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Acetylenehexacarbonyldicobalt complexes, a novel class of antitumor drugs

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Abstract

Acetylenehexacarbonyldicobalt complexes were synthesized and tested for antitumor activity. The MCF-7 and MDA-MB-231 mammary tumor cell lines and the LNCaP/FGC prostate carcinoma cell line were used as in vitro models. The structural evaluation was performed by IR and NMR spectroscopy and revealed a change of the linear acetylene core to a structure comparable to *Z*-olefins after coordination to the cobalt centers. In cell culture experiments the strongest effects were found for hexacarbonyl[2-propinylacetylsalicylate]dicobalt (**10**), which was more active than cisplatin on the human mammary tumor cell lines MCF-7 and MDA-MB-231 in each concentration tested (a 5 μM concentration of this compound even caused cytocidal effects). In contrast to this, **10** influenced the growth of the LNCaP/FGC cells only marginally, even in the highest concentration. The mode of action of the complexes tested is unknown. As the cobalt complexes show strong antiproliferative effects and their ligands do not it could be unambiguously demonstrated that complex formation is essential to achieve cytotoxic effects.

Keywords: Antitumor activity; Cobalt complexes; Carbonyl complexes

1. Introduction

Among the multitude of drugs used in the treatment of cancer *cis*-diamminedichloroplatinum(II) (cisplatin) is established as a cytotoxic[1] agent. Cisplatin has shown to be very active against testicular and ovarian tumors, tumors of the head and neck as well as against bladder tumors [1,2]. However, it is not or is insufficiently active against tumors of the gastrointestine, the lung and the mammary. Since these tumors account for the major part of cancer mortality today the chemical and clinical search for new selectively acting cisplatin derivatives or new tumor inhibiting metal complexes [3–6] goes on.

As an approach for the treatment of hormone dependent tumors (e.g. the estrogen receptor positive breast cancer), metal complexes of estradiol derivatives were synthesized [7]. Out of this class of compounds the hexacarbonyl[ethinylestradiol]dicobalt complex was also used to monitor the binding of steroids to the estrogen receptor [8]. The formation of an α-cation in the vicinity of the $Co_2(CO)_6$ moiety enabled the complex to bind covalently to nucleophilic centers in the hormone binding site of the estrogen receptor.

This strategy of drug design induced us to synthesize hexacarbonyldicobalt complexes as cytostatics. The first results were published previously [9] and demonstrated the tumor inhibiting properties of acetylenehexacarbonyldicobalt complexes. In this study the antiproliferative effects depended strongly on the structure of the bound acetylene ligand. On the H2981 lung adenocarcinoma cell line, treatment with 10 μM of hexacarbonyl[2-propinylacetylsalicylate]dicobalt (**10**) and hexacarbonyl[2-propinylsalicylate]dicobalt (**11**) led to an 80–90% inhibition of the [^{3}H]-thymidine incorpora-

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[1] Cytotoxic effect: being damaging to the cells. Cytocidal effect: reduction of initial cell mass, cells are being killed. Cytostatic effect: prevention or considerable inhibition of cell proliferation.

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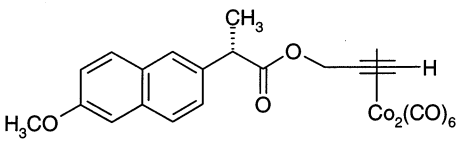
tion. To achieve the same effect hexacarbonyl[*N*-2-propinylbenzothiazolidine-3-one-1,1-dioxide]dicobalt (**9**) had to be used in a fivefold higher concentration.

Due to these positive results we tested these acetylenehexacarbonyldicobalt complexes also on further tumor cell lines (human breast cancer cell lines MCF-7 and MDA-MB-231, human prostate cancer cell line LNCaP/FGC) and elucidated their structure in detail. In this paper we present these results along with the synthesis of new compounds (see Chart 1).



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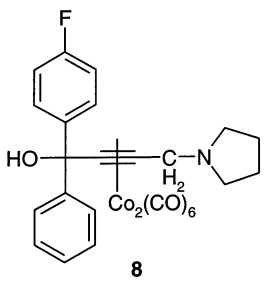

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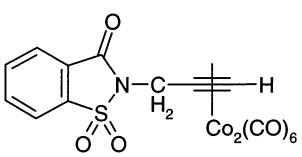

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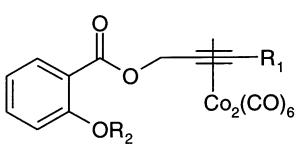


	R_1	R_2
3	methyl	acetyl
10	H	acetyl
11	H	H

(Chart 1)

2. Experimental

2.1. General procedure

Melting point: uncorrected. Elemental analysis: Perkin–Elmer. IR (KBr): Matson Genesis. ^{1}H NMR: Varian Gemini 200 (200 MHz). ^{13}C NMR: Varian Gemini 200 (50.29 MHz). MS: Varian MAT 44S, Finnigan MAT 312. Flash chromatography: silica gel 60, 230–400 mesh (Merck). (*S*)-Naproxen and acetylsalicylic acid were purchased from Sigma. Dicobaltoctacarbonyl (Fluka, moistened with 5–10% hexane) was used as purchased and presumed to be of 95% purity. THF was distilled from $LiAlH_4$ under nitrogen prior to use. CH_2Cl_2 was dried over molecular sieves (4 Å). The syntheses of **7**, **9**, **10** and **11** were published previously [9,10].

2.2. Syntheses of the alkyne ligands

2.2.1. (2-Butin-1-yl)acetylsalicylate (*2*)

A total of 900 mg (5 mmol) of acetylsalicylic acid (**1**), 1.07 g (5.2 mmol) of dicyclohexylcarbodiimide, 0.41 ml (386 mg, 5.5 mmol) of 2-butine-1-ol and 61 mg (0.5 mmol) of 4-dimethylaminopyridine were stirred in 15 ml of dry CH_2Cl_2 over night. The mixture was filtered, the precipitate was rinsed with CH_2Cl_2 and the combined filtrates were evaporated. The resulting oil was chromatographed with ethyl 1:5 acetate–hexane. The first fraction contained the product. Yield: 410 mg (12%); colorless crystals; m.p. 43°C. IR (KBr): $\bar{\nu} = 1740$ cm^{-1} (CO), 1700 cm^{-1} (CO). MS (70 eV): m/z (%) = 232 (6) [M^+], 121 (100). ^{1}H NMR ($CDCl_3$): $\delta = 1.88$ (t, $^5J = 2.2$ Hz, 3H, C–CH_3), 2.38 (s, 3H, COCH_3), 4.85 (q, $^5J = 2.2$ Hz, 2H, OCH_2), 7.09 (dd, $^3J = 8.0$ Hz, $^4J = 1.2$ Hz, 1H, 3′-*H*), 7.33 (ddd, $^3J = 7.5$ Hz, $^3J = 7.8$ Hz, $^4J = 1.2$ Hz, 1H, 5′-*H*), 7.58 (ddd, $^3J = 8.0$ Hz, $^3J = 7.5$ Hz, $^4J = 1.7$ Hz, 1H, 4′-*H*), 8.07 (dd, $^3J = 7.8$ Hz, $^4J = 1.7$ Hz, 1H, 6′-*H*). ^{13}C NMR ($CDCl_3$): $\delta = 3.73$ (C≡C–*C*H$_3$), 21.03 (CO*C*H$_3$), 53.59 (O*C*H$_2$), 83.04 (C≡*C*–CH$_3$), 83.69 (*C*≡C–CH$_3$), 122.95 (*C*–COO), 123.94 (*C*-3), 126.12 (*C*-5), 132.12 (*C*-6), 134.18 (*C*-4), 150.78 (*C*–OAc), 163.99 (*C*OOR), 169.80 (Me*C*O). *Anal.* Calc. for $C_{13}H_{12}O_4$: C, 67.2; H, 5.22. Found: C, 67.1; H, 5.26%.

2.2.2. (S)-(2-Propinyl)-2-(6-methoxy-2-naphthyl)-propionate (*5*)

A total of 921 mg (4 mmol) of (*S*)-naproxen (**4**), 908 mg (4.4 mmol) of dicyclohexylcarbodiimide, 0.26 ml (252 mg, 4.5 mmol) of propargyl alcohol and 61 mg (0.5 mmol) of 4-dimethylaminopyridine were stirred in 15 ml of dry CH_2Cl_2 over night. The mixture was filtered, the precipitate was rinsed with CH_2Cl_2 and the combined filtrates were evaporated. The resulting oil

was chromatographed with 1:3 ethyl acetate–hexane and the solid obtained was recrystallized from hexane. Yield: 870 mg (81%); colorless crystals; m.p. 69–70°C. IR (KBr): $\bar{\nu} = 1730$ cm^{-1} (CO). MS (70 eV): m/z (%) = 268 (47) [M^+], 185 (100). ^{1}H NMR ($CDCl_3$): $\delta = 1.59$ (d, $^3J = 7.1$ Hz, 3H, CH_3), 2.43 (t, $^4J = 2.5$ Hz, 1H, CH), 3.89 (s, 3H, OCH_3), 3.98 (q, $^3J = 7.1$ Hz, 1H, Ar–CH), 4.60 (dd, 1H, $^2J = 15.6$ Hz, $^4J = 2.5$ Hz, 1H, OCH_2), 4.73 (dd, 1H, $^2J = 15.6$ Hz, $^4J = 2.5$ Hz, 1H, OCH_2), 7.11–7.17 (m, 2H, Ar–H), 7.40 (dd, $^3J = 8.5$ Hz, $^4J = 1.9$ Hz, 1H, Ar–H), 7.67–7.72 (m, 3H, Ar–H). ^{13}C NMR ($CDCl_3$): $\delta = 18.61$ (CHCH$_3$), 45.25 (Ar–CH), 52.33 (OCH$_2$), 55.36 (OCH$_3$), 74.94 (C≡C–H), 77.68 (C≡C–H), 105.69 (C–7), 119.08 (C-5′), 126.09 and 126.20 (C-1′ and 3′), 127.29 (C-4′), 128.98 (C-8′a), 129.35 (C-8′), 133.82 (C-2′), 135.19 (C-4′a), 157.77 (COMe), 173.88 (CO). *Anal.* Calc. for $C_{17}H_{16}O_3$: C, 76.1; H, 6.02. Found: C, 75.6; H, 6.23%.

2.3. General method for the preparation of acetylene-hexacarbonyldicobalt complexes

A total of 1 mmol of the alkyne was dissolved in 10 ml of dry THF in an oven dried flask which had been purged with nitrogen and 377 mg (1.05 mmol) of dicobaltoctacarbonyl (Fluka, 95%) were added. Evolution of carbon monoxide immediately started upon addition. The reaction mixture was stirred for 6 h at room temperature (r.t.). 1 g of silica gel 60 was then added and the mixture was evaporated to dryness. The dark powder was subjected to flash column chromatography with the requisite solvent mixture. Dark colored product containing fractions were evaporated to dryness. Unless stated otherwise the compounds solidified and were of analytical purity without recrystallization.

2.3.1. [(2-Butin-1-yl)acetylsalicylate]hexacarbonyldicobalt (3)

General method for acetylenehexacarbonyldicobalt complexes; 232 mg (1 mmol) **2**. After chromatography with 1:10 ethyl acetate–hexane and evaporation a brown oil was obtained. Yield: 180 mg (35%). MS (70 eV): m/z (%) = 462 (8) [$M^+ - 2$CO], 434 (41) [$M^+ - 3$CO], 406 (26) [$M^+ - 4$CO], 378 (19) [$M^+ - 5$CO], 350 [$M^+ - 6$CO], 306 (100). IR (KBr): $\bar{\nu} = 2093$, 2054, 2025 cm^{-1} (Co–CO). ^{1}H NMR ($CDCl_3$): $\delta = 2.37$ (s, 3H, COCH_3), 2.66 (s, 3H, ≡C–CH_3), 5.49 (s, 2H, OCH_2), 7.13 (d, $^3J = 7.7$ Hz, Ar–H), 7.30–7.34 (m, 1H, Ar–H), 7.54–7.59 (m, 1H, Ar–H), 8.09 (1H, Ar–H). ^{13}C NMR ($CDCl_3$): $\delta = 20.28$ and 20.99 (≡C–CH$_3$ and COCH$_3$), 65.33 (OCH$_2$), 89.93 (C≡C–CH$_3$), 93.37 (C≡C–CH$_3$), 122.31 (C–COO), 123.98, 126.02, 131.52 and 134.26 (Ar–CH), 151.31 (C–OAc), 163.66 and 169.74 (COOR), 199.2 (Co–CO). *Anal.* Calc. for $C_{19}H_{12}Co_2O_{10}$: C, 44.0; H, 2.34. Found: C, 44.1; H, 2.52%.

2.3.2. Hexacarbonyl[(S)-(2-propinyl)-2-(6-methoxy-2-naphthyl)propionate]dicobalt (6)

General method for acetylenehexacarbonyldicobalt complexes; 268 mg (1 mmol) **5**. After chromatography with 1:10 ethyl acetate–hexane and evaporation a brown oil was obtained. Yield: 310 mg (56%). MS (70 eV): m/z (%) = 470 (62) [$M^+ - 3$CO], 442 (6) [$M^+ - 4$CO], 414 (64) [$M^+ - 5$CO], 386 (100) [$M^+ - 6$CO]. IR (KBr): $\bar{\nu} = 2097$, 2058, 2029 cm^{-1} (Co–CO). ^{1}H NMR ($CDCl_3$): $\delta = 1.62$ (d, $^3J = 6.3$ Hz, 3H, CH_3), 3.92 (s, 4H, Ar–CH and OCH_3), 5.12 (d, 1H, $^2J = 14$ Hz, OCH_2), 5.40 (d, 1H, $^2J = 14$ Hz, OCH_2), 6.00 (s, 1H, C–H), 7.08–7.16 (m, 2H, Ar–H), 7.40–7.45 (m, 1H, Ar–H), 7.69 (s, 3H, Ar–H). ^{13}C NMR ($CDCl_3$): $\delta =$ 18.51 (CH$_3$), 45.46 (Ar–CH), 55.39 (OCH$_3$), 65.49 (OCH$_2$), 72.04 (C≡C–H), 88.66 (OCH$_2$–C), 105.73 (C-7), 119.06 (C-5), 126.25 (C-1 and C-3), 127.31 (C-4), 129.08 (C-8a), 129.34 (C-8), 133.89 (C-2), 135.43 (C-4a), 157.78 (COMe), 174.47 (CO), 199.0 (Co–CO). *Anal.* Calc. for $C_{23}H_{16}Co_2O_9$: C, 49.8; H, 2.92. Found: C, 50.1; H, 3.06%.

2.3.3. [(R,S)-1-(4-Fluorophenyl)-1-phenyl-4-pyrrolidinyl-2-butine-1-ol]hexacarbonyldicobalt (8)

General method for acetylenehexacarbonyldicobalt complexes; 309 mg (1 mmol) **7**. After chromatography with ethyl acetate/hexane and evaporation brown–red crystals were obtained. Yield: 390 mg (66%); m.p. 125–126°C. MS (70 eV): m/z (%) = 511 (18) [$M^+ - 3$CO], 483 (39) [$M^+ - 4$CO], 455 (16) [$M^+ - 5$CO], 427 (15) [$M^+ - 6$CO], 84 (100). IR (KBr): $\bar{\nu} = 2091$, 2052, 2035, 2024, 2013 cm^{-1} (Co–CO). ^{1}H NMR ($CDCl_3$): $\delta = 1.92$ (s, 4H, NCH$_2$CH_2), 2.86 (s, 4H, NCH_2CH$_2$), 3.91 (s, 2H, CH_2N), 6.97–7.05 (m, 2H, Ar–H), 7.21–7.35 (m, 3H, Ar–H), 7.59–7.70 (m, 4H, Ar-H), 8.04 (s, 1H, OH). *Anal.* Calc. for $C_{26}H_{20}Co_2FNO_7$: C, 52.5; H, 3.39; N, 2.35. Found: C, 52.7; H, 3.35; N, 2.61%.

2.4. Biological methods

2.4.1. 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, USA). Cell line banking and quality control were performed according to the seed stock concept reviewed by Hay [11]. The MCF-7 cells were maintained in L-glutamine containing Eagle's MEM (Sigma, Germany), supplemented with $NaHCO_3$ (2.2 g l^{-1}), sodium pyruvate (110 mg l^{-1}), gentamycin (50 mg l^{-1}) and 10% fetal calf serum (FCS; Gibco, Germany) using 75 cm^2 culture flasks in a humidified atmosphere (95% air/5% CO_2) at 37°C. The MDA-MB-231 cells (McCoy's 5A medium supplemented with $NaHCO_3$ (2.2 g

l^{-1}), sodium pyruvate (110 mg l^{-1}), gentamycin (50 mg l^{-1}) and 5% FCS) and the LNCaP/FGC cells (L-glutamine containing RPMI 1640, supplemented with $NaHCO_3$ (2.0 g l^{-1}), gentamycin (50 mg l^{-1}) and 7.5% FCS) were maintained under the same conditions. The cell lines were passaged weekly after treatment with trypsin (0.05%)/ethylenediaminetetraacetic acid (0.02%; EDTA; Boehringer, Germany). Mycoplasma contamination was routinely monitored and only mycoplasma-free cultures were used.

2.4.2. 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 [12,13]. Briefly, in 96-well microtiter plates, 100 μl of a cell suspension at 7700 cells ml^{-1} culture medium (MCF-7) at 3200 cells ml^{-1} (MDA-MB-231) and at 2700 cells ml^{-1} (LNCaP/FGC), respectively, were plated into each well and incubated at 37°C for 3 or 5 days, (LNCaP/FGC) 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. For each test concentration and for the control, which contained the corresponding amount of DMF, 16 wells were used. After the proper incubation time the medium was removed, the cells were fixed with a glutardialdehyde solution and stored under phosphate buffered saline (PBS) at 4 °C. Cell biomass was determined by a crystal violet staining technique as described earlier [11,12]. The effectiveness of the complexes is expressed as corrected percentage T/C_{corr} values according to the following equations: cytostatic effect: T/C_{corr} [%] = $[(T - C_o)/(C - C_o)] \times 100$, where T (test) and C (control) are the optical densities at 578 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_o is the optical density of the crystal violet extract immediately before treatment. Cytocidal effect: τ [%] = $[(T - C_o)/C_o] \times 100$. For the automatic estimation of the optical density of the crystal violet extract in the wells a Microplate EL 309 Autoreader was used.

3. Results and discussion

3.1. Synthesis and structural characterization

3.1.1. Synthesis

For the syntheses of the drugs **3**, **6** and **8** we used the course of reaction described by Jung et al. [9] (see Chart 2).

In the first step acetylsalicylic acid (**1**) was reacted with 2-butine-1-ol to yield (2-butin-1-yl)acetylsalicylate (**2**) while esterification of (*S*)-naproxen (**4**) with propargyl alcohol resulted in (*S*)-(2-propinyl)-2-(6-methoxy-2-naphthyl)propionate (**5**) (see Chart 2).

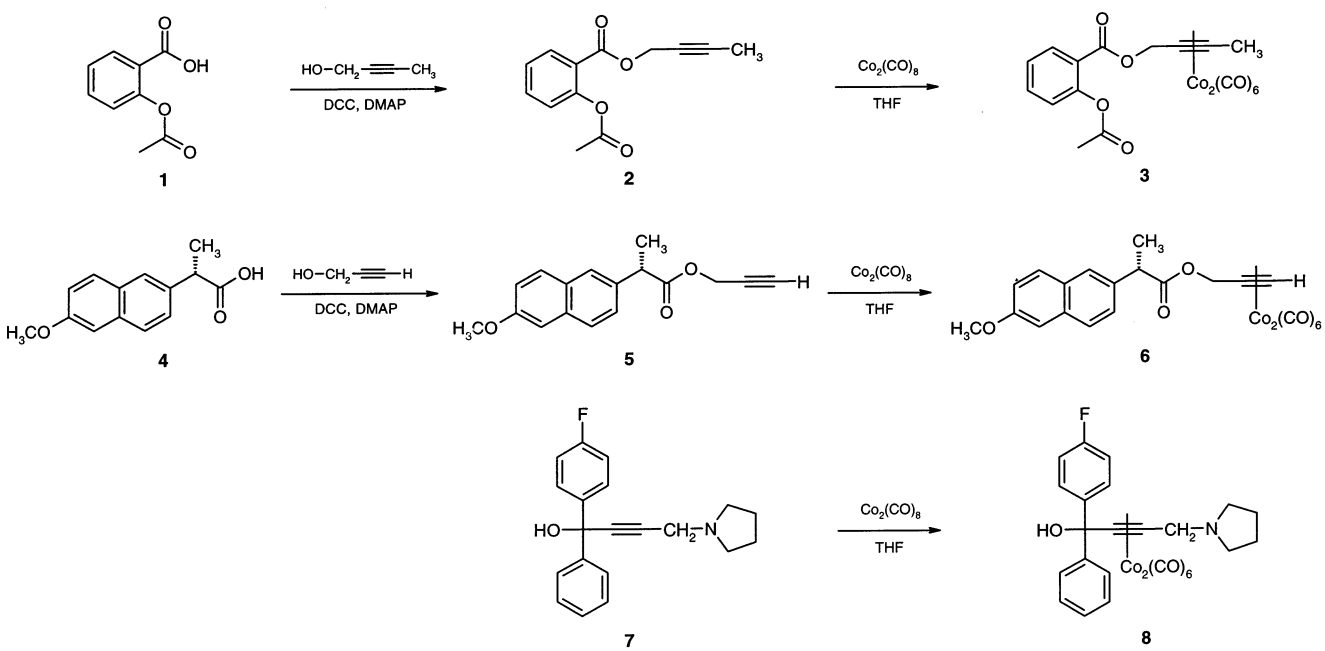


Chart 2

For coordination, the acetylenes were stirred with $Co_2(CO)_8$ at r.t. The separation of the acetylenehexacarbonyldicobalt complexes from their reaction mixtures was performed by column chromatography on SiO_2.

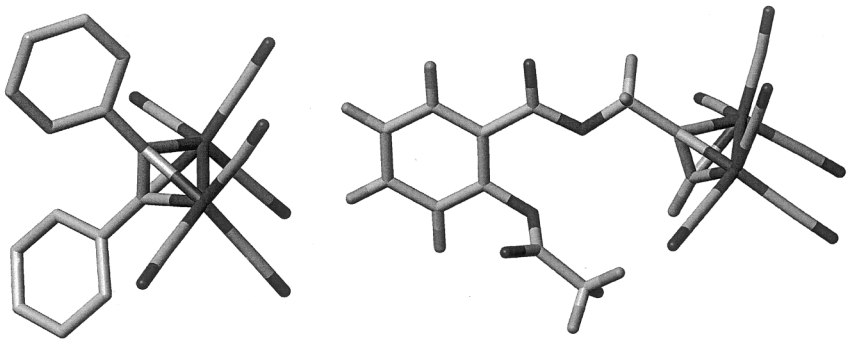


Fig. 1. X-ray structure of hexacarbonyl[diphenylacetylene]dicobalt [14] (left) and a low-energy conformation of **10** (right) generated by Sybyl 6.6 (Tripos, Inc.) using the Tripos force field and Gasteiger–Huckel charges.

3.1.2. Structural characterization

For the characterization of the acetylenehexacarbonyldicobalt complexes and the evaluation of their spatial structure IR and NMR spectroscopy were used. Due to the binding to the cobalt atoms the spatial structure of the acetylene as well as the binding mode of the carbonyl ligands changed. The triple bond of the acetylene moiety with two perpendicular oriented π-orbitals makes the coordination to $Co_2(CO)_8$ possible accompanied by elimination of two CO ligands from their coordination places. The binding mode should be comparable to that of hexacarbonyl[diphenylacetylene]dicobalt [14]. Fig. 1 represents the X-ray structure of this complex together with the low energy conformation of **10**.

In both complexes the X–C≡C–X moiety of the acetylene is oriented to the Co–Co-axis in an angle of 90° with π-bonds to the cobalt atoms. Consequently, the triple bond character is lowered. Compared to the free acetylene the C–C-distance increased from about 1.2 to about 1.5 Å. Furthermore, the linearity of the alkyne is lost (X–C≡C–X dihedral angle < 180°).

The binding mode of the carbonyl ligands in the hexacarbonyldicobalt complexes was evaluated by IR-spectroscopy. The comparison of the spectra of the complexes **3**, **6**, **8**–**11** with that of $Co_2(CO)_8$ indicates that the CO ligands in the complex are exclusively terminally oriented. In $Co_2(CO)_8$ two carbonyl groups are bridged between the metal atoms. In accordance with this, the IR-spectra exhibits stretching vibrations ($\bar{\nu}_{CO}$) at 1832 and 1846 cm^{-1} for the bridged CO.

From the asymmetrical structure of the acetylenehexacarbonyldicobalt complexes result six different stretching vibrations [15]. In the case of complex **10** (see Fig. 2), the CO absorptions are well separated at 2008, 2018, 2033, 2040, 2059 and 2097 cm^{-1}.

The coordination to cobalt changes the ^1H and ^{13}C NMR resonances of the acetylenes characteristically [16]. In the ^1H NMR spectra of the free acetylenes the resonances for C≡C–H^B ($\delta = 2.39$–2.56), C≡C–CH_2^A ($\delta = 3.43$–4.95) or C≡C–CH_3^C ($\delta = 1.88$) protons are split by long range couplings (see Table 1 and data in the experimental part). After coordination to cobalt the signals are shifted to lower field (C–H^A, C–H^C about 0.5–0.8 ppm and C–H^B about 3.5–3.8 ppm) and are simplified due to the loss of the long range couplings. These results indicate a reduction of the triple bond character of the acetylenes and the change of the linear geometry to that of a *Z*-olefin [17].

The coordination changes not only the geometry of the ligand but also the electronic environment at C^2 and C^3, well documented by the resonances in the ^{13}C

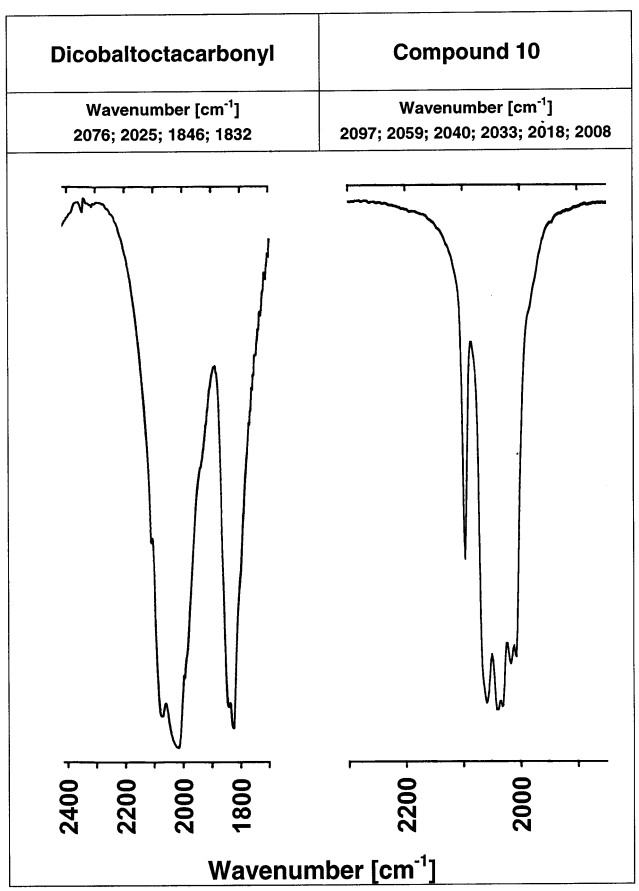
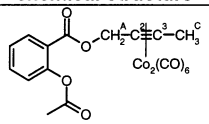
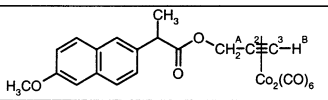
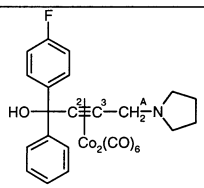
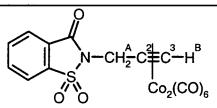
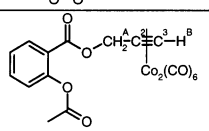
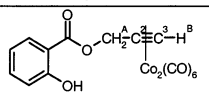


Fig. 2. IR spectra of $Co_2(CO)_8$ and **10**.

Table 1
NMR data[a] of acetylenehexacarbonyldicobalt complexes

	chemical structure	^{1}H-NMR	free ligand	complex	^{13}C-NMR	free ligand	complex	$\Delta\delta_1$[b]	$\Delta\delta_2$[b]	$\Delta\Delta\delta$[c]
3		H^A	4.85	5.49	C^2	83.69	93.37	0.65	3.44	2.79
		H^C	1.88	2.66	C^3	83.04	89.93			
6		H^A	4.60; 4.73	5.12; 5.40	C^2	77.68	88.66	2.74	16.62	13.88
		H^B	2.43	6.0	C^3	74.94	72.04			
8		H^A	3.43	3.91	C^2	87.92	106.24	4.56	10.80	6.24
					C^3	83.36	95.44			
9		H^A	4.55	5.10	C^2	75.79	87.33	2.76	13.14	10.38
		H^B	2.39	6.12	C^3	73.14	74.19			
10		H^A	4.88	5.47	C^2	77.52	88.42	2.08	16.14	14.06
		H^B	2.53	6.11	C^3	75.44	72.28			
11		H^A	4.95	5.55	C^2	75.70	87.69	0.06	15.59	15.53
		H^B	2.56	6.14	C^3	75.64	72.10			

a Experimental conditions see Experimental part
b $\Delta\delta = \delta(C^2) - \delta(C^3)$; $\Delta\delta_1$: free ligand; $\Delta\delta_2$: complex
c $\Delta\Delta\delta = \Delta\delta_2 - \Delta\delta_1$

NMR-spectra [18]. The chemical shift of the ^{13}C atoms depends on the configuration, as well as on the adjacent substituents.

In case of the acetylenes presented in this paper, the different substituents at the sp-carbons cause separate resonances for C^2 and C^3. In the ^{13}C NMR spectra of

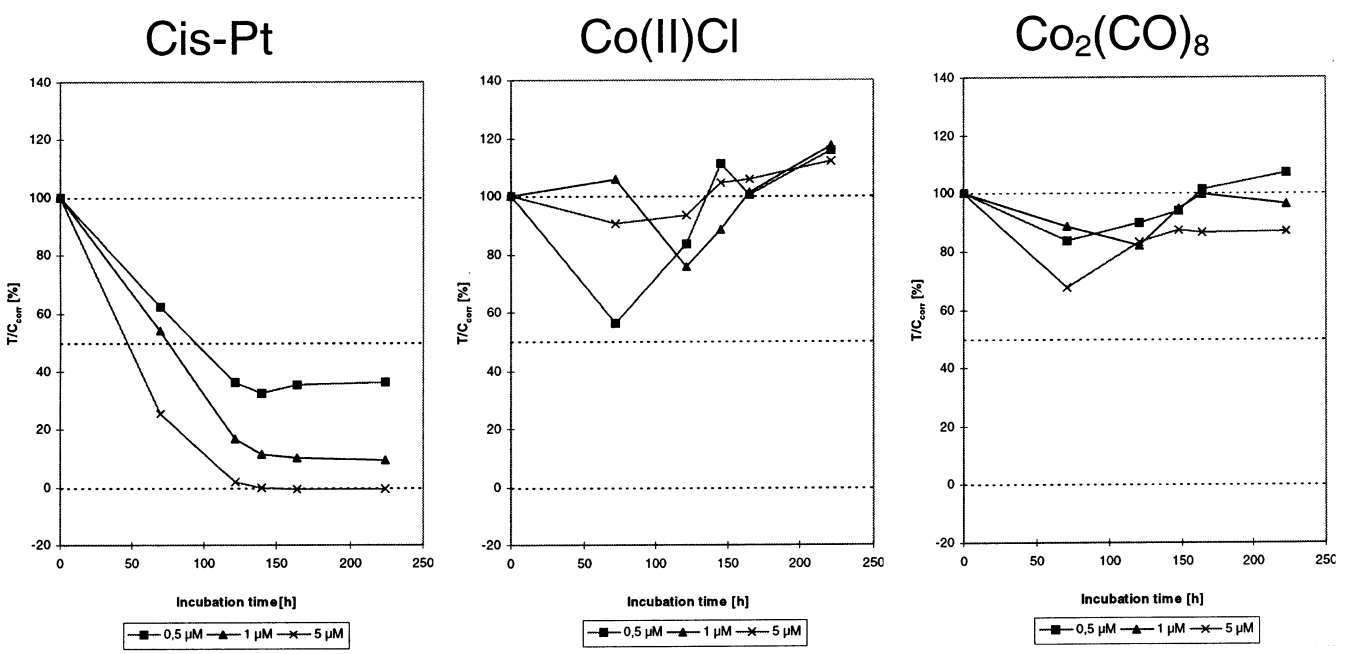


Fig. 3. Effect of cisplatin, $CoCl_2$ and $Co_2(CO)_8$ (0.5, 1.0, 5.0 μM) on the proliferation of human MCF-7 cells.

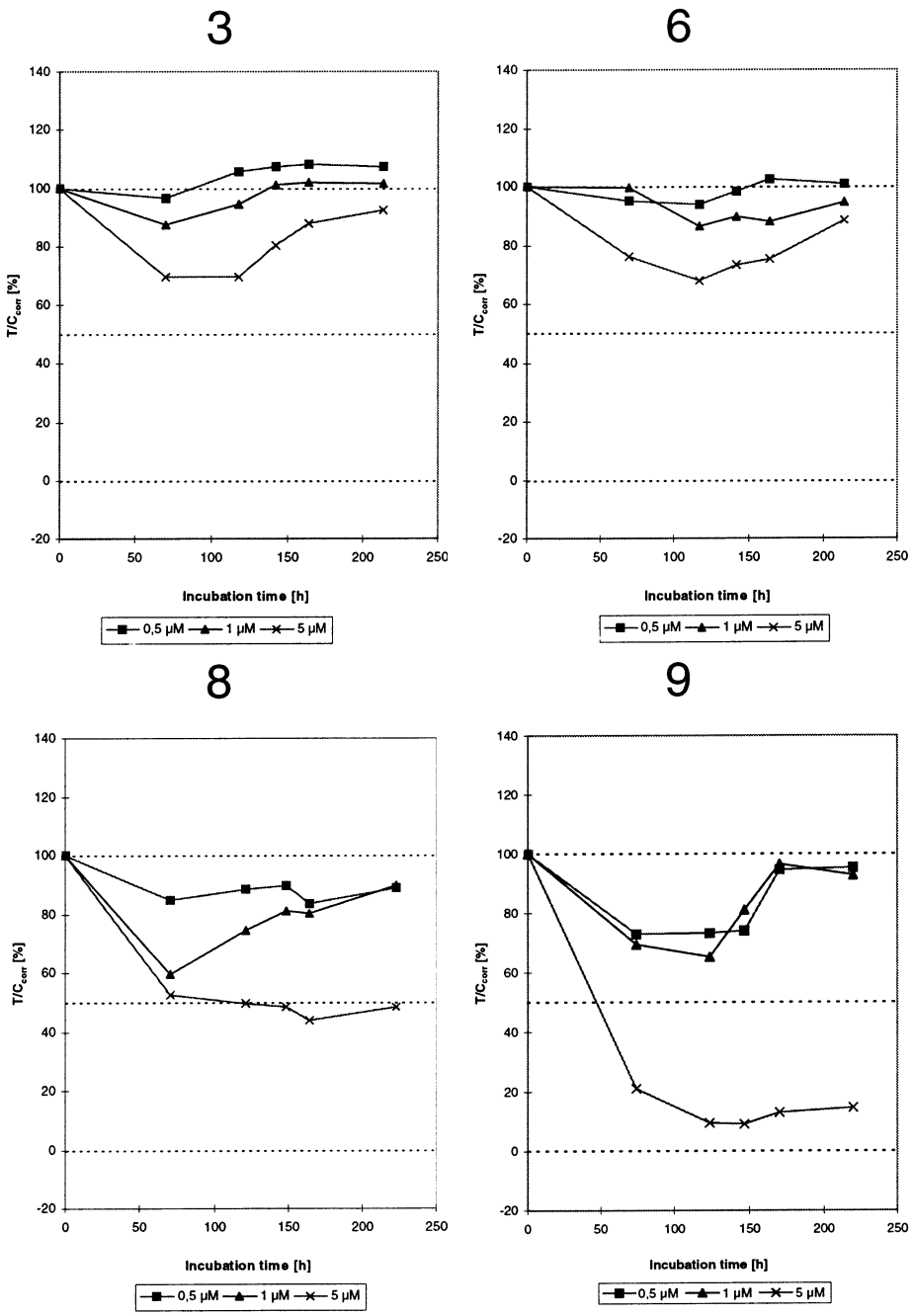


Fig. 4. Effect of **3**, **6**, **8** and **9** (0.5, 1.0, 5.0 μM) on the proliferation of human MCF-7 cells.

the free ligands the separation amounts to $\Delta\delta_1 = \delta(C^2) - \delta(C^3) = 0.06–4.56$ (see Table 1). This effect is more marked for the hexacarbonyldicobalt complexes **6**, **9**–**11** ($\Delta\delta_2 = 13.14–16.62$, see Table 1). The π-bond to the metal atom leads at C^2 to a strong deshielding and therefore to a low-field shift of this signal. The magnitude of this 'coordination effect' is expressed by $\Delta\Delta\delta = \Delta\delta_2 - \Delta\delta_1$ and amounts to 10.38–15.53 (see Table 1). This means, that the C≡C core of the acetylene is more polarized in the coordinated molecule than in the free state. Complexes **3** and **8** show the same effect, however, to a smaller extent ($\Delta\Delta\delta$ (**3**) = 2.79; $\Delta\Delta\delta$ (**8**) = 6.24).

3.2. Antiproliferative effects

The complexes **3**, **6**, **8**–**11** were tested on the mammary carcinoma cell lines MCF-7 and MDA-MB-231 and the LNCaP/FGC prostate cancer cell line. As reference cisplatin was used. Figs. 3–5 present the results of the tests on the MCF-7 cell line. Cisplatin reduced the growth of the tumor cells dependent on the

concentration used. In a concentration of 5 μM it suppressed the cell proliferation completely ($T/C_{corr} = 0\%$, see Fig. 3). The antiproliferative potency of the acetylenehexacarbonyldicobalt complexes depends on the structure of the coordinated alkyne. [(2-Butin-1-yl)acetylsalicylate]hexacarbonyldicobalt (**3**) and hexacarbonyl[(*S*)-(2-propinyl)-2-(6-methoxy-2-naphthyl)-propionate]dicobalt (**6**) reduced the cell growth only slightly in the highest concentration used (Fig. 4). Antiproliferative effects ($T/C_{corr} = 50\%$) were determined by [1-(4-fluorophenyl)-1-phenyl-4-pyrrolidinyl-2-butine-1-ol]hexacarbonyldicobalt (**8**) in a concentration of 5 μM. The most active compounds were the complexes **9**–**11**. After incubation for about 125 h hexacarbonyl[*N*-2-propinylbenzothiazolidine-3-one-1,1-dioxide]-dicobalt (**9**) reached a T/C_{corr} of 8% (Fig. 4) while the complexes hexacarbonyl[2-propinylacetylsalicylate]-dicobalt (**10**) and hexacarbonyl[2-propinylsalicylate]-dicobalt (**11**) caused cytocidal effects in a concentration of 5 μM (see Fig. 5). Furthermore, complex **10** was more active than cisplatin in all concentrations used (compare Figs. 3 and 5).

The same trends in activity were found for the complexes on the MDA-MB-231 cell line. The complexes **3** and **6** influenced the growth of the MDA-MB-231 cells only marginally ($T/C_{corr} \approx 75\%$ at 5 μM) (not shown), while **8** and **9** caused a 50% reduction of the proliferation in a concentration of 5 μM (Fig. 6). Analogously

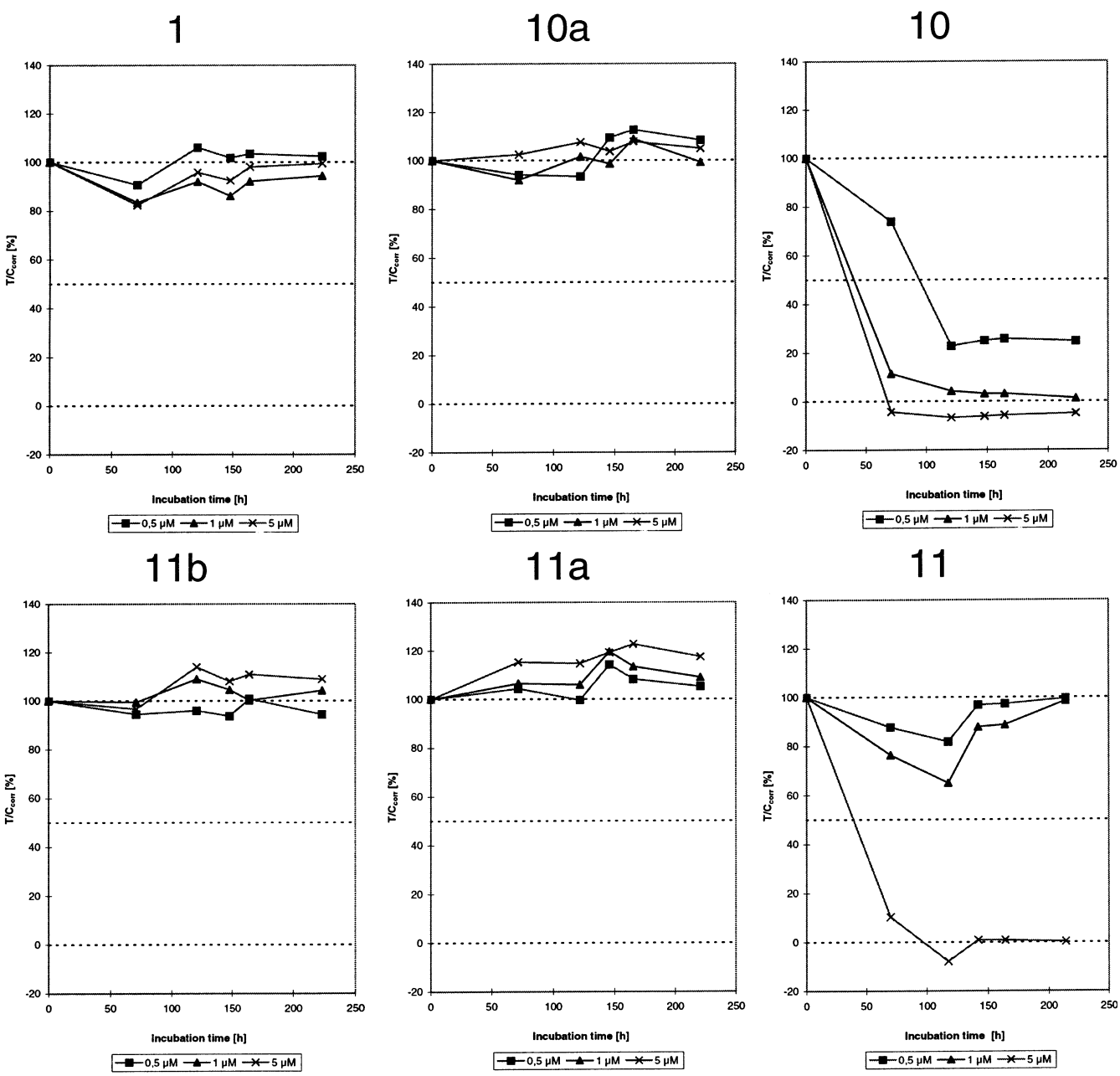


Fig. 5. Effect of **11b**, **11a**, **11** and **1**, **10a**, **10** (0.5, 1.0, 5.0 μM) on the proliferation of human MCF-7 cells.

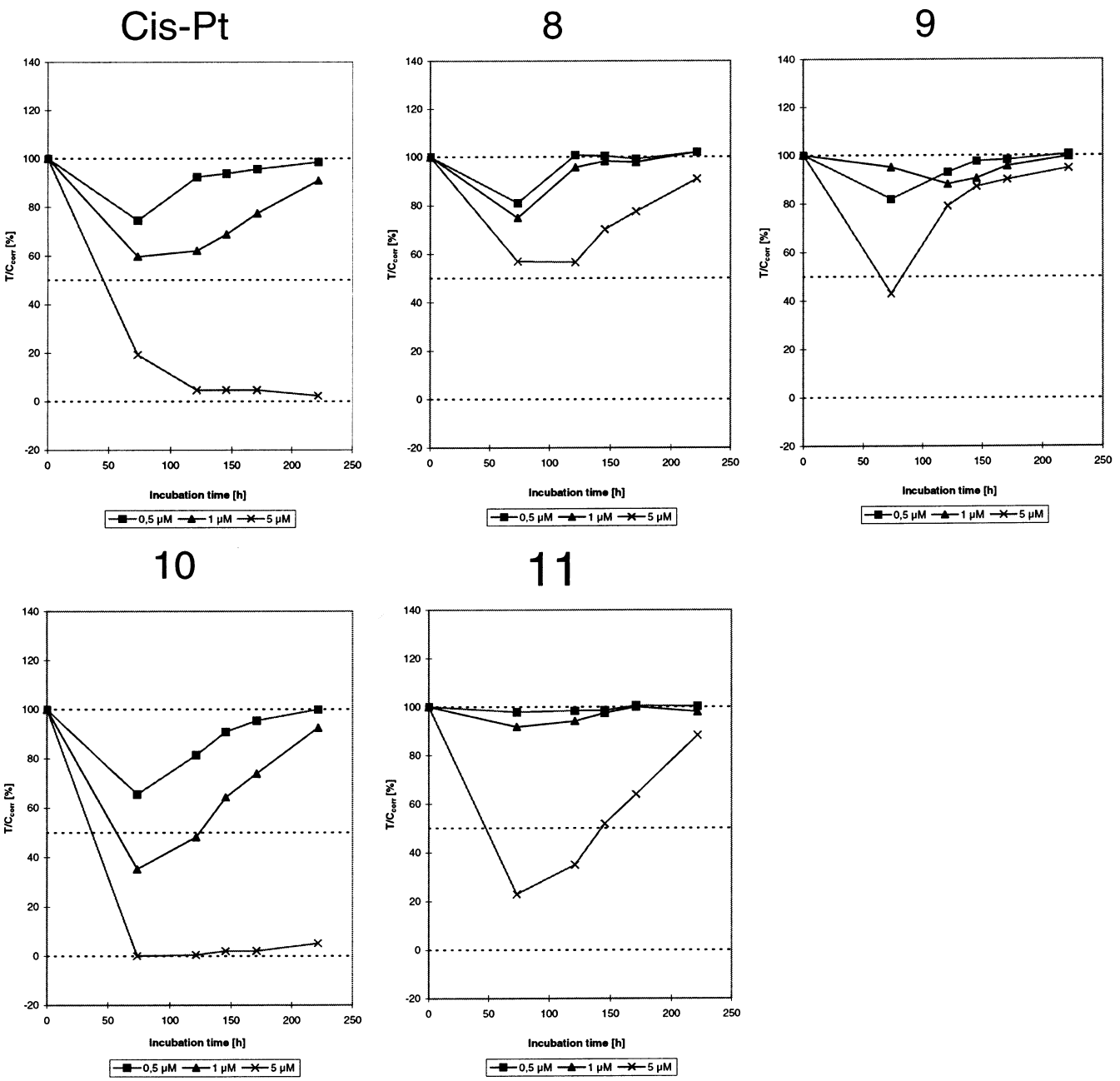


Fig. 6. Effect of **8**, **9**, **10** and **11** (0.5, 1.0, 5.0 μM) on the proliferation of human MDA-MB-231 cells.

to the results on the MCF-7 cell line **10** and **11** were the most active compounds. In a concentration of 5 μM **10** suppressed the cell growth completely ($T/C = 0\%$) while **11** showed a maximum effect of $T/C_{corr} = 20\%$.

As a third in vitro test we used the human LNCaP/FGC prostate cancer cell line. However, complex **10** with the highest cytotoxic potency was only half as active as cisplatin (see Fig. 7). In a 5 μM concentration the growth of LNCaP/FGC prostate cancer cells was reduced to $T/C_{corr} = 48\%$.

3.3. Discussion

The results of this paper demonstrate clearly that acetylenehexacarbonyldicobalt complexes represent a novel class of antitumor drugs. Their cytotoxic potency depends on the structure of the used acetylene ligand. Among the complexes tested, the hexacarbonyl[2-propinylacetylsalicylate]dicobalt (**10**) possesses the highest antitumor activity. **10** reduced the growth of H2981 lung adenocarcinoma and 3677 human melanoma cells [9] as well as the proliferation of human

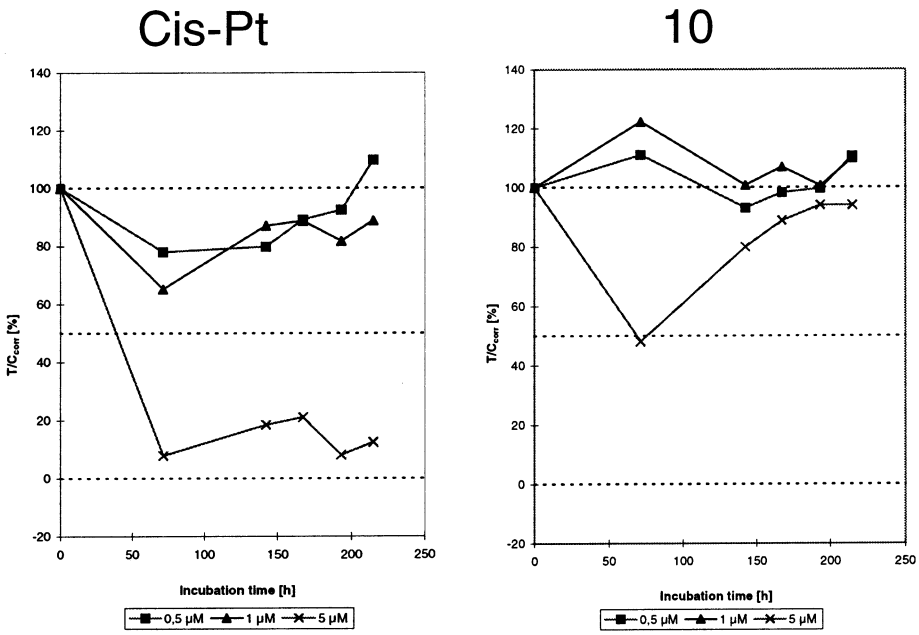


Fig. 7. Effect of **10** (0.5, 1.0, 5.0 μM) on the proliferation of human LNCaP/FGC cells.

mammary (MCF-7 and MDA-MB-231) and prostate carcinoma cells (LNCaP/FGC). On the MCF-7 and MDA-MB-231 cell line it is more active than cisplatin (compare Figs. 4–6) while it is only half as active against LNCaP/FGC cells (compare Fig. 7). The growth of 3677 human melanoma and H2981 lung adenocarcinoma cells was influenced by **10** in a 10 times higher concentration than the phenylenediamine mustard reference substance.

From these results it could be deduced that the 2-propinylsalicylate residue mediated the high activity for mammary carcinoma cells. The 2-propinylsalicylate complex **11** as well as the 2-propinylacetylsalicylate complex **10** exhibit the highest activity against mammary carcinoma cell lines.

To estimate the significance of the binding of the alkynes to the hexacarbonyldicobalt moiety for the cytotoxic effects, we tested possible decomposition products of an oxidative cleavage and subsequent hydrolysis of the complexes **10** and **11** under identical conditions on the MCF-7 cell line (see Fig. 5). As presented in Fig. 5, the 2-propinylsalicylate (**11a**), the 2-propinylacetylsalicylate (**10a**), the salicylic acid (**11b**) as well as the acetylsalicylic acid (**1**) are completely inactive, although acetylsalicylic acid (**1**) is known to have antitumor activity [19]. These results and the inactivity of $CoCl_2$ and $Co_2(CO)_8$ (see Fig. 3) indicate clearly that the antitumor properties are mediated by the intact acetylenehexacarbonyldicobalt complexes.

The mode of action, however, is unknown. But we assume an interaction of the acetylenehexacarbonyldicobalt moiety with biological targets, since substitution reactions at the bound ligands are possible.

As a first hypothetical mode of action a nucleophilic attack at the carbonyls must be discussed. In acetylenehexacarbonyldicobalt complexes these ligands have a partial positive charge at the carbon atoms (documented by the unusually low-field resonances of the Co–CO at $\delta \approx 199$) which allows a nucleophilic attack at these centers. Such reactions are well known in the literature and were investigated intensively. The group of Beck could demonstrate a bimolecular reaction of the S_N2-type for the interaction of hexacarbonyl complexes with *N*-nucleophiles (NH_3 or NHR^-) [20]. In a first reaction step the nucleophile binds to CO and a rearrangement reaction leads to an isocyanato derivative. Based on MO-calculations they concluded that the reaction rate depends on the magnitude of the positive charge at the C-atom.

A second possible center for a nucleophilic attack is represented by the alkyne ligand. Due to the π-bond the C–C triple bond becomes electron deficient. This leads e.g. to a deshielding of the C^2 carbon atoms as demonstrated by the low field shift of the resonances in the ^{13}C NMR-spectra. It can be assumed that especially C^2 may be subject to a nucleophilic attack.

As a third mode of action, an exchange of the ligands at the metal atoms by bionucleophiles is conceivable. For example the CO-ligands could be substituted by other electron donors such as amine- or R_2S-groups.

The results of this study revealed that also steric effects must be taken into consideration, since the spectroscopic studies proved comparable electronic environments at the conceivable reaction centers of the complexes. Large substituents at C^2 or methyl groups at C^3 lower the antitumor activity as documented by the effects of the complexes **3**, **10** and **11**.

4. Conclusions

Although their mode of action is unknown, acetylenehexacarbonyldicobalt complexes represent a new class of antitumor active metal complexes. Hexacarbonyl[2-propinylacetylsalicylate]dicobalt (**10**) was found to be the most active derivative among the new complexes with high activity against mammary carcinoma cell lines.

Acknowledgements

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