C*H*), 6.83 (m, 6H, *ArH*-3, Ar*H*-5, Ar′*H*-5), 6.94 (dd, ^4J = 1.4 Hz, ^3J = 7.5 Hz, 2H, Ar*H*-4), 7.27 (m, 6H, Ar*H*-6, Ar′*H*-4, Ar′*H*-6), 8.38 (s, 2H, NC*H*), 13.62 (s, 2H, O*H*).

***meso*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(3-methoxyphenyl)-2,5-diazahexa-1,5-diene (m-2). m-2** was obtained from *meso*-1,2-diamino-1,2-bis(3-methoxyphenyl)ethane (1.00 mmol, 272.0 mg) and salicylaldehyde (2.1 mmol, 256.2 mg). Yield: 0.93 mmol (448.4 mg), 93%, pale-yellow crystals, mp 198 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2836 m (OCH_3), 2600 m, br (OH), 1623 s (C=N), 1533 m, 1489 m, 1465 m, 1363 m, 1322 m, 1260 s (OH), 1226 s, 1165 m, 1123 m, 1040 m, 996 w, 976 m, 847 m, 790 m, 747 w, 700 m. ^{1}H NMR (DMSO-d_6): δ = 3.68 (s, 6H, OCH_3), 5.03 (s, 2H, C*H*), 6.79 (dd, ^4J = 2.0 Hz, ^3J = 8.05 Hz, 2H, Ar*H*-3), 6.85 (m, 4H, Ar′*H*-2, Ar′*H*-6), 7.22 (dd, ^3J = 8.13, ^3J = 8.05 Hz, 2H, Ar′*H*-5), 7.31 (m, 4H, Ar*H*-4, Ar*H*-6), 8.43 (s, 2H, NC*H*), 13,19 (s, 2H, O*H*).

***d,l*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(3-methoxyphenyl)-2,5-diazahexa-1,5-diene (d,l-2). d,l-2** was obtained from *d,l*-1,2-diamino-1,2-bis(3-methoxyphenyl)ethane (1.11 mmol, 302.8 mg) and salicylaldehyde (2.75 mmol, 335.8 mg). Yield: 0.93 mmol (449.7 mg), 84%, yellow powder, mp 116 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2835 m (OCH_3), 2600 m, br (OH), 1627 s (C=N), 1601 s, 1584 s, 1491 m, 1276 s (OH), 1152 m, 1117 w, 1041 m, 924 w, 785 m, 757 m, 706 m. ^{1}H NMR (DMSO-d_6): δ = 3.66 (s, 6H, OCH_3), 5.06 (s, 2H, C*H*), 6.75 (dd, ^4J = 2.3 Hz, ^3J = 8.1 Hz, 2H, Ar*H*-3), 6.87 (m, 8H, Ar*H*-5, Ar′*H*-2, Ar′*H*-4, Ar′*H*-6), 7.17 (dd, ^3J = 8.0 Hz, ^4J = 2.3 Hz, Ar*H*-4), 7.31 (m, 4H, Ar*H*-6, Ar′*H*-5), 8.50 (s, 2H, NC*H*), 13.30 (s, 2H, O*H*).

***meso*-1,6-Bis(2-hydroxyphenyl-3,4-bis(4-methoxyphenyl)-2,5-diazahexa-1,5-diene (m-3). m-3** was obtained from *meso*-1,2-diamino-1,2-bis(4-methoxyphenyl)ethane (1.00 mmol, 272.0 mg) and salicylaldehyde (2.10 mmol, 256.2 mg). Yield: 0.89 mmol (427.2 mg), 89%, pale-yellow crystals, mp 179 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2837 m (OCH_3), 2600 m, br (OH), 1628 vs (C=N), 1582 m, 1511 s, 1461 m, 1414 m, 1278 s (OH), 1249 vs, 1177 m, 1152 m, 1115 m, 1032 s, 830 m, 760 s. ^{1}H NMR (DMSO-d_6): δ = 3.68 (s, 6H, OCH_3), 4.98 (s, 2H, C*H*), 6.86 (m, 8H, Ar*H*-3, Ar*H*-5, Ar′*H*-3), 7.27 (m, 8H, Ar*H*-4, Ar*H*-6, Ar′*H*-2, Ar′*H*-6), 8.42 (s, 2H, NC*H*), 13.23 (s, 2H, O*H*).

***d,l*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(4-methoxyphenyl)-2,5-diazahexa-1,5-diene (d,l-3). d,l-3** was obtained from *d,l*-1,2-diamino-1,2-bis(4-methoxyphenyl)ethane (1.26 mmol, 311.6 mg) and salicylaldehyde (2.74 mmol, 333.7 mg). Yield: 0.93 mmol (444.6 mg), 74%, yellow needles, mp 189 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2836 m (OCH_3), 2600 m, br (OH), 1625 s (C=N), 1581 s, 1512 s, 1460 s, 1415 m, 1606 s, 1277 s (OH), 1248 s, 1177 s, 1151 m, 1117 m, 1059 m, 1033 s, 935 w, 905 m, 833 s, 755 s, 666 m. ^{1}H NMR (DMSO-d_6): δ = 3.68 (s, 6H, OCH_3), 5.01 (s, 2H, C*H*), 6.83 (m, 8H, *ArH*-3, Ar*H*-5, Ar′*H*-2, Ar′*H*-4), 7.28 (m, 8H, Ar*H*-4, Ar*H*-6, Ar′*H*-2, Ar′*H*-6), 8.52 (s, 2H, NC*H*), 13.33 (s, 2H, O*H*).

***meso*-1,3,4,6-Tetrakis(2-hydroxyphenyl)-2,5-diazahexa-1,5-diene (m-4). m-4** was obtained from *meso*-1,2-diamino-1,2-bis(2-hydroxyphenyl)ethane (1.00 mmol, 244.3 mg) and salicylaldehyde (2.10 mmol, 256.4 mg). Yield: 0.85 mmol (384.6 mg), 85%, pale-yellow powder, mp 232 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2600 m, br (OH), 1627 s (C=N), 1528 m, 1490 s, 1458 s, 1393 m, 1337 m, 1278 s (OH), 1223 m, 1152 m, 1117 m, 1038 m, 853 w, 755 s. ^{1}H NMR (DMSO-d_6): δ = 5.51 (s, 2H, C*H*), 6.79 (m, 4H, Ar*H*-3, Ar*H*-5, Ar′*H*-3, Ar′*H*-5), 7.02 (d, ^3J = 7.7 Hz, 2H, Ar′*H*-6), 7.28 (m, 6H, Ar*H*-4, Ar*H*-6, Ar′*H*-4), 8.35 (s, 2H, NC*H*), 9.59 (s, br, 2H, Ar′-O*H*), 13.34 (s, br, 2H, Ar-O*H*).

***d,l*-1,3,4,6-Tetrakis(2-hydroxyphenyl)-2,5-diazahexa-1,5-diene (d,l-4). d,l-4** was obtained from *d,l*-1,2-diamino-1,2-bis(2-hydroxyphenyl)ethane (0.76 mmol, 186.1 mg) and salicylaldehyde (1.57 mmol, 191.7 mg). Yield: 0.30 mmol (132.5 mg), 39%, yellow powder, mp 217 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 1627 s (C=N), 1530 m, 1489 m, 1456 s, 1397 m, 1335 m, 1277 s (OH), 1221 m, 1151 m, 1119 w, 1040 m, 854 w, 753 s. ^{1}H NMR (DMSO-d_6): δ = 5.53 (s, 2H, C*H*), 6.66 (dd, ^3J = 7.5 Hz, ^3J = 8.4 Hz, 2H, Ar*H*-5), 6.90 (m, 6H, Ar*H*-3, Ar′*H*-3, Ar′*H*-5), 6.98 (ddd, ^4J = 1.4 Hz, ^3J = 8.4 Hz, ^3J = 7.5 Hz, 2H, Ar*H*-4), 7.28 (m, 6H, Ar*H*-5, Ar′*H*-4, Ar′*H*-6), 8.43 (s, 2H, NC*H*), 9.63 (s, 2H, Ar′-O*H*), 13.61 (s, 2H, Ar-O*H*).

***meso*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(3-hydroxyphenyl)-2,5-diazahexa-1,5-diene (m-5). m-5** was obtained from *meso*-1,2-diamino-1,2-bis(3-hydroxyphenyl)ethane (4.08 mmol, 987.6 mg) and salicylaldehyde (8.24 mmol, 1004.7 mg). Yield: 2.24 mmol (1010.3 mg), 55%, yellow powder, mp 232 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2600 m, br (OH), 1627 s(C=N), 1491

m, 1456 m, 1043 w, 1276 m (OH), 1216 m, 1154 m, 997 w, 758 m, 700 w. ^{1}H NMR (DMSO-d_6): δ = 4.89 (s, 2H, C*H*), 6.58 (dd, ^4J = 1.7 Hz, ^3J = 7.4 Hz, 2H, Ar*H*-3), 6.81 (m, 8H, Ar*H*-5, Ar′*H*-2, Ar′*H*-4, Ar′*H*-6), 7.05 (dd, ^3J = 7.8 Hz, ^4J = 1.7 Hz, Ar*H*-6), 7.30 (m, 4H, Ar*H*-4, Ar′*H*-5), 8.37 (s, 2H, Ar-O*H*), 13.22(s, 2H, Ar-O*H*).

***d,l*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(3-hydroxyphenyl)-2,5-diazahexa-1,5-diene (d,l-5). d,l-5** was obtained from *d,l*-1,2-diamino-1,2-bis(3-hydroxyphenyl)ethane (0.59 mmol, 143.2 mg) and salicylaldehyde (1.33 mmol, 162.8 mg). Yield: 0.51 mmol (231.5 mg), 87%, yellow powder, mp 218 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 1627 s (C=N), 1532 m, 1489 s, 1457 s, 1399 m, 1275 s (OH), 1218 s, 1152 s, 1118 m, 1053 m, 1030 m, 758 s, 703 m. ^{1}H NMR (DMSO-d_6): δ = 4.92 (s, 2H, C*H*), 6.57 (dd, ^4J = 2.0 Hz, ^3J = 8.1 Hz, 2H, Ar*H*-3), 6.75 (m, 4H, Ar′*H*-2, Ar′*H*-4), 6.84 (m, 4H, Ar*H*-5, Ar′*H*-6), 7.01 (m, 2H, Ar*H*-6), 7.30 (m, 4H, Ar*H*-4, Ar′*H*-5), 8.51 (s, 2H, NC*H*), 9.33 (s, 3H, Ar′-O*H*), 13.34 (s, 2H, Ar-O*H*).

***meso*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(4-hydroxyphenyl)-2,5-diazahexa-1,5-diene (m-6). m-6** was obtained from *meso*-1,2-diamino-1,2-bis(4-hydroxyphenyl)ethane (4.05 mmol, 980.8 mg) and salicylaldehyde (8.14 mmol, 993.0 mg). Yield: 2.41 mmol (1090.6 mg), 60%, orange powder, mp 224 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2600 w, br (OH), 1625 s (C=N), 1513 m, 1459 m, 1274 m (OH), 1221 m, 1173 m, 1115 w, 1051 w, 1017 w, 837 m, 759 m. ^{1}H NMR (DMSO-d_6): δ = 4.85 (s, 2H, C*H*), 6.67 (d, ^3J = 8.4 Hz, 4H, Ar′*H*-3, Ar′*H*-5), 6.84 (m, 4H, Ar*H*-3, Ar*H*-5), 7.09 (d, ^3J = 8.4 Hz, 4H, Ar′*H*-2, Ar′*H*-6), 7.30 (m, 4H, Ar*H*-4, Ar*H*-6), 8.38 (s, 2H, NC*H*), 9.31 (s, 2H, Ar′-O*H*), 13.41 (s, 2H, Ar-O*H*).

***d,l*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(4-hydroxyphenyl)-2,5-diazahexa-1,5-diene (d,l-6). d,l-6** was obtained from *d,l*-1,2-diamino-1,2-bis(4-hydroxyphenyl)ethane (0.53 mmol, 129.8 mg) and salicylaldehyde (1.16 mmol, 141.4 mg). Yield: 0.18 mmol (82.0 mg), 34%, yellow powder, mp 203 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2600 m, br (OH), 1625 s (C=N), 1514 s, 1456 s, 1386 m, 1276 s (OH), 1219 s, 1152 s, 1117 m, 1030 m, 904 m, 833 s, 755 s. ^{1}H NMR (DMSO-d_6): δ = 4.88 (s, 2H, C*H*), 6.62 (d, ^3J = 8.5 Hz, 4H, Ar′*H*-3, Ar′*H*-5), 6.83 (m, 4H, Ar*H*-3, Ar*H*-5), 7.07 (d, ^3J = 8.5 Hz, 4H, Ar′*H*-2, Ar′*H*-6), 7.29 (m, 4H, Ar*H*-4, Ar*H*-6), 8.48 (s, 2H, NC*H*), 9.28 (s, 2H, Ar′-O*H*), 13.41 (s, 2H, Ar-O*H*).

General Procedure for the Synthesis of [1,6-Bis(2-hydroxyphenyl)-3,4-diaryl-2,5-diazahexa-1,5-dienes]cobalt(II). An amount of 0.3 mmol of the respective 1,6-bis(2-hydroxyphenyl)-3,4-diaryl-2,5-diazahexa-1,5-diene was suspended in 5 mL of ethanol and heated to 60−80 °C under an argon atmosphere. The suspension of 0.3 mmol of cobalt(II) acetate in 5 mL of ethanol was heated on a water bath till the formation of a precipitate was completed. Subsequently, both mixtures were combined and stirred for 1 h. The colored crystals were sucked off under nitrogen, dried over $CaSO_4$, and stored under an argon atmosphere.

[*meso*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(2-methoxyphenyl)-2,5-diazahexa-1,5-diene]cobalt(II) (m-7). m-7 was obtained from *meso*-1,6-bis(2-hydroxyphenyl)-3,4-bis(2-methoxyphenyl)-2,5-diazahexa-1,5-diene (**m-1**) (0.30 mmol, 145.8 mg) and (cobalt(II) acetate)·4H_2O (0.33 mmol, 82.0 mg). Yield: 0.20 mmol (106.3 mg), 65%, red crystals, mp 275 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2837 w (OCH_3), 1602 s (C=N), 1527 s, 1491 m, 1462 s, 1443 s, 1382 w, 1314 m, 1246 s, 1208 m, 1150 m, 1051 m, 1025 m, 957 w, 910 w, 870 w, 756 w, 756 s, 647 w. MS (EI, 120 °C): *m/z* (%) = 537 (51) [M]$^{+\bullet}$. Anal. ($C_{30}H_{26}N_2O_4$·Co·0.5H_2O) C, H, N.

[*d,l*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(2-methoxyphenyl)-2,5-diazahexa-1,5-diene]cobalt(II) (d,l-7). d,l-7 was obtained from *d,l*-1,6-bis(2-hydroxyphenyl)-3,4-bis(2-methoxyphenyl)-2,5-diazahexa-1,5-diene (**d,l-1**) (0.42 mmol, 200.1 mg) and (cobalt(II) acetate)·4H_2O (0.44 mmol, 110.6 mg). Yield: 0.28 mmol (152.2 mg), 66%, red powder, mp >300 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2836 w (OCH_3), 1602 s (C=N), 1528 s, 1491 s, 1464 m, 1444 s, 1315 m, 1246 s, 1150 m, 1051 m, 1025 m, 912 w, 850 w, 755 s. MS (EI, 300 °C): *m/z* (%) = 537 (48) [M]$^{+\bullet}$. Anal. ($C_{30}H_{26}N_2O_4$·0.5H_2O) C, H, N.

[*meso*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(3-methoxyphenyl)-2,5-diazahexa-1,5-diene]cobalt(II) (m-8). m-8 was obtained from *meso*-1,6-bis(2-hydroxyphenyl)-3,4-bis(3-methoxyphenyl)-2,5-diazahexa-1,5-diene (**m-2**) (0.32 mmol, 144.3 mg) and (cobalt(II) acetate)·4H_2O (0.36 mmol, 88.7 mg). Yield: 0.23 mmol (124.9 mg), 72%, orange powder, mp 272 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2982 w (OCH_3), 1603 s (C=N), 1528 m, 1489 m, 1444 m, 1316 m, 1262 m, 1209 w, 1153 m, 1042 m, 911 w, 760 m, 717 m. MS (EI, 215 °C): *m/z* (%) = 537 (100) [M]$^{+\bullet}$, 298 (50) [(½Lig)Co]$^+$. Anal. ($C_{30}H_{26}N_2O_4$Co·0.5H_2O) C, H, N.

[*d,l*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(3-methoxyphenyl)-2,5-diazahexa-1,5-diene]cobalt(II) (d,l-8). d,l-8 was obtained from *d,l*-1,6-bis(2-hydroxyphenyl)-3,4-bis(3-methoxyphenyl)-2,5-diazahexa-1,5-diene (**d,l-2**) (0.28 mmol, 73.9 mg) and (cobalt(II) acetate)·4H_2O (0.30 mmol, 73.9 mg). Yield: 0.22 mmol (118.8 mg), 79%, pale-red powder, mp 260 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2834 w (OCH_3), 1604 s (C=N), 1527 m, 1491 m, 1447 m, 1320 m, 1263 m, 1209 w, 1151 m, 1043 m, 915 w, 760 m. MS (EI, 250 °C): *m/z* (%) = 537 (100) [M]$^{+\bullet}$, 298 (57) [(½Lig)Co]$^+$. Anal. ($C_{30}H_{26}N_2O_4$Co) C, H, N.

[*meso*-1,6-Bis(2-hydroxyphenyl-3,4-bis(4-methoxyphenyl)-2,5-diazahexa-1,5-diene]cobalt(II) (m-9). m-9 was obtained from *meso*-1,6-bis(2-hydroxyphenyl-3,4-bis(4-methoxyphenyl)-2,5-diazahexa-1,5-diene (**m-3**) (0.69 mmol, 332.8 mg) and (cobalt(II) acetate)·4H_2O (0.72 mmol, 180.2 mg). Yield: 0.47 mmol (255.1 mg), 68%, red powder, mp 295 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2835 w (OCH_3), 1606 s (C=N), 1514 s, 1442 s, 1382 w, 1308 m, 1252 s, 1208 m, 1179 s, 1150 m, 1033 m, 989 w, 908 m, 826 m, 807 m, 759 m, 629 w. MS (EI, 280 °C): *m/z* (%) = 537 (100) [M]$^{+\bullet}$, 298 (59) [(½Lig)Co]$^+$. Anal. ($C_{30}H_{26}N_2O_4$Co·0.5H_2O) C, H, N.

[*d,l*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(4-methoxyphenyl)-2,5-diazahexa-1,5-diene]cobalt(II) (d,l-9). d,l-9 was obtained from *d,l*-1,6-bis(2-hydroxyphenyl)-3,4-bis(4-methoxyphenyl)-2,5-diazahexa-1,5-diene (**d,l-3**) (277.0 mmol, 133.0 mg) and (cobalt(II) acetate)·4H_2O (0.29 mmol, 72.2 mg). Yield: 0.21 mmol (114.1 mg), 77%, red powder, mp 269−275 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2835 w (OCH_3), 1606 s (C=N), 1585 s, 1513 s, 1466 m, 1444 s, 1308 m, 1252 s, 1177 m, 1150 m, 1030 m, 912 w, 833 w, 759 m, 617 w. MS (EI, 240 °C): *m/z* (%) = 537 (100) [M]$^{+\bullet}$. Anal. ($C_{30}H_{26}N_2O_4$Co·0.5H_2O) C, H, N.

[*meso*-1,3,4,6-Tetrakis(2-hydroxyphenyl)-2,5-diazahexa-1,5-diene]cobalt(II) (m-10). m-10 was obtained from *meso*-1,3,4,6-tetrakis(2-hydroxyphenyl)-2,5-diazahexa-1,5-diene (**m-4**) (0.50 mmol, 227.5 mg) and (cobalt(II) acetate)·4H_2O (0.54 mmol, 75.0 mg). Yield: 0.39 mmol (177.2 mg), 78%, brownish powder, mp 270 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 1636 s, 1601 s (C=N), 1540 s, 1474 s, 1450 s, 1408 m, 1286 s, 1240 m, 1152 m, 1129 m, 1038 m, 903 m, 855 m, 754 s, 658 w. MS (FAB, negative (CH_3OH)): *m/z* (%) = 509 (100) [M]$^{-\bullet}$. Anal. ($C_{28}H_{22}N_2O_4$Co·1.5H_2O) C, H, N.

[*d,l*-1,3,4,6-Tetrakis(2-hydroxyphenyl)-2,5-diazahexa-1,5-diene]cobalt(II) (d,l-10). d,l-10 was obtained from *d,l*-1,3,4,6-tetrakis(2-hydroxyphenyl)-2,5-diazahexa-1,5-diene (**d,l-4**) (0.18 mmol, 79.8 mg) and (cobalt(II) acetate)·4H_2O (0.19 mmol, 47.1 mg). Yield: 0.12 mmol (62.7 mg), 69%, red powder, mp >300 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2600 m, br (OH), 1641 s, 1601 s (C=N), 1536 m, 1472 s, 1450 s, 1513 s, 1283 s, 1207 m, 1152 m, 1130 m, 1036 w, 903 m, 753 s. MS (EI, 300 °C): *m/z* (%) = 509 (2) [M]$^{+\bullet}$, 283 (3) [(½Lig)Co]$^+$, 225 (24) [(½Lig)]$^+$. Anal. ($C_{28}H_{22}N_2O_4$Co) C, H, N.

[*meso*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(3-hydroxyphenyl)-2,5-diazahexa-1,5-diene]cobalt(II) (m-11). m-11 was obtained from *meso*-1,6-bis(2-hydroxyphenyl)-3,4-bis(3-hydroxyphenyl)-2,5-diazahexa-1,5-diene (**m-5**) (0.70 mmol, 315.4 mg) and (cobalt(II) acetate)·4H_2O (0.73 mmol, 181.1 mg). Yield: 0.51 mmol (257.1 mg), 72%, red powder, mp >300°C. IR (KBr, cm^{-1}): $\bar{\nu}$= 1604 s (C=N), 1530 s, 1448 s, 1381 w, 1303 m, 1272 m, 1212 m, 1153 m, 1049 w, 928 w, 908 w, 760 m, 719 w, 628 w. MS (EI, 340 °C): *m/z* (%) = 509 (23) [M]$^{+\bullet}$, 284 (23) [(½Lig)Co]$^+$. Anal. ($C_{28}H_{22}N_2O_4$Co) C, H, N.

[*d,l*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(3-hydroxyphenyl)-2,5-diazahexa-1,5-diene]cobalt(II) (d,l-11). d,l-11 was ob-

tained from *d,l*-1,6-bis(2-hydroxyphenyl)-3,4-bis(3-hydroxyphenyl)-2,5-diazahexa-1,5-diene (**d,l-5**) (0.33 mmol, 151.3 mg) and (cobalt(II) acetate)·$4H_2O$ (0.36 mmol, 88.7 mg). Yield: 0.27 mmol (140.8 mg), 81%, orange powder, mp >300 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 1602 s (C=N), 1531 s, 1446 s, 1381 w, 1446 s, 1302 m, 1210 m, 1152 m, 999 w, 916 w, 757 m. MS (EI, 340 °C): *m/z* (%) = 509 (100) $[M]^{+\bullet}$, 283 (60) $[(^1/_2\text{Lig})\text{Co}]^+$. Anal. ($C_{28}H_{22}N_2O_4Co\cdot 0.5H_2O$) C, H, N.

[*meso*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(4-hydroxyphenyl)-2,5-diazahexa-1,5-diene]cobalt(II) (m-12). m-12 was obtained from *meso*-1,6-bis(2-hydroxyphenyl)-3,4-bis(4-hydroxyphenyl)-2,5-diazahexa-1,5-diene (**m-6**) (0.71 mmol, 321.8 mg) and (cobalt(II) acetate)·$4H_2O$ (0.75 mmol, 186.0 mg). Yield: 0.56 mmol (283.3 mg), 78%, red powder, mp >300 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 1606 s (C=N), 1516 s, 1443 s, 1381 w, 1304 m, 1256 m, 1233 m, 1175 m, 1152 m, 1028 w, 907 w, 814 m, 760 m, 658 w. MS (EI, 400 °C): *m/z* (%) = 509 (100) $[M]^{+\bullet}$, 284 (95) $[(^1/_2\text{Lig})\text{Co}]^+$, 225 (26) $[^1/_2\text{Lig}]^+$. Anal. ($C_{28}H_{22}N_2O_4Co\cdot 0.5H_2O$) C, H, N.

[*d,l*-1,6-Bis(2-hydroxyphenyl)-3,4-bis(4-hydroxyphenyl)-2,5-diazahexa-1,5-diene]cobalt(II) (d,l-12). d,l-12 was obtained from *d,l*-1,6-bis(2-hydroxyphenyl)-3,4-bis(4-hydroxyphenyl)-2,5-diazahexa-1,5-diene (**d,l-12**) (0.17 mmol, 78.1 mg) and (cobalt(II) acetate)·$4H_2O$ (0.18 mmol, 45.2 mg). Yield: 0.14 mmol (73.6 mg), 82%, pale-red powder, mp >300 °C. IR (KBr, cm^{-1}): $\bar{\nu}$ = 2600 w, br (OH), 1604 s (C=N), 1531 s, 1469 m, 1445 s, 1304 m, 1264 m, 1209 s, 1173 m, 1152 m, 997 w, 912 w, 838 m, 758 m, 621 w. MS (EI, 330 °C): *m/z* (%) = 509 (100) $[M]^{+\bullet}$, 284 (83) $[(^1/_2\text{Lig})\text{Co}]^+$. Anal. ($C_{28}H_{22}N_2O_4Co\cdot 0.5H_2O$) C, H, N.

Assay To Verify the Catalytic Property of Cobalt(3,4-diarylsalen) Complexes. O_2 was triggered for 2 h in a methanol solution consisting of 2,6-di-*tert*-butylphenol and the cobalt(3,4-diarylsalen) complex. The analysis took place after having separated the solvent with the help of the *tert*-butyl group integrals in the ^{1}H NMR spectrum (δ(BQ) = 1.28, δ(DPQ) = 1.37, δ(DP) = 1.45).

Biological Methods. Cell Culture. The human MCF-7 and MDA-MB 231 breast cancer cell lines as well as the LNCaP/FGC cell line were obtained from the American Type Culture Collection (ATCC). Cell line banking and quality control were performed according to the seed stock concept reviewed by Hay.[26] The MCF-7 cells were maintained in L-glutamine containing Eagle's MEM (Sigma, Germany), supplemented with $NaHCO_3$ (2.2 g/L), sodium pyruvate (110 mg/L), gentamycin (50 mg/L), and 10% fetal calf serum (FCS; Gibco, Germany) using 75 cm^2 culture flasks in a humidified atmosphere (5% CO_2) at 37 °C. The MDA-MB 231 cells (McCoy's 5A medium supplemented with $NaHCO_3$ (2.2 g/L), sodium pyruvate (110 mg/L), gentamycin (50 mg/L), and 5% FCS) and the LNCaP/FGC cells (L-glutamine containing RPMI 1640, supplemented with $NaHCO_3$ (2.0 g/L), gentamycin (50 mg/L), and 7.5% FCS) were maintained under the same conditions. The cell lines were passaged weekly after previous treatment with trypsin (0.05%)/ethylenediaminetetraacetic acid (0.02% EDTA; Boehringer, Germany). Mycoplasma contamination was routinely monitored, and only mycoplasma-free cultures were used.

In Vitro Chemosensitivity Assays. The in vitro testing of the cobalt complexes for antitumor activity was carried out on exponentially dividing human cancer cells according to a previously published microtiter assay.[17,27] Exponential cell growth is guaranteed during the whole time of incubation. Briefly, by utilization of 96-well microtiter plates, 100 μL of a cell suspension were plated into each well at 7700 cells/mL culture medium (MCF-7), at 3200 cells/mL (MDA-MB 231), and at 2700 cells/mL (LNCaP/FGC), incubated at 37 °C for 3 or 5 days in the case of LNCaP/FGC cells in a humidified atmosphere (5% CO_2). By addition of an adequate volume of a stock solution of the respective compound (solvent: DMF) to the medium, the desired test concentration was obtained. Sixteen wells were used for each test concentration and for the control, which contained the corresponding amount of DMF. The medium was removed after reaching the proper incubation time. Subsequently, the cells were fixed with a glutaric dialdehyde solution and stored under phosphate buffered saline (PBS) at 4 °C. Cell biomass was determined by means of a crystal violet staining technique as described earlier.[17,27] The effectiveness of the complexes is expressed as corrected % T/C_{corr} or % τ values according to the following equations:

$$\text{cytostatic effect:} \quad T/C_{corr}\,[\%] = \frac{T - C_0}{C - C_0} \times 100$$

$$\text{cytocidal effect:} \quad \tau\,[\%] = \frac{T - C_0}{C_0} \times 100$$

where T (test) and C (control) are the optical densities at 590 nm of the crystal violet extract of the cells in the wells (i.e., the chromatin-bound crystal violet extracted with ethanol 70%), and C_0 is the density of the cell extract immediately before treatment.

For the automatic estimation of the optical density of the crystal violet extract in the wells, a Microplate EL 309 autoreader was used.

Accumulation Studies. The accumulation studies were performed according to methods already described by us.[4,20] Briefly, for the cobalt accumulation studies, cells were grown in 75 cm^2 culture flasks (Falcon Plastics 3028) in a humidified atmosphere at 37 °C. When the cells had approached confluency, the culture medium was aspirated and replaced with a fresh medium containing 5 μM cobalt complex or 0.1% DMF (blank) (50 μL of stock solution was added to 50 mL of culture medium). Twelve culture flasks were used for the blanks as well as for the incubation range 20−360 min. At appropriate intervals (0, 20, 40, 60, 80, 100, 120, 180, 240, 300, and 360 min), the flasks were removed from the incubator, the medium was aspirated, and the cells were washed with PBS and harvested after treatment with 1−2 mL of trypsin (0.05%) and EDTA (0.02%) in PBS. For the complete removal of the cells, the culture flasks were rinsed with an additional 7 mL of PBS. At each time point, the cells were pooled and centrifuged at 2000 U/min for 5 min at 15 °C. The pellet was twice resuspended in 8 mL of PBS and centrifuged. Finally, the washing solution was decanted from the cell pellet which was treated with 900 μL of TA solution (200 μL of Triton X-100 + 200 μL of Antifoam B (Sigma) diluted in 100 mL of water). After sonification, the homogeneous cell suspensions (about 800 μL) were used for cobalt determination and protein measurement.

The Co determinations were performed by flameless atomic absorption spectroscopy (wavelength, 240.7 nm; apparatus, Philips PU 9100x atomic absorption spectrophotometer with graphite furnace TJA-Unicam). An amount of 20 μL of an appropriately diluted sample was introduced into the atomizer.

As Co standard, we used dilutions of $Co(NO_3)_2\cdot H_2O$ in 0.05 M HNO_3 (c(Co) = 1.00 g/L). The calibration curve showed a linear run in the low-concentration range of 1.0−10.0 μM. The Co values were calculated by means of this calibration curve. Each Co analysis was based on no less than three individual determinations. The detectability was about 0.08 μM Co for a 10 μL probe, and the precision of the method was ±3.0%. Correlation of cell number and protein content and the calculation of cellular cobalt concentration were performed according to Reile et al.[20] For this purpose, 5 μL of a cell suspension and 95 μL of water were combined with 1 mL of Bradford reagent.[28] After 20 min incubation at room temperature, the extinction was measured in a UV spectrometer at 595 nm. For calibration, BSA (0.1, 0.2, ..., 1.0 μg/μL) was dissolved in PBS. The diameters of trypsinized MCF-7 cells (n = 50) as determined by Reile et al.[20] were used for the calculation of cellular cobalt concentration.

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Supporting Information Available: IR-spectra of **d,l-3** and **d,l-9**, typical mass spectra of the cobalt(3,4-diarylsalen) on **d,l-9**, and graphite furnace atomic absorption temperature program. This material is available free of charge via the Internet at http://pubs.acs.org.

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