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Original article

[Cyclopentadienyl]metalcarbonyl complexes of acetylsalicylic acid as neo-anticancer agents

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ABSTRACT

[(Prop-2-ynyl)-2-acetoxybenzoate]dicobalthexacarbonyl (Co-ASS), a derivative of the nonsteroidal anti-inflammatory drug aspirin® (ASS), demonstrated high cytotoxic potential against various tumor cells. The [acetylene]$Co_2(CO)_6$ cluster strongly increased the biological effects compared to aspirin®. In this study we evaluated the use of [cyclopentadienyl]metalcarbonyl as cytotoxic moiety with a broader series of metals: molybdenum, manganese, cobalt and rhodium. All compounds were tested for cytotoxicity against breast (MCF-7, MDA-MB-231) and colon cancer (HT-29) cell lines. Their COX-1 and COX-2 inhibitory effects were evaluated at isolated isoenzymes. Additionally, the influence on the level of the major COX metabolite prostaglandin E_2 (PGE_2) was quantified in MDA-MB-231 breast cancer cells. Whereas the pure ligands or ASS did not show any cytotoxic effect, all metal complexes inhibited the tumor cell growth. The inhibitory effects at COX-1 and COX-2 enzymes were low. Only the Prop-Cp-ASS-Rh complex (10 μM) caused an important inhibition of COX-1 by 60% and COX-2 by 30%. ASS showed at the same concentration only a marginal repression of COX-1 activity (30%) and no effect on COX-2.

1. Introduction

Despite the success of cisplatin and closely related platinum agents in tumor therapy [1,2], there is an increasing interest in new metal-based drugs for the second line therapy of platinum resistant tumors, e.g. the mammary carcinoma using carrier ligands [3–8]. Especially with complexes of ruthenium, rhodium or manganese promising results were generated during the last years [9,10].

Furthermore, some nonsteroidal anti-inflammatory drugs (NSAIDs), mainly aspirin® (ASS), have already demonstrated their activity in prevention of colon cancer. ASS mediates its effects via interaction with the cyclooxygenase enzymes COX-1 and COX-2 without showing cytotoxic effects. In order to optimize ASS for the treatment of cancer we focused our attention on its combination with a metal moiety. [(Prop-2-ynyl)-2-acetoxybenzoate]hexacarbonyldicobalt (Co-ASS) was already identified as a cytotoxic COX inhibitor [11]. The cobalt cluster mediated cytotoxic properties and higher COX inhibition compared to ASS [12]. Whether the inactivation of the COX caused cell death is still unclear. However, it has been already verified that the interaction with the DNA (as known from other metal complexes) did not contribute to the mode of action [13].

In a structure–activity relationship (SAR) study we tried to verify the significance of the metal and its binding mode to the ASS moiety for cytotoxic and COX inhibitory effects. In the first part of this study [14], we demonstrated that the [alkyne]$Co_2(CO)_6$ cluster caused maximal effects. Exchange of the $Co_2(CO)_6$ by Ru- or Fe-carbonyls and the use of higher metal carbonyl cluster (e.g. $Co_4(CO)_{10}$) led to active compounds but did not enhance the biological activity compared to Co-ASS [14]. Interestingly, the ferrocen analogous was completely inactive.

In the present study, we used a cyclopentadiene moiety and combined it with metalcarbonyls of cobalt, manganese, rhodium and molybdenum. These compounds were easily available using η^5-[(cyclopentadienyl)alkyl-2-acetoxybenzoate]thallium as intermediate [15]. Furthermore, the alkyl chain was varied in length in order to evaluate the significance of the distance between the NSAID and the metal for cytotoxicity and COX inhibition.

The cytotoxic effects were investigated in vitro using HT-29 colon carcinoma cells as well as MDA-MB-231 and MCF-7 breast cancer cells. MCF-7 cells have a basal level of COX-1 and a barely detectable and transient COX-2 inducible expression, whereas MDA-MB-231 cells show a low expression of COX-1 but a constitutive level of COX-2 [16]. Further experiments included the COX inhibitory properties

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at isolated enzymes by ELISA and the quantification of the major intracellular metabolite prostaglandine E_2 (PGE_2) in MDA-MB-231 cells after treatment with the drugs.

2. Chemistry

The (cyclopenta-1,3-dienyl)alkyl-2-acetoxybenzoates were prepared by esterification of 2-acetylsalicoyl chloride, synthesized according to Riegel and Wittcoff [17] or Weizmann et al. [18] with the respective cyclopentadienyl alcohol [19] obtained from freshly cracked cyclopentadiene after deprotonation with NaH in THF and reaction with the chloroalcohol at 0 °C (see Scheme 1).

Attempts failed to achieve 2-cyclopentadienylethanol by reaction of 2-chloroethanol and sodocen. Only bis(cyclopentadienyl) ethane could be isolated. Therefore, we modified the procedure described by Schroeder et al. [20]. Potassium-*tert*-butylat was used as base and ethylene oxide as reactant. Cyclopentadienylethanol was obtained as clear oil by vacuum distillation. It should be noted that it was impossible up to now to get the methylene derivative.

The Alkyl-Cp-ASS compounds were characterized by ^1H NMR spectroscopy. Due to the tautomerism of the double bond, an isomeric mixture was present in each case. A rapid degradation at room temperature as described for related compounds [21] could not be detected. Only 10% of the compounds degraded by Diels–Alder-reactions within 4 months, while they were stable at −20 °C for more than a year.

Reaction of Alkyl-Cp-ASS with one equivalent of thallium ethoxide in n-hexane at −30 °C afforded the corresponding thallium complex (Alkyl-Cp-ASS-Tl, see Scheme 1), which was isolated as beige solid and characterized by HR-EI-MS and elemental analyses.

The variation of the metal was performed on the example of Prop-Cp-ASS. Treatment of Prop-Cp-ASS-Tl with $MnBr(CO)_5$ in THF yielded the manganese complex Prop-Cp-ASS-Mn as yellow oil, reaction with $[Rh_2(CO)_4Cl_2]$ in THF at −50 °C gave Prop-Cp-ASS-Rh as a dark brown solid (Scheme 2). TlBr or TlCl precipitated during the reaction and were sucked off prior to the evaporation of the solvent.

Prop-Cp-ASS-Co was obtained by treatment of two equivalents of Prop-Cp-ASS-Tl in THF with the deep green reaction mixture of I_2 and $Co_2(CO)_8$ in THF at room temperature (Scheme 2) [22]. The resulting deep yellow solid could be purified by column chromatography.

Finally, Prop-Cp-ASS was reacted with $Mo(CO)_3(NCMe)_3$ in toluene to yield Prop-Cp-ASS-Mo, which was treated with a stoichiometric amount of CHI_3 in dichloromethane giving Prop-Cp-ASS-Mo-I as dark red crystalline solid (see Scheme 2).

In order to clarify the importance of the aspirin-moiety for the biological effects of Alkyl-Cp-ASS-Tl complexes, thallocen (cyclopentadienylthallium) was synthesized as further reference compound [23].

3. Results and discussion

3.1. Cytotoxicity

Antitumor activity was determined in vitro using MCF-7 and MDA-MB-231 breast cancer cell lines as well as the colon carcinoma cell line HT-29 (Table 1) in a crystal violet assay. In this test, the cell biomass was quantified after an incubation time of 96 h under the influence of vehicle (DMSO) or increasing amounts (0.1–10 μM) of complexes. For each concentration the T/C_{corr} value was calculated (see Section 5.2.2). The IC_{50} value represents the concentration causing 50% inhibition of growth inhibition ($T/C_{corr} = 50\%$).

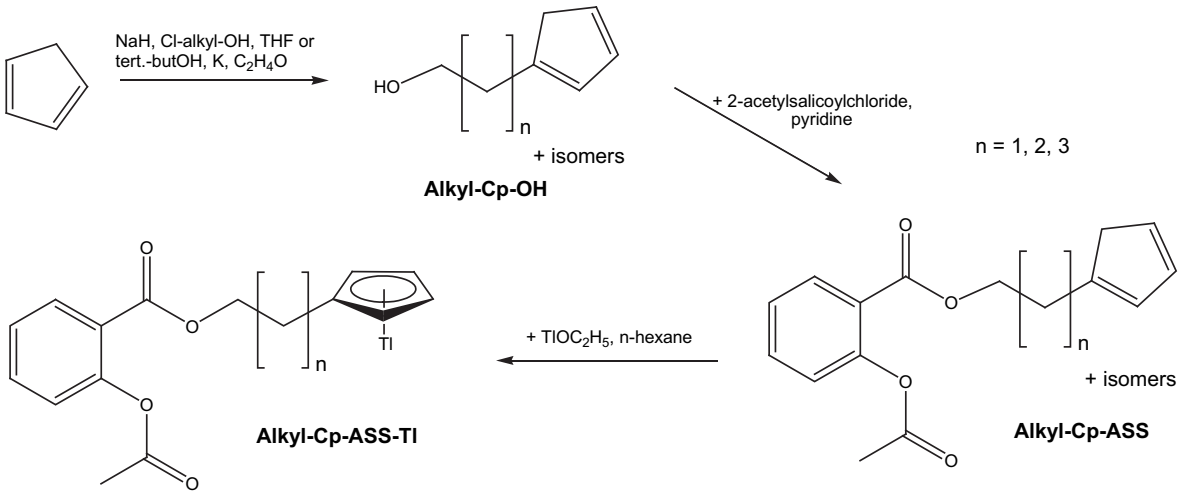
Cisplatin as a reference reduced the growth of all used cell lines with IC_{50} values in the range of 2.0–3.3 μM. Co-ASS showed a clear selectivity for mammary carcinoma cells (MCF-7: $IC_{50} = 1.4$ μM; MDA-MB-231: $IC_{50} = 1.9$ μM) and was more active than cisplatin (MCF-7: $IC_{50} = 2.0$ μM; MDA-MB-231: $IC_{50} = 3.3$ μM). Against HT-29 cells an IC_{50} value of only 9.8 μM was calculated. The cytotoxicity of Co-ASS was clearly mediated by the combination of ASS and the $[propargyl]Co_2(CO)_6$ moiety. Both, ASS and the cobalt cluster alone were inactive ($IC_{50} > 50$ μM).

Alkyl-Cp-ASS-metal complexes showed a comparable trend. The free ligands and the reactants $Mn(CO)_5Br$, $Rh_2(CO)_4Cl_2$ and $Mo(CO)_3NCMe$ did not cause cytotoxicity. Only thallocen was marginally active at HT-29 cells ($IC_{50} = 23.9$ μM).

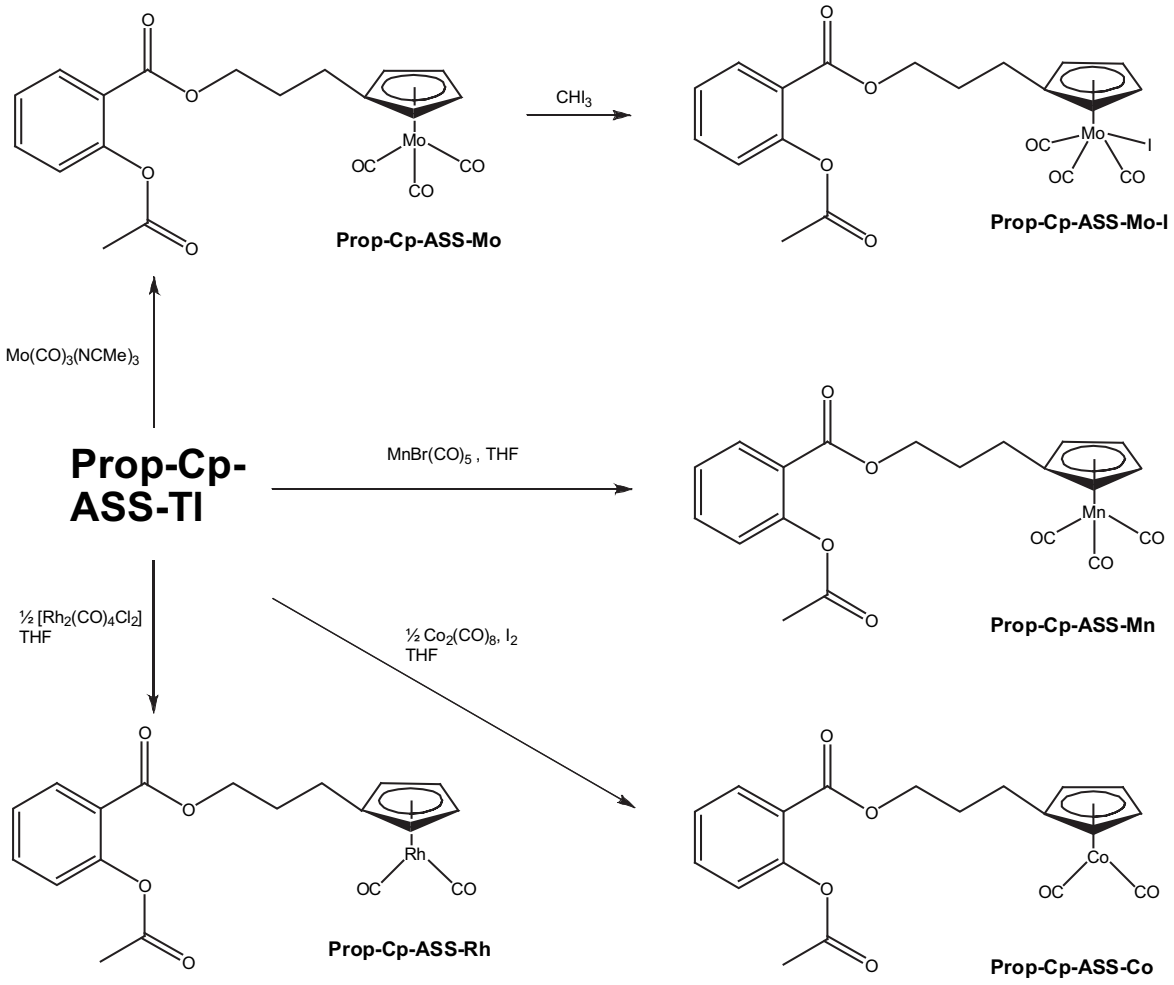
Thallium complexes demonstrated cytotoxic properties at the MCF-7 cell line dependent on the length of the alkyl spacer (Et ($IC_{50} = 9.8$ μM) < Prop ($IC_{50} = 7.3$ μM) < But ($IC_{50} = 5.4$ μM)). At the MDA-MB-231 cell line But-Cp-ASS-Tl ($IC_{50} = 20.7$ μM) was less active than its ethyl ($IC_{50} = 11.4$ μM) and propyl ($IC_{50} = 11.7$ μM) derivatives, while at the HT-29 cell line Et-Cp-Ass-Tl showed high cytotoxicity ($IC_{50} = 4.6$ μM).

Prop-Cp-ASS-Tl was selected for metal-variations. The exchange of Tl by Co-, Mn-, Rh- and Mo-carbonyls slightly reduced the activity at MCF-7 cells ($IC_{50} = 10–15$ μM). An exception was Prop-Cp-ASS-Mn which was distinctly less active at MCF-7 ($IC_{50} = 24.8$ μM) cells and completely inactive at the MDA-MB-231 cell line.

Generally, these compounds are less cytotoxic against MDA-MB-231 cells. The IC_{50} values amounted to only 26–50 μM. Effects at HT-29 and MCF-7 cells were nearly identical (Table 1).



Scheme 1. Synthesis of Alkyl-Cp-ASS and related thallium complexes.



Scheme 2. Metal exchange at Prop-ASS-Tl.

3.2. Determination of COX inhibitory effects

Since it was very likely that the interference in the arachidonic acid pathway does contribute to the mode of action, the effects of the organometallic compounds on isolated COX-1 and COX-2 enzymes were quantified by ELISA. In an MDA-MB-231 cell based assay we also quantified the PGE_2 level as an indicator of interaction with intracellular cyclooxygenase enzymes.

Aspirin® was only marginally active in the ELISA at a concentration of 10 μM (COX-1: 29% inhibition; COX-2: no effects). At the same concentration, none of the free ligands were able to inhibit the enzymes.

Prop-Cp-ASS-metal complexes showed inhibitory effects at COX-1 very similar to aspirin®. Only Prop-Cp-ASS-Mo-I was less active, while with Prop-Cp-ASS-Rh inhibitory effects of 57.3% were achieved.

At the COX-2 isoenzyme all Prop-Cp-ASS-metal complexes were more active than aspirin® (Chart 1). Prop-Cp-ASS-Tl (42.3%

Table 1
Cytotoxic effects (IC_{50} values given in μM) of Alkyl-Cp-ASS-metal complexes.

Compound	MCF-7	MDA-MB-231	HT-29
cisplatin	2.0 ± 0.3	3.3 ± 0.5	2.4 ± 0.4
Co-ASS	1.4 ± 0.3	1.9 ± 0.3	9.8 ± 3.0
thallocen	>50	>50	23.9 ± 2.7
$Mn(CO)_5Br$, $Rh(CO)_4Cl_2$	>50	>50	>50
$Mo(CO)_3NCMe$	>50	>50	>50
aspirin	>50	>50	>50
Alkyl-Cp-ASS	>50	>50	>50
Et-Cp-ASS-Tl	9.8 ± 0.2	11.4 ± 0.7	4.6 ± 0.5
Prop-Cp-ASS-Tl	7.3 ± 2.0	11.7 ± 0.9	12.1 ± 2.3
But-Cp-ASS-Tl	5.4 ± 0.2	20.7 ± 0.1	10.5 ± 0.2
Prop-Cp-ASS-Mo	14.0 ± 3.6	26.1 ± 0.2	13.8 ± 2.6
Prop-Cp-ASS-Mo-I	13.7 ± 2.4	31.3 ± 2.6	12.0 ± 1.6
Prop-Cp-ASS-Mn	24.8 ± 3.6	>50	30.4 ± 4.0
Prop-Cp-ASS-Co	10.8 ± 2.3	34.4 ± 7.3	12.3 ± 2.6
Prop-Cp-ASS-Rh	10.2 ± 3.4	26.6 ± 0.5	9.2 ± 1.7

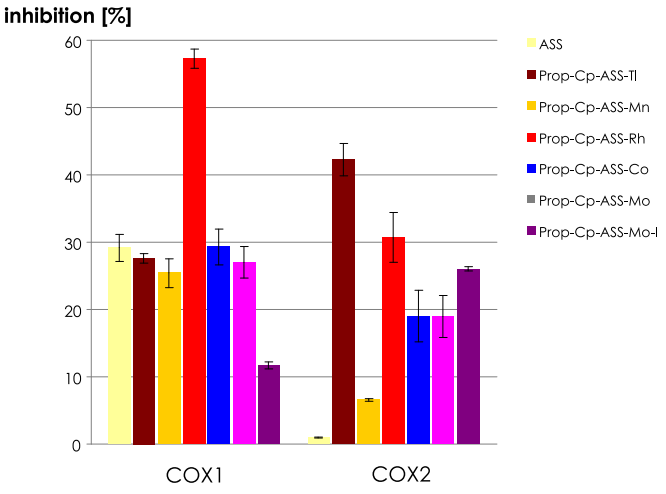


Chart 1. COX inhibition of Prop-Cp-ASS-metal compounds.

Table 2
COX and PGE_2 assay results of selected compounds.

Compound	COX-1	COX-2	PGE
aspirin®	29.2% ± 2.0	1% ± 0.1	0
Co-ASS	68.0% ± 5.4	63.0% ± 5.0	40.0% ± 5.0
Et-Cp-ASS-Tl	19.3% ± 1.0	14.5% ± 0.5	26.5% ± 8.0
Prop-Cp-ASS-Tl	27.6% ± 0.7	42.3% ± 2.4	35.6% ± 5.0
But-Cp-ASS-Tl	17.2% ± 1.2	12.3% ± 0.7	12.5% ± 1.0
Prop-Cp-ASS-Mo	27.0% ± 2.3	24.0% ± 3.1	3.8% ± 0.4
Prop-Cp-ASS-Mo-I	11.7% ± 0.5	26.0% ± 0.3	4.5% ± 0.2
Prop-Cp-ASS-Mn	25.4% ± 2.1	6.6% ± 0.2	2.6% ± 0.1
Prop-Cp-ASS-Co	29.3% ± 2.7	19.0% ± 3.8	11.3% ± 0.8
Prop-Cp-ASS-Rh	57.3% ± 1.4	30.7% ± 3.7	55.1% ± 4.4

inhibition) and Prop-Cp-ASS-Mo-I (26.0% inhibition) even exceeded their effects on COX-1 – (Table 2). The elongation or reduction of the chain length reduced the effects at both isoenzymes.

The same trend was observed for Alkyl-Cp-ASS-Tl complexes on the PGE_2 level in MDA-MB-231 cells. Prop-Cp-ASS-Tl lowered the PGE_2 level more effectively (35.6%) than its ethyl (Et-Cp-ASS-Tl: 26.5%) or butyl derivative (But-Cp-ASS-Tl: 12.5%). Exchange of the Tl by Mo, Mn or Co terminated the effects (0–5% inhibition). Only the Prop-Cp-ASS-Rh complex showed increased inhibition (55.1%), even higher than those obtained with Co-ASS.

The COX inhibitory effects of the Alkyl-Cp-ASS-metal complexes did not correlate with the cytotoxicity against MDA-MB-231 and MCF-7 cells. Therefore, it is very likely that the interference of Alkyl-Cp-ASS-metal complexes in the arachidonic acid cascade plays a subordinate role in the mode of action.

The high COX inactivation observed by Prop-Cp-ASS-Rh did not lead to strong cytotoxicity.

However, there have been several reports utilizing HT-29 cells in which NSAIDs have been found to inhibit proliferation, cause cell cycle quiescence, and induce apoptosis. There exist most probably different pathways involving both COX-mediated and non-COX-mediated mechanisms in the induction of apoptosis. Detailed pharmacological studies are in progress and will be published in a forthcoming paper.

4. Conclusion

In a previous SAR study we already demonstrated that esterification of aspirin® with a [propargyl]$Co_2(CO)_6$ moiety increased the cytotoxicity and COX inhibitory properties. Co-ASS was identified as lead structure, which was more active than cisplatin against MCF-7 and MDA-MB-231 cells. Not only the [alkyne]$Co_2(CO)_6$ moiety but also a Cp-metal part increased the cytotoxicity of aspirin®. However, compared to [alkyne]$Co_2(CO)_6$ in most cases lower cytotoxicity and COX inhibition were observed. A clear conclusion whether the COX enzyme inhibition participates in the mode of action cannot be deduced from the results. Nevertheless, a series of very interesting cytotoxic metal complexes were identified with preference for MCF-7 breast cancer and HT-29 colon carcinoma cells. Their mode of action will be investigated in continuation of this study.

5. Experimental

5.1. Chemistry

Commercially available chemicals were used without further purification. C_5H_5Tl [21], $Mo(CO)_3(NCMe)_3$ [24] and $MnBr(CO)_5$ [25] were prepared by published procedures. Solvents were purified by distillation from an appropriate drying agent: Tetrahydrofuran, diethyl ether, toluene, pentane and n-hexane were dried over sodium/potassium alloy and distilled under argon atmosphere. Pyridine was dried over KOH, dichloromethane over P_2O_5 and both were stored over molecular sieves (4 Å and 3 Å). *tert*-Butanol was dried over $MgSO_4$ prior to use. Products were purified by flash chromatography on silica gel (230–400 mesh, Merck). Melting points: 510 Büchi (Flawil/Schweiz) capillary melting point apparatus. IR spectra (KBr pellets): Perkin–Elmer Model 580 A (Rodgau-Jügesheim/Germany). ^1H and ^{13}C NMR: Avance DPX-400 spectrometer (Bruker, Karlsruhe/Germany) at 400 MHz (^1H) or 100 MHz (^{13}C); (internal standard: TMS). Elemental analyses: Microlaboratory of the Freie Universität Berlin on Perkin–Elmer 240C. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values. MS and HR-MS spectra: Finnigan MAT 711 (EI, 70 eV), MAT CH7A (EI, 80 eV, 3 kV), CH5DF (FAB, 80 eV, 3 kV) and Agilent ESI-TOF 6210 (4 μl/min, 1 bar, 4000 V). Microplate reader: FLASHscan S12 (AnalytikJena AG/Germany).

5.1.1. Preparation of cyclopentadienyl alcohol derivatives

5.1.1.1. 2-(Cyclopenta-1,3-dienyl)ethanol (Cp-Et-OH). In 100 ml of dry *tert*-butanol, 2.0 g of elemental potassium were dissolved under hydrogen production in a wash-flask equipped with magnetic stirrer and a ceramic frit in a glass tube. Temperature was controlled by a water bath adjusted at 25–28 °C to prevent crystallisation of the alcohol. After dissolution was completed, argon was bubbled through the apparatus for 30 min to create an inert atmosphere. Afterwards 0.5 mol of freshly cracked cyclopentadiene (41.2 ml) was added. The colour of the solution turned to red. About 20 g of ethylene oxide were bubbled through the solution over a period of 4 h while the colour changed to dark gray. The main fraction of *tert*-butanol was then evaporated on a rotavap. The residue was given in a separating funnel and diluted with water and neutralized with 5% H_2SO_4 and extracted twice with 100 ml of diethyl ether. The extracts were washed once with water and dried over Na_2SO_4. Solvents were removed under reduced pressure, leaving yellow oil which was purified by vacuum distillation. The desired clear oil was obtained at 35 °C at 5×10^{-3} T (3.8 g, 6.9%). MS (EI, 70 eV, 35 °C): m/z (%) = 110 (12) [M^+], 80 (48) [M–(CH_2OH)], 66 (100) [Cp]. ^1H NMR ($CDCl_3$): δ = 2.74 (m, 2H, –C**H**$_2$-Cp), 2.90 (m, 2H, =CH–C**H**$_2$–CH=), 3.60 (m, 2H, HO–C**H**$_2$–), 6.06–6.45 (m, 3H, Cp–R (alkenyl)).

5.1.1.2. 3-(Cyclopenta-1,3-dienyl)propanol (Cp-Prop-OH). To a mixture of 0.25 mol of NaH (10 g of 60% dispersion in oil) in 100 ml of THF in a large Schlenk tube one equivalent of freshly distilled cyclopentadiene (20.7 ml) was added under argon atmosphere. The mixture was stirred and cooled during the addition with an ice bath. One equivalent of 3-chloropropanol (20.9 ml) was added to the sodocen solution and stirred for 6 h at room temperature. The main fraction of THF was distilled off at 30 °C and the resulting solution was diluted with 100 ml of ether, followed by slow addition of 150 ml of water. The aqueous layer was separated and was extracted twice with 50 ml of ether. Organic layers were combined and washed twice with 50 ml of water and dried over Na_2SO_4. The solvent was removed under reduced pressure followed by a vacuum distillation. The clear oil was collected at 65 °C at 10^{-2} Torr. Yield: 4.7 g (15%). MS (EI, 70 eV, 35 °C): m/z (%) = 124 (44) [M^+], 80 (59) [Cp-CH_2], 66 (43) [Cp], 58 (100) [C_3H_6–O]. ^1H NMR ($CDCl_3$): δ = 1.91 (m, 2H, –C**H**$_2$–CH_2-Cp), 2.42 (m, 2H, –C**H**$_2$-Cp), 2.92 (m, 2H, =CH–C**H**$_2$–CH=), 3.62 (m, 2H, HO–C**H**$_2$–), 6.06–6.45 (m, 3H, Cp-R (alkenyl)).

5.1.1.3. 4-(Cyclopenta-1,3-dienyl)butanol (Cp-But-OH). Analogously to the procedure described above (Section 5.1.1.2). 4-Chlorobutanol: 27.2 g (25 ml); cyclopentadiene: 21 ml. A clear oil was obtained at 105 °C and 10^{-2} Torr. Yield: 3.1 g (9%). MS (EI, 35 °C): m/z (%) = 138 (42) [M^+], 120 (9) [M–(OH)], 105 (22) [M–(CH_2OH)], 91 (76) [Cp-C_2H_4], 79 (100) [Cp-CH_2], 66 (56) [Cp]. ^1H NMR ($CDCl_3$): δ = 1.67 (m, 2H, –C**H**$_2$–CH_2-Cp), 1.78 (m, 2H, –C**H**$_2$–CH_2–O–), 2.45 (m, 2H,

–C**H$_2$**-Cp), 2.94 (m, 2H, =CH–C**H$_2$**–CH=), 3.57 (t, ^{3}J = 6.5 Hz, 2H, HO–C**H$_2$**-), 6.19–6.45 (m, 3H, Cp-R (alkenyl)).

5.1.2. General preparation of (cyclopentadienyl)alkyl-2-acetoxybenzoate

In an ice-cooled 100 ml round-bottom flask 0.025 mol of cyclopentadienyl alcohol was mixed under stirring with 5 ml of abs. pyridine. An amount of 0.03 mol of 2-acetylsalicoyl chloride (ASS-Cl) [18] was solved in 40 ml of diethyl ether and added dropwise to the reaction mixture over a period of 1 h. Then, the flask was allowed to warm up to room temperature. In a separating funnel the organic layer was washed with 1 N HCl and with saturated sodium bicarbonate solution. The organic layer was dried over Na_2SO_4 and the solvent was removed in vacuo. The crude product was purified on a flash column with eluent petrol ether/diethyl ether 5:1.

5.1.2.1. 2-(Cyclopenta-1,3-dienyl)ethyl-2-acetoxybenzoate (Et-Cp-ASS). Cp-Et-OH: 2.8 g (0.026 mol), ASS-Cl: 6.0 g (0.03 mol). Yield: 6.3 g (89%) as colorless oil. MS (EI, 70 eV, 35 °C): *m/z* (%) = 272 (2) [M$^+$], 180 (10) [M-(Cp-C_2H_4)], 163 (13) [M-(Cp)-(Ac)], 121 (36) [(C_6H_4)–COO], 93 (100) [Cp-C_2H_4], 66 (54) [Cp], 43 (87) [Ac]. ^{1}H NMR ($CDCl_3$): δ = 2.26 (s, 3H, O=C–C**H$_3$**), 2.74 (m, 2H, –C**H$_2$**-Cp), 2.91 (m, 2H, =CH–C**H$_2$**–CH=), 4.36 (t, ^{3}J = 7.0 Hz, 2H, –O–C**H$_2$**–), 6.06–6.45 (m, 3H, Cp-R (alkenyl)), 7.04 (d, ^{3}J = 8.1 Hz, 1H, 3′-H), 7.23 (ddd, ^{3}J = 7.6 Hz, ^{3}J = 7.6 Hz, ^{4}J = 0.9 Hz, 1H, 5′-H), 7.48 (ddd, ^{3}J = 8.0 Hz, ^{3}J = 7.5 Hz, ^{4}J = 1.6 Hz, 1H, 4′-H), 7.92 (dd, ^{3}J = 7.9 Hz, ^{3}J = 7.9 Hz, 1H, 6′-H).

5.1.2.2. 3-(Cyclopenta-1,3-dienyl)propyl-2-acetoxybenzoate (Prop-Cp-ASS). Cp-Prop-OH: 3.1 g (0.025 mol), ASS-Cl: 5.2 g (0.026 mol). Yield: 5.6 g (78%) as colorless oil. MS (EI, 100 °C): *m/z* (%) = 286 (3) [M$^+$], 163 (18) [M-(Ac)-(Cp-CH_2)], 121 (100) [(C_6H_4)–COO], 105 (96) [(C_6H_4)–CO], 43 (26) [Ac]. ^{1}H NMR ($CDCl_3$): δ = 2.01 (m, 2H, –C**H$_2$**–CH_2-Cp), 2.36 (s, 3H, O=C–C**H$_3$**), 2.54 (m, 2H, –C**H$_2$**-Cp), 2.95 (m, 2H, =CH–C**H$_2$**–CH=), 4.31 (t, ^{3}J = 6.6 Hz, 2H, –O–C**H$_2$**–), 6.06–6.45 (m, 3H, Cp-R (alkenyl)), 7.11 (dd, ^{3}J = 8.0 Hz, ^{4}J = 1.0 Hz, 1H, 3′-H), 7.31 (ddd, ^{3}J = 7.9 Hz, ^{3}J = 7.5 Hz, ^{4}J = 1.0 Hz, 1H, 5′-H), 7.56 (ddd, ^{3}J = 7.9 Hz, ^{3}J = 7.6 Hz, ^{4}J = 1.7 Hz, 1H, 4′-H), 8.01 (dd, ^{3}J = 7.8 Hz, ^{4}J = 1.8 Hz, 1H, 6′-H).

5.1.2.3. 4-(Cyclopenta-1,3-dienyl)butyl-2-acetoxybenzoate (But-Cp-ASS). Cp-But-OH: 2.8 g (0.02 mol), ASS-Cl: 4.4 g (0.022 mol). Yield: 4.9 g (82%) as colorless oil. MS (EI, 100 °C): *m/z* (%) = 300 (4) [M$^+$], 257 (1) [M-(Ac)], 163 (15) [M-(Ac)-(Cp-CH_2)], 120 (100) [(C_6H_4)–COO], 105 (9) [(C_6H_4)–CO], 43 (26) [Ac]. ^{1}H NMR ($CDCl_3$): δ = 1.70 (m, 2H, –C**H$_2$**–CH_2-Cp), 1.80 (m, 2H, –C**H$_2$**–CH_2–O–), 2.36 (s, 3H, O=C–C**H$_3$**), 2.49 (m, 2H, –C**H$_2$**-Cp), 2.95 (m, 2H, =CH–C**H$_2$**–CH=), 4.31 (t, ^{3}J = 6.6 Hz, 2H, –O–C**H$_2$**–), 6.19–6.45 (m, 3H, Cp-R (alkenyl)), 7.11 (dd, ^{3}J = 8.1 Hz, 1H, 3′-H), 7.33 (ddd, ^{3}J = 7.6 Hz, 1H, 5′-H), 7.58 (ddd, ^{3}J = 7.4 Hz, ^{4}J = 1.6 Hz, 1H, 4′-H), 8.03 (dd, ^{3}J = 7.8 Hz, ^{4}J = 1.5 Hz, 1H, 6′-H).

5.1.3. General procedure for the preparation of η^5-[(cyclopentadienyl)alkyl-2-acetoxybenzoate]thallium

Thallium ethoxide (10–17 mmol) was added dropwise at −30 °C under argon atmosphere to a pre-cooled solution of Cp-Alkyl-OH (10–17 mmol) in n-hexane (200 ml). After stirring for 1 h at −30 °C, the reaction mixture was allowed to warm to room temperature and stirred for further 16 h. A greyish yellow coloured precipitate was separated by filtration and washed with diethyl ether to yield the title compounds.

5.1.3.1. η^5-[2-((Cyclopentadienyl)ethyl)-2-acetoxybenzoate]thallium (Et-Cp-ASS-Tl). Et-Cp-ASS: 1.40 g (5.1 mmol), thallium ethoxide: 0.36 ml. Yield: 2.00 g (82%). MS (EI, 150 °C): *m/z* (%) = 434 (6) [M-(CH_2–C=O)], 314 (18) [O-C_2H_4-Cp-Tl], 283 (2) [H_3C-Cp-Tl], 269 (2) [Cp-Tl], 205 (100) [Tl], 121 (4) [(C_6H_4)–COO], 106 (1) [(C_6H_4)–CO]. HR-MS (EI, 70 eV, 140 °C): [M- CH_3-CO-] calcd. 434.06088; found 434.06037. Anal. $C_{16}H_{15}O_4Tl$ (C, H).

5.1.3.2. η^5-[3-((Cyclopentadienyl)propyl)-2-acetoxybenzoate]thallium (Prop-Cp-ASS-Tl). Prop-Cp-ASS: 4.72 g (16.5 mmol), thallium ethoxide: 1.16 ml. Yield: 7.02 g (87%). MS (EI, 200 °C): *m/z* (%) = 490 (6) [M$^+$], 446 (2) [M-(CH_2–C=O)], 355 (8) [O=C–O–C_3H_6-Cp-Tl], 326 (1) [O–C_3H_6-Cp-Tl], 269 (3) [Cp-Tl], 205 (22) [Tl], 121 (100) [(C_6H_4)–COO], 106 (92) [(C_6H_4)–CO]. MS (EI2, HR-MS, 140 °C): [M–CH_3–CO–] calcd. 446.07446; found 446.07413. Anal. $C_{17}H_{17}O_4Tl$ (C, H).

5.1.3.3. η^5-[4-((Cyclopentadienyl)butyl)-2-acetoxybenzoate]thallium (But-Cp-ASS-Tl). But-Cp-ASS: 2.70 g (16.5 mmol), thallium ethoxide: 0.62 ml. Yield 4.17 g (92%). MS (EI, 200 °C): *m/z* (%) = 488 (3) [M–CH_3], 462 (10) [M–(CH_2–C=O)], 342 (3) [O–C_4H_8-Cp-Tl], 325 (2) [C_4H_8-Cp-Tl], 297 (2) [C_2H_4-Cp-Tl], 205 (95) [Tl], 121 (89) [(C_6H_4)–COO], 106 (20) [(C_6H_4)–CO], 92 (100) [(C_6H_4)–O]. MS (EI2, 200 °C): *m/z* (%) = 504 (1) [M$^+$], 462 (4) [M–(CH_2–C=O)], 342 (6) [O–C_4H_8-Cp-Tl], 258 (2) [C_4H_4-Tl], 205 (65) [Tl], 120 (100) [(C_6H_4)–COO], 105 (36) [(C_6H_4)–CO], 92 (74) [(C_6H_4)–O], 44 (76) [CH_2–C=O]. MS (EI2, HR-MS, 200 °C): [M–CH_3–CO–] calcd. 462.09219; found 462.09184. Anal. $C_{18}H_{19}O_4Tl$ (C, H).

5.1.4. Preparation of η^5-[3-((cyclopentadienyl)propyl)-2-acetoxybenzoate]tricarbonylmolybdenum (Prop-Cp-ASS-Mo) resp. η^5-[3-((cyclopentadienyl)propyl)-2-acetoxybenzoate] iodotricarbonylmolybdenum (Prop-Cp-ASS-Mo-I)

A solution of Prop-Cp-ASS (0.58 g, 2.0 mmol) in toluene (40 ml) was added to a solution of $Mo(CO)_3(NCMe)_3$ (0.54 g, 2.0 mmol) in toluene (40 ml) at 25 °C. The reaction mixture was stirred for 2 h and the resulting orange solution was filtered through celite. The filtrate was concentrated to dryness. Half of the residue was put on a silica gel column and was eluted with petrol ether/diethyl ether 5:1. Yield: 0.31 g (67%). The second half was dissolved in dichloromethane (50 ml). Solid CHI_3 (0.39 g, 1.0 mmol) was added to the dichloromethane solution and the colour immediately turned to deep red. The reaction mixture was stirred for a further 30 min to ensure completion of the reaction and the solvent was removed under vacuum. After chromatography with the same solvent mentioned above, Prop-Cp-ASS-Mo-I was isolated as a dark red solid. Yield: 0.42 g (71%). Prop-Cp-ASS-Mo: MS (EI2, 75 °C): *m/z* (%) = 467 (6) [M$^+$], 437 (8) [M–CO], 411 (1) [M–2(CO)], 381 (6) [M–3(CO)], 341 (16) [M–3(CO)–(CH_2–C=O)], 121 (41) [(C_6H_4)–COO], 106 (100) [(C_6H_4)–CO]. Prop-Cp-ASS-Mo-I: MS (EI2, 90 °C): *m/z* (%) = 594 (15) [M$^+$], 566 (47) [M–CO], 510 (82) [M–3(CO)], 467 (60) [M–(I)], 440 (15) [M–(CO)–(I)], 394 (100) [M–(CH_2=C=O)–(CO)–(I)], 340 (23) [M–(CH_2=C=O)-3(CO)–(I)], 120 (11) [(C_6H_4)–COO], 106 (5) [(C_6H_4)–CO]. MS (EI2, HR-MS, 90 °C): [M$^+$] calcd. 587.90869; found 587.90871. Anal. $C_{20}H_{17}O_7MoI$ (C, H).

5.1.5. Preparation of η^5-[3-((cyclopentadienyl)propyl)-2-acetoxybenzoate]tricarbonylmanganese (Prop-Cp-ASS-Mn)

Solid $MnBr(CO)_5$ (0.19 g, 0.7 mmol) was added to a stirred solution of Prop-Cp-ASS-Tl (0.34 g, 0.7 mmol) in THF (40 ml) at room temperature. The reaction mixture was stirred overnight and the solvent was removed under vacuum. The residue was extracted on a silica column with petrol ether/diethyl ether 5:1 to yield the title compound as a waxy dark yellow solid. Yield: (81%). MS (EI, 150 °C): *m/z* (%) = 424 (6) [M$^+$], 340 (11) [M–3(CO)], 298 (2) [M–3(CO)–(CH_2–C=O)], 222 (23) [M–(Cp-Mn-3CO)], 163 (2) [H_3C–CO_2–(C_6H_4)–CO], 121 (42) [(C_6H_4)–COO], 106 (34) [(C_6H_4)–CO], 28 (100) [CO]. MS (EI2, HR-MS, 80 °C): [M$^+$] calcd. 424.03546; found 424.03533. Anal. $C_{20}H_{17}O_7Mn$ (C, H).

5.1.6. Preparation of η^5-[3-((cyclopentadienyl)propyl)-2-acetoxybenzoate]dicarbonylcobalt (Prop-Cp-ASS-Co)

Into an argon-purged 500 ml Schlenk tube equipped with a magnetic stirrer was added 340 mg (1.0 mmol) of dicobaltoctacarbonyl and 300 ml of THF. To this mixture was slowly added 250 mg (1.0 mmol) of iodine crystals, and the solution was stirred at 25 °C for 3 h. To the resulting green solution, 980 mg (2.0 mmol) of Prop-Cp-ASS-Tl was added, and stirring was maintained for additional 24 h. The reaction mixture was filtered through celite, and the solvent was removed in vacuo. The residue was mixed with silica in diethyl ether, the solvent was removed, and the coated product was placed on a 2 × 35 cm dry-packed column of silica gel. Elution of the column with petrol ether/diethyl ether 5:1 ceded the desired deep yellow product with a yield of 510 mg (64%). MS (EI, 150 °C): *m/z* (%) = 400 (4) [M^+], 372 (6) [M-CO], 344 (27) [M-2(CO)], 302 (28) [M-2(CO)–(CH_2–C=O)], 121 (8) [(C_6H_4)–COO], 106 (25) [(C_6H_4)–CO], 42 (100) [CH_3–CO]. 28 (66) [CO]. MS (EI2, HR-MS, 90 °C): [M-CO] calcd. = 372.04080; found = 372.04042. ^{1}H NMR ($CDCl_3$): δ = 1.93 (m, 2H, -C**H$_2$**–CH_2-Cp), 2.28 (s, 3H, O=C–**C**H_3), 2.61 (t, ^{3}J = 7.9 Hz, 2H, -C**H$_2$**-Cp), 4.26 (t, ^{3}J = 6.2 Hz, 2H, –O–C**H$_2$**–), 5.32 (t, ^{3}J = 2.1 Hz, 2H, Co(C_5H_4), 3′4′-H), 5.46 (t, ^{3}J = 2.1 Hz, 2H, Co(C_5H_4), 2′5′-H), 7.05 (d, ^{3}J = 7.6 Hz, 1H, 3′-H), 7.26 (dd, ^{3}J = 7.9 Hz, ^{3}J = 7.6 Hz, ^{4}J = 1.0 Hz, 1H, 5′-H), 7.53 (ddd, ^{3}J = 7.9 Hz, ^{3}J = 7.6 Hz, ^{4}J = 1.5 Hz, 1H, 4′-H), 7.94 (dd, ^{3}J = 7.8 Hz, ^{4}J = 1.4 Hz, 1H, 6′-H). Anal. $C_{19}H_{17}O_6Co$ (C, H).

5.1.7. Preparation of η^5-[3-((cyclopentadienyl)propyl)-2-acetoxybenz]dicarbonylrhodium (Prop-Cp-ASS-Rh)

Into an argon-purged 250 ml Schlenk tube was added 75 ml of THF and 0.20 g (0.5 mmol) of dichlorotetracarbonyldirhodium [26]. After cooling the reaction mixture to −70 °C, 0.49 g (1.0 mmol) of Prop-ASS-Tl was added. The reaction mixture was allowed to warm to room temperature and was stirred for additional 21 h. At the end of this period, the mixture was filtered through celite and the filtrate treated with 5 g of silica. The THF was removed in vacuo and the coated product placed on a dry-packed silica column. Elution of the column was performed with petrol ether/diethyl ether 5:1. Evaporation of the solvent in vacuo gave 250 mg (56%) of yellow oil. MS (EI, 150 °C): *m/z* (%) = 416 (3) [M–CO], 388 (15) [M–2(CO)], 374 (27) [M–CO–(CH_2–C=O)], 346 (75) [M–2(CO)–(CH_2–C=O)], 120 (100) [(C_6H_4)–COO], 106 (90) [(C_6H_4)–CO], 42 (23) [CH_3–CO], 28 (74) [CO]. MS (EI, HR-MS, 150 °C): [M–CO] calcd. = 416.01310; found = 416.01292. MS (EI, HR-MS, 150 °C): [M–2CO] calcd. = 388.01819; found = 388.01803. ^{1}H NMR ($CDCl_3$): δ = 2.00 (m, 2H, –C**H$_2$**–CH_2-Cp), 2.36 (s, 3H, O=C–**C**H_3), 2.68 (t, ^{3}J = 7.6 Hz, 2H, –C**H$_2$**-Cp), 4.33 (t, ^{3}J = 6.3 Hz, 2H, –O–C**H$_2$**–), 5.39 (t, ^{3}J = 1.9 Hz, 2H, Rh(C_5H_4), 3′4′-H), 5.53 (t, ^{3}J = 1.9 Hz, 2H, Rh(C_5H_4), 2′5′-H), 7.12 (d, ^{3}J = 8.0 Hz, 1H, 3′-H), 7.33 (dd, ^{3}J = 7.6 Hz, ^{3}J = 7.6 Hz, ^{4}J = 1.0 Hz, 1H, 5′-H), 7.56 (ddd, ^{3}J = 8.0 Hz, ^{3}J = 7.6 Hz, ^{4}J = 1.3 Hz, 1H, 4′-H), 8.01 (dd, ^{3}J = 7.8 Hz, ^{4}J = 1.2 Hz, 1H, 6′-H). Anal. $C_{19}H_{17}O_6Rh$ (C, H).

5.2. Biological methods

5.2.1. Cell culture

The human MCF-7 and MDA-MB-231 breast cancer cell lines as well as the HT-29 colon cancer 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 [27]. All three cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g/l glucose and L-glutamine (PAA, Germany), supplemented with 5% fetal bovine serum (FBS; Biochrom, Germany) at 37 °C in a humidified atmosphere with 5% CO_2.

5.2.2. Cytotoxicity assay

In 96 well plates 100 μl of a cell suspension in culture medium at 7500 cells/ml (MCF-7 and MDA-MB-231) or 2500 cells/ml (HT-29) were plated into each well and incubated for three days under culture conditions. After the addition of various concentrations of the test compounds, cells were incubated for another 96 h. Then the medium was removed, the cells were fixed with glutardialdehyde solution 1% and stored under phosphate buffered saline (PBS) at 4 °C. Cell biomass was determined by a crystal violet staining, followed by extracting of the bound dye with ethanol and a photometric measurement at 590 nm. Mean values were calculated and the effects of the compounds were expressed as % Treated/Control$_{corr}$ values according to the following equations:

$$T/C_{corr}[\%] = \frac{T - C_0}{C - C_0} \cdot 100$$

(C_0 is the biomass of control cells at the time of compound addition; C is the biomass of control cells at the time of the test end; T is the biomass of probes/samples at the time of the test end).

The IC_{50} value was determined as the concentration causing 50% inhibition of cell proliferation and calculated as mean of at least two independent experiments.

5.2.3. Inhibition of COX-enzymes

The inhibition of isolated ovine COX-1 and human recombinant COX-2 at 10 μM of the respective compounds was determined by ELISA ("COX Inhibitor Screening Assay", Cayman Chemical). The experiments were performed according to the manufacturer's instructions. Absorption was measured at 415 nm (Flashscan S12, AnalytikJena AG).

5.2.4. PGE_2-assay

MDA-MB-231 cells were grown in 24 well plates until at least 70% confluency. The medium was then removed and replaced by fresh DMEM containing the respective substances (10 μM); control cells were maintained in the absence of any compound. The cells were incubated for 23 h followed by the addition of 100 μM arachidonic acid. 24 h after compound addition, the drug-containing medium with the including prostaglandin E_2 was removed and tested by ELISA ("Prostaglandin E_2 EIA Kit-Monoclonal", Cayman Chemicals). Experiments were performed according to the manufacturer's instructions. Absorption was measured at 415 nm (Flashscan S12, AnalytikJena AG).

6. Elemental analyses

6.1. η^5-[2-((Cyclopentadienyl)ethyl)-2-acetoxybenzoate]thallium (Et-Cp-ASS-Tl)

Anal. calcd. for $C_{16}H_{15}O_4Tl$: C, 40.40; H, 3.20; found: C, 40.29; H, 3.51%.

6.2. η^5-[3-((Cyclopentadienyl)propyl)-2-acetoxybenzoate]thallium (Prop-Cp-ASS-Tl)

Anal. calcd. for $C_{17}H_{17}O_4Tl$: C, 41.70; H, 3.50; found: C, 41.49; H, 3.61%.

6.3. η^5-[4-((Cyclopentadienyl)butyl)-2-acetoxybenzoate]thallium (But-Cp-ASS-Tl)

Anal. calcd. for $C_{18}H_{19}O_4Tl$: C, 42.92; H, 3.80; found: C, 42.67; H, 3.81%.

6.4. η^5-[3-((Cyclopentadienyl)propyl)-2-acetoxybenzoate] tricarbonylmolybdenum (Prop-Cp-ASS-Mo)

Anal. calcd. for $C_{20}H_{17}O_7Mo$: C, 51.63; H, 3.68; found: C, 51.67; H, 3.95%.

6.5. η^5-[3-((Cyclopentadienyl)propyl)-2-acetoxybenzoate] iodotricarbonylmolybdenum (Prop-Cp-ASS-Mo-I)

Anal. calcd. for $C_{20}H_{17}O_7MoI$: C, 40.56; H, 2.89; found: C, 40.51; H, 3.22%.

6.6. η^5-[3-((Cyclopentadienyl)propyl)-2-acetoxybenzoate] tricarbonylmanganese (Prop-Cp-ASS-Mn)

Anal. calcd. for $C_{20}H_{17}O_7Mn$: C, 56.62; H, 4.04; found: C, 56.46; H, 4.17%.

6.7. η^5-[3-((Cyclopentadienyl)propyl)-2-acetoxybenzoate] dicarbonylcobalt (Prop-Cp-ASS-Co)

Anal. calcd. for $C_{19}H_{17}O_6Co$: C, 57.01; H, 4.28; found: C, 57.12; H, 4.42%.

6.8. η^5-[3-((Cyclopentadienyl)propyl)-2-acetoxybenzoate] dicarbonylrhodium (Prop-Cp-ASS-Rh)

Anal. calcd. for $C_{19}H_{17}O_6Rh$: C, 51.37; H, 3.86; found: C, 51.44; H, 4.01%.

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