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Synthesis, cytotoxicity, cellular uptake and influence on eicosanoid metabolism of cobalt–alkyne modified fructoses in comparison to auranofin and the cytotoxic COX inhibitor Co-ASS

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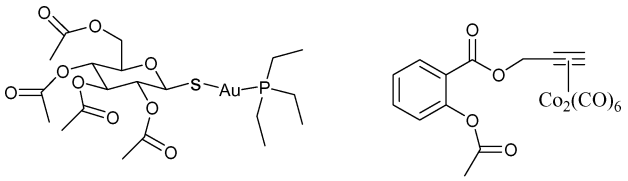
Propargylhexacarbonyldicobalt complexes with fructopyranose ligands were prepared and investigated for cytotoxicity in the MCF-7 human breast cancer cell line. The antiproliferative effects depended on the presence of isopropylidene protecting groups in the carbohydrate ligand and correlated with the cellular concentration of the complexes. IC_{50} values of > 20 μM demonstrated that the fructose derivatives were only moderately active compared to the references auranofin and the aspirin (ASS) derivative [2-acetoxy(2-propynyl)benzoate]hexacarbonyldicobalt (Co-ASS). In continuation of our studies on the mode of action of cobalt–alkyne complexes we studied the influence of the compounds on the formation of 12-HHT (COX-1 product) and 12-HETE (12-LOX product) by human platelets as an indication of the interference in the eicosanoid metabolism, which is discussed as a target system of cytostatics. Co-ASS was an efficient COX-1 inhibitor without LOX inhibitory activity and auranofin inhibited both COX-1 and 12-LOX eicosanoid production. The missing activity of the fructopyranose complexes at the 12-LOX and the only moderate effects at COX-1 indicate that COX/LOX inhibition may be in part responsible for the pharmacological effects of auranofin and Co-ASS but not for those of the fructopyranose complexes.

Introduction

The gold–glucose complex, auranofin, and the propargylhexacarbonyldicobalt derivative, Co-ASS, of the non-steroidal anti-inflammatory drug (NSAID) acetylsalicylic acid (aspirin, ASS) are useful lead structures for the design of new metal based cytostatics (for structures see Fig. 1).



auranofin Co-ASS

Fig. 1 Formulas of the reference substances auranofin and Co-ASS.

Auranofin was initially developed for the treatment of rheumatoid arthritis. Unfortunately, clinical trials documented not only its therapeutic efficiency but also manifested side effects, which limited its therapeutic use.[1,2,3,4] The pharmacological effects resulted from several immunosuppressive actions,[4] induction of mitochondrial permeability transition,[5] inhibition of thioredoxin reductase[5,6] or cathepsins,[7] as well the interference of eicosanoid metabolism *via* cyclooxygenase (COX) and lipoxygenase (LOX) enzymes.[8,9,10]

The interaction with COX and LOX enzymes might be a possible explanation for the antiproliferative effects of auranofin observed in diverse tumor models.

Such mode of action was recently postulated for Co-ASS, which possessed high cytotoxicity and represented by far a more efficient COX inhibitor than aspirin. Cisplatin-like effects were not observed in pharmacological studies.[11]

The inhibition of the eicosanoid producing enzymes of the cyclooxygenase and lipoxygenase class by NSAIDs is considered a promising strategy for the development of new cytostatics.[12,13,14]

Therefore, we tried to develop new cytotoxic COX/LOX inhibitors by a combination of essential structural features of auranofin and Co-ASS.

The decision to link a sugar moiety to the [propargyl]-$Co_2(CO)_6$ cluster was stimulated by the promising *in vitro* and *in vivo* antitumor results of certain carbohydrate complexes of palladium and platinum[15,16,17,18,19] and the findings that other metal–carbonyl complexes (iron containing nucleosides) exhibited cytotoxicity and triggered apoptotic effects.[20]

Concretely, we chose fructose as a substructure because it was reported that this sugar induced apoptosis at high millimolar concentrations.[21] Three new derivatives were prepared and investigated for cytotoxicity in the MCF-7 human breast cancer cell line. These cells are very sensitive to cobalt–alkyne complexes of the Co-ASS type[22,23] and exhibit high levels of COX-1 and 12-LOX.[24,25] The interaction with COX-1 and 12-LOX as a possible mode of action was studied in detail in an established human platelet assay by quantification of the eicosanoids 12(*S*)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid (12-HHT; COX-1 product) and 12(*S*)-hydroxy-5,8-*cis*-10-*trans*-14-*cis*-eicosatetraenoic acid (12-HETE; 12-LOX product).

Results and discussion

Chemistry

The synthesis of the target compounds is depicted in Scheme 1. The propargylic ether **3a** was prepared in a two step procedure starting from β-D-fructopyranose (**1**). In the first step, the hydroxy groups in positions 1,2,4 and 5 had to be protected by acetalation with acetone in order to guarantee the exclusive ether building at C3 in the second step.[26,27]

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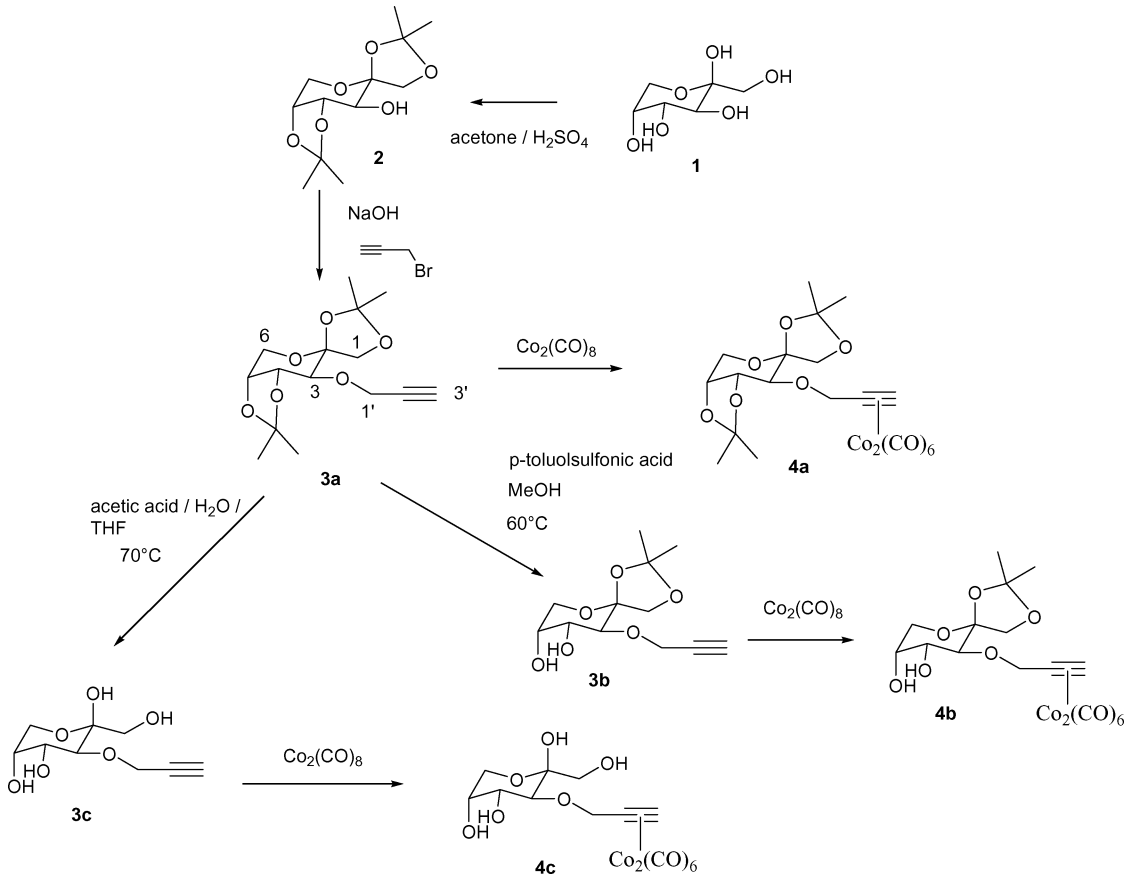

Scheme 1 Synthesis of cobalt–alkyne complexes with fructopyranose ligands.

The heating of **3a** with *para*-toluolsulfonic acid in methanolic solution for 3 h afforded the hydrolysis of the acetal at C4/C5 (**3b**, see Scheme 1), while heating of **3a** in an acetic acid–THF mixture for 18 h led to a complete loss of the protecting groups (**3c**, see Scheme 1). Interestingly, it was impossible to cleave the acetal at C1/C2.

The characterization of the propargylether derivatives was performed by NMR spectroscopy. Singlet resonances for the CH_3 groups appeared in the ^{1}H-NMR spectra of **3a** in the range of $\delta = 1.3$ to 1.6, while in the ^{13}C-NMR spectra the isopropylidene groups caused four CH_3 signals in the range of $\delta = 26$ to 29 and two $C(CH_3)_2$ signals at $\delta = 109$ and 112.[27] Cleavage of the acetal at C4/C5 reduced the number of these signals by half (for data see Experimental).

The location of the isopropylidene moiety in **3b** at the C1/C2 standing hydroxy groups can be deduced from the ^{13}C-NMR spectra due to a significant low field shift of the C2 ($\delta = 105.2$) and C1 ($\delta = 69.8$) resonances compared to **3c** ($\delta = 98.2$ and 68.6, respectively)

The coordination of the propargyl ethers **3a**–**3c** to obtain the corresponding cobalt–alkyne complexes **4a**–**4c** was performed by reaction with an excess of dicobaltoctacarbonyl in dry THF. Elemental analyses of all presented substances were within 0.4% of the calculated values and documented the high purity.

All complexes were characterized by spectroscopic methods. In the MS spectra a very characteristic fragmentation was observed. The compounds showed a consecutive loss of CO ($m/z = 28$, see Experimental), indicating the high stability of the cobalt–alkyne bond. The CO ligands also showed significant signals in the IR spectra. Intensive bands appeared in the range from 2000–2100 cm^{-1}, however, with only low resolution. Unfortunately, the ^{1}H-NMR spectra of **4a**–**4c** could only be used with some restrictions because the $Co_2(CO)_6$ cluster caused a broadening of the signals and made a coupling analysis impossible. Nevertheless, the coordination of the alkyne was unequivocally demonstrated by the characteristic shifts of the 3′-H from approx. $\delta = 2.5$ in the case of **3a**–**3c** to approx. $\delta = 6.1$ in the spectra of **4a**–**4c**.

It should be noted that the above mentioned effects are typical for cobalt–alkyne complexes.[22]

Cytotoxicity and cellular uptake

The cytotoxic activity of the target and the reference compounds (Co-ASS and auranofin) was evaluated using the MCF-7 human breast cancer cell line (see Fig. 2). Auranofin and Co-ASS caused IC_{50} values of 1.1 (± 0.3) and 1.4 (± 0.3) μM, respectively, and were even more potent cytostatics than cisplatin ($IC_{50} = 2.0$ (± 0.3) μM).[11]

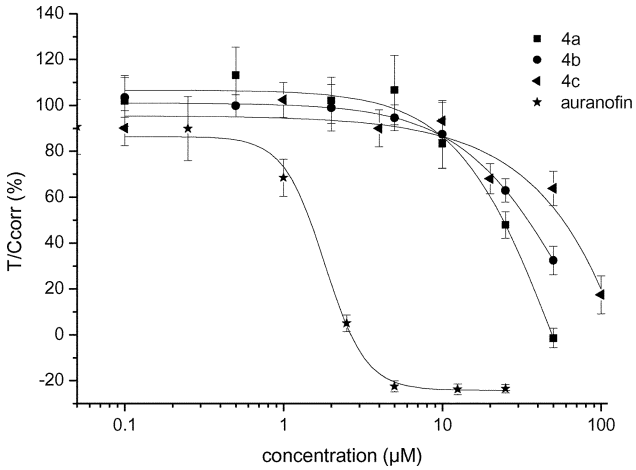

Fig. 2 Concentration dependent cytotoxicity (representative single experiments) of **4a**–**4c** and auranofin in MCF-7 cells.

The cobalt–alkyne complexes **4a**–**4c** significantly influenced the proliferation of the cells ($T/C_{corr} < 80\%$) only in concentrations higher than 10 μM. The degree of this effect depended on the protection of the hydroxy groups in the sugar moiety. Compound **4c**, with four hydroxy groups, showed only very low activity (IC_{50} = 63.4 (± 7.2) μM). Acetalation of the C1/C2 standing OH groups decreased the IC_{50} value about 1.8-fold to 35.3 (± 0.9) μM. Finally, **4a**, without free hydroxy groups, influenced the cell growth 1.6 times more efficiently than **4b** (IC_{50} = 21.4 (± 2.3) μM).

This effect might be the consequence of an increased lipophilicity of the protected compounds, leading to a higher cellular uptake. In order to confirm this assumption, we quantified the cellular cobalt concentration after 24 h of incubation with **4a**–**4c** at non toxic concentrations (2 μM and 10 μM). This protocol was used because we already demonstrated for cobalt–alkyne complexes a fast accumulation in MCF-7 cells, which remained constant over 24 h of incubation.[11]

As depicted in Fig. 3, the lowest intracellular concentrations were found for the least cytotoxic **4c** and each additional isopropylidene group led to an approx. two-fold increase of cellular uptake. The correlation between cytotoxicity (IC_{50} value) and the cellular uptake (pmol μg^{-1} value) was very good, with r^2 = 0.9050 for the 2 μM and r^2 = 0.9209 for the 10 μM experiments.

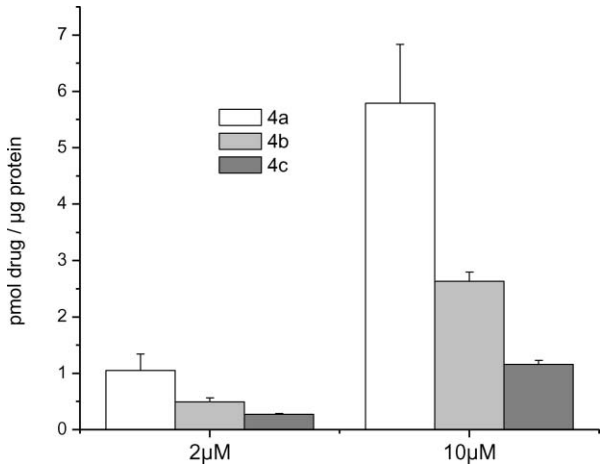

Fig. 3 Cellular uptake of cobalt–alkyne fructopyranose complexes into MCF-7 cells.

The accumulation grade in the tumor cells can be calculated from the mean cellular diameter, the mean cellular protein content and the intracellular molar concentration.[28,29] As exemplarily demonstrated for the most active compound **4a**, a saturation in the tumor cells was not achieved. Incubation with 2 μM as well as 10 μM of **4a** led to accumulation grades of about 60-fold.

It should be noted that the fructopyranose complexes were strongly enriched in MCF-7 cells, but not as high by far as other cobalt–alkyne derivatives. The reference Co-ASS showed an accumulation grade of up to 150-fold.[11]

These high cellular drug concentrations of cobalt–alkyne complexes compared to other metal-based drugs, *e.g.* cisplatin or carboplatin (accumulation grade in MCF-7 cells: < 6[28,29]) are supposedly due to their generally higher lipophilicity and might control the selectivity for breast cancer cells.[11,30,31] The number of free hydroxy groups in the compounds **4a**–**4c** correlates with the cellular uptake and the cytotoxicity. In leukemia cells, however, for Co-ASS and related derivatives distinctly lower intracellular concentrations and decreased antiproliferative effects were found.[31] This allows the assumption that the selectivity of cobalt–alkyne complexes for breast cancer cells is caused by a mechanism based on the lipophilicity of metal complexes.

In this context, it is of interest to note that the same tendency was observed for platinum complexes. Exchange of the NH_3 ligands of cisplatin by the very hydrophobic 1,2-bis(4-fluorophenyl)ethylenediamine increased the accumulation grade in MCF-7 cells from 1.1 (cisplatin) to 20-fold. Exchange of the 4-F substituents by hydroxy groups terminated this effect.[32]

Influence on eicosanoid metabolism

In contrast to platinum complexes which achieved antiproliferative effects *via* DNA-binding, the mode of action of cobalt–alkyne complexes is unclear.

However, for Co-ASS and related compounds an interference in the arachidonic acid cascade was recently demonstrated. The inhibition of these enzymes is a new strategy for the design of selective antitumor agents. Therefore, we studied the influence of the fructopyranose complexes as well as the references Co-ASS and auranofin on the production of the COX-1 product 12-HHT and the 12-LOX product 12-HETE in human platelets (see Table 1). The compounds were used in lower (10 μM) and higher (100 μM) concentrations compared to the IC_{50} values determined in the cytotoxicity experiments.

On one hand compounds **4a**–**4c** and Co-ASS did not influence the production of 12-HETE, neither in the 10 μM nor in the 100 μM concentrations. On the other hand, **4c** (22% inhibition at 100 μM) and Co-ASS (49% inhibition at 100 μM) were active against COX-1. Interestingly, **4a**, as the most active compound in the antiproliferation assay, stimulated 12-HHT production (128% at 100 μM). The formation of 12-HHT in the presence of **4b** could not be evaluated under the chromatographic conditions described in the "Experimental section".

It should be mentioned that auranofin was effective against both COX-1 and 12-LOX and inhibited eicosanoid formation by approx. 35–55%.

Conclusion

Cobalt–alkyne complexes with fructopyranose ligands were prepared and investigated for their cytotoxicity in MCF-7 human breast cancer cells. The antiproliferative effects depended on the presence of the isopropylidene protecting groups in the carbohydrate ligand. Cellular uptake studies document that the high cellular drug concentrations depended on the protection of the hydroxy groups in the fructopyranose moiety. Compared to auranofin, established cytostatics and the cytotoxic COX-inhibitor Co-ASS, the antiproliferative activity of the target compounds was only moderate. The evaluation of the influence on eicosanoid metabolism demonstrated no notable influence on 12-LOX activity and only low influence (inhibition or induction) on COX-1 activity. The reference compound Co-ASS was by far a more active COX-1 inhibitor without activity on 12-LOX, while auranofin inhibited both COX-1 and 12-LOX. Therefore, the inhibition of COX and LOX as a mode of drug action becomes reasonable for auranofin and Co-ASS but not for the cobalt–alkyne fructopyranose derivatives.

Table 1 Influence of cobalt–alkyne fructopyranoses and the reference compounds auranofin and Co-ASS on COX-1 and 12-LOX product formation in human platelets;— not determined

	12-HHT (%)		12-HETE (%)	
Compound	10 μM	100 μM	10 μM	100 μM
4a	110 (± 2)	128 (± 18)	90 (± 4)	89 (± 7)
4b	—	—	97 (± 8)	96 (± 15)
4c	83 (± 16)	78 (± 8)	95 (± 3)	89 (± 26)
Auranofin	61 (± 6)	48 (± 11)	65 (± 17)	47 (± 22)
Co-ASS	92 (± 15)	51 (± 10)	108 (± 5)	93 (± 10)

Experimental

General

Chemicals were purchased from Sigma, Fluka or Acros. 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose **2** and 1,2:4,5-di-*O*-isopropylidene-3-*O*-(2-propinyl)-β-D-fructopyranose **3a** were prepared by literature procedures.[26,27] MCF-7 cells were maintained as described.[22] Drugs were freshly prepared as stock solutions in dimethylformamide (DMF) and diluted with cell culture medium when used for the biochemical experiments (DMF 0.1 v/v). HPLC: Kontron HPLC system equipped with a diode array detector. Elemental analysis: Perkin-Elmer 240 C. IR spectra: ATI Mattson Genesis. NMR spectra: Avance/DPX 400 (Bruker), MS spectra: CH-/A-Varian MAT (70 eV).

Synthesis

1,2-*O*-Isopropylidene-3-*O*-(2-propinyl)-β-D-fructopyranose (3b). A solution of 400 mg (2.3 mmol) of *para*-toluolsulfonic acid in 12.0 mL of methanol was added to a solution of 200 mg (0.67 mmol) of **3a** in 22.5 mL of methanol. After stirring for 3 h at 60–65 °C the reaction was stopped by addition of 126 mL of saturated $NaHCO_3$. The product was extracted with dichloromethane, the solvent was removed and the product was isolated by column chromatography (mobile phase: dichloromethane–methanol, 19 : 1). Yield: 155 mg (0.60 mmol, 90%) light yellow oil.

^{1}H-NMR ($CDCl_3$): $\delta = 1.43$, 1.49 (2s, 6H, 2 CH_3), 2.52 (t, $J =$ 2.2 Hz, 1H, 3′-H), 3.78 (m, 2H, ring-CH), 4.00 (m, 4H, ring-CH), 4.21 (d, $J = 8.7$ Hz, 1H, 1-Hb), 4.38 (dd, $J = 2.4$ Hz, $J = 16.1$ Hz, 1H, 1′-Ha), 4.55 (dd, $J = 2.3$ Hz, J = 16.1 Hz, 1H, 1′-Hb). ^{13}C-NMR ($CDCl_3$): $\delta = 26.0$, 26.9 (CH_3), 60.0 (1′-C), 63.5 (6-C), 69.8 (1-C), 71.4, 71.8 (3-C, 5-C), 75.4 (3′-C), 75.7 (4-C), 79.8 (2′-C), 105.2 (2-C), 112.0 (C(CH_3)$_2$). MS (EI, 80 °C): m/z (%) = 258(0.7) [M$^+$], 243(25.0) [M$^+$ − CH_3], 103(100). CHN (found: C, 55.48; H, 6.99%. Calc. for $C_{12}H_{18}O_6$ C, 55.81; H, 7.02%).

3-*O*-(2-Propinyl)-β-D-fructopyranose (3c). An amount of 1.00 g (3.36 mmol) of **3a** was dissolved in 165 mL of 60% acetic acid–THF, 8 : 2 (v/v) and stirred for 18 h at 65 °C. The solvent was removed, the residue was taken up in toluol and evaporated to dryness again (three times) to remove any water. The product was isolated by column chromatography (mobile phase: CH_2Cl_2–methanol, 3 : 1). Yield: 350 mg (1.61 mmol, 48%) light yellow oil.

^{1}H-NMR ($CDCl_3$): $\delta = 2.53$ (t, $J = 2.3$ Hz, 1H, 3′-H), 3.35 (d, $J = 16.5$ Hz, 1H, 3-H), 3.79 (m, 3H, 1-Ha, 6-H), 4.07 (m, 1H, 5-H), 4.11 (d, $J = 3.0$ Hz, 1H, 1-Hb), 4.40 ($J = 2.3$ Hz, $J = 16.2$ Hz, 1H, 1′-Ha), 4.56 (dd, $J = 2.3$ Hz, J = 16.2 Hz, 1H, 1′-Hb). ^{13}C-NMR ($CDCl_3$): $\delta = 60.1$ (1′-C), 62.5 (6-C), 68.6 (1-C), 71.3, 73.4 (3-C, 5-C), 75.8 (3′-C), 76.7 (4-C), 78.1 (2′-C), 98.2 (2-C). MS (EI, 200 °C): m/z (%) = 201(84.6) [M$^+$ − OH], 111(100). CHN (found: C, 49.92; H, 6.81 %. Calc. for $C_9H_{14}O_6$ C, 49.54; H, 6.47%).

General method for the preparation of cobalt–alkyne complexes 4a–4c

Cobalt–alkyne complexes were prepared according to a previously described procedure:[22] the alkyne (0.3–1.2 mmol) was dissolved in 10–15 mL of dry THF. Dicobaltoctacarbonyl was added in excess and the reaction mixture was stirred at rt until no further product formation was observed by thin layer chromatography. Silica gel (1 g) was added and the solution was evaporated to dryness. The dark colored products were isolated by flash column chromatography on silica gel (mobile phase: diethylether–petrol ether). Yields were not optimized.

Hexacarbonyl[1,2:4,5-di-*O*-isopropylidene-3-*O*-(2-propinyl)-β-D-fructopyranose]dicobalt (4a). 298 mg (1.00 mmol) **3a**, 377 mg (1.10 mmol) dicobaltoctacarbonyl. Yield: 485 mg (0.83 mmol, 83%) dark red oil.

^{1}H-NMR ($CDCl_3$): $\delta = 1.40$, 1.50 (2 s, br, 12H, 4 CH_3), 3.62–5.04 (m, 9H, 1–6H, 1′-H), 6.05 (s, 1H, 3′-H). MS (EI, 75 °C): m/z (%) = 569(2.7) [M$^+$ − CH_3], 556(0.7) [M$^+$ − CO], 528(8.9) [M$^+$ − 2CO], 500(24.1) [M$^+$ − 3CO], 472(37.7) [M$^+$ − 4CO], 444(7.2) [M$^+$ − 5CO], 416(18.9) [M$^+$ − 6CO], 28(100) [CO]. IR (KBr): $\nu_{max}/cm^{-1} = 2095$, 2054, 2027 (Co–CO). CHN (found: C, 43.00; H, 3.92%. Calc. for $C_{21}H_{22}Co_2O_{12}$ C, 43.17; H, 3.80%).

Hexacarbonyl[1,2-*O*-isopropylidene-3-*O*-(2-propinyl)-β-D-fructopyranose]dicobalt (4b). 80 mg (0.31 mmol) of **3b**, 189 mg (0.55 mmol) of dicobaltoctacarbonyl. Yield: 141 mg (0.26 mmol, 84%) dark red crystals (mp 72 °C).

^{1}H-NMR ($CDCl_3$): $\delta = 1.27$ (s, br, 6H, 2 CH_3), 1.99 (s, br, OH), 2.56 (s, br, OH), 3.37–4.86 (m, 9H, 1–6H, 1′-H), 6.08 (s, br, 1H, 3′-H). MS (EI, 170 °C): m/z (%) = 488(2.7) [M$^+$ − 2CO], 460(3.8) [M$^+$ − 3CO], 432(9.4) [M$^+$ − 4CO], 404(2.1) [M$^+$ − 5CO], 376(5.1) [M$^+$ − 6CO], 28(100) [CO]. IR (KBr): $\nu_{max}/cm^{-1} = 2095$, 2052, 2023 (Co–CO). CHN (found: C, 39.77; H, 3.63%. Calc. for $C_{18}H_{18}Co_2O_{12}$ C, 39.73; H, 3.33%).

Hexacarbonyl[3-*O*-(2-propinyl)-β-D-fructopyranose]dicobalt (4c). 250 mg (1.15 mmol) of **3c**, 566 mg (1.65 mmol) of dicobaltoctacarbonyl. Yield: 470 mg (0.93 mmol, 81%) dark red crystals (mp 76 °C).

^{1}H-NMR($CDCl_3$): $\delta = 0.78$–2.59 (m, br, 4H, 4 OH), 2.76–5.15 (m, 9H, 1–6H, 1′-H), 6.06 (s, br, 1H, 3′-H). MS (EI, 75 °C): m/z (%) = 487(0.6) [M$^+$ − OH], 462(2.2) [M$^+$ − CH_2/CO], 434(3.9) [462 − CO], 406(11.6) [462 − 2CO], 378(1.1) [462 − 3CO], 350(9.0) [462 − 4CO], 28(100) [CO]. IR (KBr): $\nu_{max}/cm^{-1} =$ 2097, 2052, 2021 (Co–CO). CHN (found: C, 35.41; H, 2.42%. Calc. for $C_{15}H_{14}Co_2O_{12}$ C, 35.74; H, 2.80%).

Biological methods

Cytotoxicity. The cytotoxicity assay was performed as described previously.[11] Briefly, MCF-7 cells were incubated with cell culture medium containing the drugs in graded concentrations for 96 h and the influence on cell growth was determined as a T/C_{corr}(%) value. In a single experiment each compound concentration was tested six-fold. The IC_{50} value was calculated as the concentration reducing the proliferation of the cells by 50% and is presented as a mean (± SEM) of two or three independent experiments.

Cellular uptake. The cellular uptake was determined as described previously.[11] Briefly, at least 70% confluent MCF-7 cells were incubated with cell culture medium containing the drugs in concentrations of 2 μM and 10 μM for 24 h. The medium was removed, the cells were washed twice with phosphate buffered saline, lysed and the cellular lysates were investigated for their cobalt content by atomic absorption spectroscopy and for their protein content. Each experiment was performed in triplicate and the results were expressed as pmol drug per μg protein value (± SEM) calculated from the data of three independent experiments.

Determination of 12-LOX and COX-1 product formation. The formation of 12-HTT and 12-HETE was performed according to a previously described assay[33] with some modifications. Briefly, venous blood was taken from healthy adult donors and diluted 1 : 1 with phosphate buffered saline (pH 7.4). Each 10 mL lymphocyte separation medium (PAA, density: 1.077 g mL^{-1}) were overlayed with 20 mL of the diluted cell suspension. For isolation of the platelets the two layer system was centrifuged at 800 g at rt for 10 min, the supernatant was isolated and centrifuged at 2100 g for 15 min at rt. The drugs were freshly prepared as stock solutions in DMF for each experiment. To determine 12-LOX and COX-1 product formation in intact cells, platelets were resuspended in 5.0 mL phosphate buffered saline (pH 7.4) and each 1.0 mL aliquot

was incubated with 10 μM and 100 μM of test compound or vehicle for 60 min at 37 °C (final DMF concentration 0.1% v/v). Arachidonic acid (final concentration 40 μM) and calcium ionophore (final concentration 10 μM) were added and the reaction was stopped after 15 min at 37 °C by addition of 1.0 mL MeOH. 30 μL 1 N HCl, 1.2 μg of PGB1 (internal standard) and 500 μL phosphate buffered saline were added. After centrifugation (800 g, 10 min) 12-HETE and 12-HHT were extracted using Strata C18-E (500 mg/3 ml) solid phase extraction columns (Phenomenex) and analyzed by HPLC using the internal standard calibration method. The chromatographic conditions were as follows. Mobile phase: methanol–water–trifluoroacetic acid: 72 : 28 : 0.007 or 65 : 35 : 0.007; flow rate: 0.7 mL min^{-1}; stationary phase: Nucleosil C18 (25 cm, 5 μM, 4 mm id). Wavelengths of 235 nm (12-HHT, 12-HETE) and 280 nm (PGB1) were used for detection. The influence on the product formation was calculated as percentage of the untreated control and is presented as means (± SEM) of two or three independent experiments.

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