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R. Kaczmarek, R. Dembinski et al.
Organometallic Nucleosides: Synthesis and Biological Evaluation of Substituted Dicobalt Hexacarbonyl 2'-Deoxy-5-oxopropynyluridines
Organometallic Nucleosides: Synthesis and Biological Evaluation of Substituted Dicobalt Hexacarbonyl 2′-Deoxy-5-oxopropynyluridines


1. Introduction

Nucleoside analogues are molecules of high pharmacological interest for the treatment of various conditions, especially cancer and viral diseases.[1–4] These agents behave as antimetabolites and compete with physiologic nucleosides, and consequently, they interact with a large number of intracellular targets to induce cytotoxicity. Substitution at the C-5 position of the uracil base provides a common framework for potent biological properties.[5]

In parallel, biorganometallic chemistry provides new tools to influence biological interactions.[6–13] Among a variety of organometallic compounds, transition-metal carbonyls forged their presence in medicinal chemistry.[14] For example, a rhenium carbonyl complex, a protein kinase inhibitor, activates p53 and induces apoptosis in human melanoma cells.[15] Related complexes were designed distinctly: as tamoxifen-based anticancer drug derivatives,[16,17] as inhibitors of human carbonic anhydrase (hCA),[18] as antibacterial agents,[19] and as triazoles displaying antitrypanosomal activity.[20] Representative nucleoside-related examples include rhenium tricarbonyl complexes such as 1 (Figure 1)[21] and manganese and chromium tricarbonyl with 2′-deoxy-5-alkynyluridines led to a significant increase in the cytotoxic potency. The cobalt compounds displayed antiproliferative activities with median inhibitory values (IC50) in the range of 20 to 80 μM for the HeLa cell line and 18 to 30 μM for the K562 cell line. Coordination of an acetyl-substituted cobalt nucleoside was expanded by using the 1,1-bis(diphenylphosphino)methane (dppm) ligand, which exhibited cytotoxicity at comparable levels. The formation of reactive oxygen species in the presence of cobalt compounds was determined in K562 cells. The results indicate that the mechanism of action for most antiproliferative cobalt compounds may be related to the induction of oxidative stress.

Reactions of dicobalt octacarbonyl [Co₂(CO)₈] with 2′-deoxy-5-oxopropynyluridines and related compounds gave dicobalt hexacarbonyl nucleoside complexes (83–31 %). The synthetic outcomes were confirmed by X-ray structure determination of dicobalt hexacarbonyl 2′-deoxy-5-(4-hydroxybut-1-yn-1-yl)uridine, which exhibits intermolecular hydrogen bonding between a modified base and ribose. The electronic structure of this compound was characterized by the DFT calculations. The growth inhibition of HeLa and K562 cancer cell lines by organometallic nucleosides was examined and compared to that by alkynyl nucleoside precursors. Coordination of the dicobalt carbonyl moiety to the 2′-deoxy-5-alkynyluridines led to a significant increase in the cytotoxic potency. The cobalt compounds displayed antiproliferative activities with median inhibitory values (IC50) in the range of 20 to 80 μM for the HeLa cell line and 18 to 30 μM for the K562 cell line. Coordination of an acetyl-substituted cobalt nucleoside was expanded by using the 1,1-bis(diphenylphosphino)methane (dpdm) ligand, which exhibited cytotoxicity at comparable levels. The formation of reactive oxygen species in the presence of cobalt compounds was determined in K562 cells. The results indicate that the mechanism of action for most antiproliferative cobalt compounds may be related to the induction of oxidative stress.

Figure 1. Structures of representative metal–carbonyl complexes with medicinal potential.

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boryl arylalkynyl nucleosides 2 (Figure 1).\textsuperscript{[22]} Nucleoside–iron carbonyl complex 3 (Figure 1) was reported to bestow significant apoptosis-inducing activity against BJAB tumor cells\textsuperscript{[23]} and specific cytotoxicity to reactive oxygen species (ROS)-stressed cancer cells.\textsuperscript{[24]}

Covalnt possesses a diverse array of properties that can be manipulated to yield promising drug candidates.\textsuperscript{[25]} The medicinal potential of cobalt carbonyl complexes has been reported.\textsuperscript{[26]} Peptide labeling by using a dicobalt hexacarbonyl alkynyl complex\textsuperscript{[27]} and cobalt carbonyl complexes encapsulated in a micelle structure (not illustrated), aiming to deliver cobalt pharmaceuticals,\textsuperscript{[28]} has been investigated. The activity of a cobalt derivative of 17-ethynyltestosterone (4; Figure 1) has also been explored.\textsuperscript{[29]}

Recently, two cobalt-based hybrid molecules that combine an Nrf2 (a basic leucine zipper protein) inducer with a releaser of carbon monoxide (an anti-inflammatory product of heme oxygenase-1) were reported to increase Nrf2/H-O1 expression markedly and to exert anti-inflammatory activity in vitro. Compounds 5 and 6 (Figure 1) also up-regulate tissue heme oxygenase-1 and deliver CO to the blood after administration in vivo, which supports their potential anti-inflammatory properties.\textsuperscript{[30]}

The antiproliferative properties of dicobalt hexacarbonyl complexes have been noted,\textsuperscript{[31–33]} and aspin–cobalt carbonyl derivative 7 containing a propargyl alcohol unit (Figure 1) was identified as a lead compound during in vitro studies against MCF-7 and MDA-MB-231 human mammary breast cancer cells.\textsuperscript{[34]} Additional studies have suggested a mode of action in which cytochrome-c oxidase inhibition plays a major role.\textsuperscript{[35]} The aspin–cobalt carbonyl derivative also exhibited antiangiogenic effects in a zebrafish embryo assay, as well as significant inhibition of matrix metalloproteinase-7 (MMP-7).\textsuperscript{[36]} Among its many other demonstrated biological effects was a strong induction of caspase-3, which is an important activator in apoptosis. A related aromatic compound with a nitro substituent, that is, 8 (Figure 1), was shown to strongly induce apoptosis, arrest the cell cycle at the S phase, increase cellular oxidative stress levels, and induce permeability of the mitochondrial membrane.\textsuperscript{[37]} Whereas its non-cobalt-containing precursor also caused an increase in mitochondrial membrane permeability, it did not produce an increase in oxidative stress levels, nor did it have apoptosis-inducing or antiproliferative effects.

A review of structures 4–8 (Figure 1) revealed a common cobalt carbonyl propargyloxy ligand (\(-\text{C}_2\text{H}_2\text{O}_2\)), which appears in the form of its ester or free alcohol. Thus, we were intrigued as to whether the combination of a nucleoside with the presence of a propargyloxy structural motif would increase the potency of such conjugates. Consequently, the design of the investigated compounds included several propynoxy (propargyloxy) and related units, as discussed below.

Despite numerous advances, the repertoire of metallo-nucleosides is still limited. Interest in the use of the ethynyl (acetylenic) fragment for the modification of nucleoside bases has resulted in a great number of applications.\textsuperscript{[38]} Our earlier studies confirmed activity against MCF-7 and MDA-MB-231 human mammary breast cancer cells of alkyl- and aryl-substituted cobalt hexacarbonyl nucleosides,\textsuperscript{[39]} as well as their precursors, 5-alkynyl uridines.\textsuperscript{[40]} We were intrigued to investigate further 2'-deoxy-5-alkynyluridines and their hexacarbonyl dicobalt adducts in particular to pursue the synthesis and biological evaluation of a combination of oxopropynyl (oxopropargyl), cobalt carbonyl, and nucleoside structural features as new target compounds. Enhancement of action through installment of the acetyl group was also explored.

## 2. Results and Discussion

### 2.1. 2'-Deoxy-5-alkynyluridines

2'-Deoxy-5-alkynyluridines 9a–h (Scheme 1), containing a conjugated alkyne function, represent versatile materials for reactions that lead, among others, to furopyrimidines,\textsuperscript{[41]} halofuropyrimidines,\textsuperscript{[42]} and alkynyl dimers.\textsuperscript{[43]}

![Scheme 1. Synthesis of 2'-deoxy-5-alkynyluridines 9a–h from 2'-deoxy-5-iodouridine (I-DU).](image)

Sonogashira coupling of alkynes offers an atom-efficient pathway toward modification of nucleosides.\textsuperscript{[39, 43]} Preparative synthesis of a series of 2'-deoxy-5-alkynyluridines 9 was performed from 2'-deoxy-5-iodouridine (I-DU) and the appropriate terminal alkyne in the presence of catalytic amounts of Pd(PPh\textsubscript{3})\textsubscript{4} copper(I) iodide, and triethylamine in DMF in a tightly controlled temperature regime (40 °C, oil bath, 22 h; Scheme 1) to avoid subsequent cyclization to furopyrimidines; this protocol did not require protection of the hydroxy groups.\textsuperscript{[39]} To explore the structure-reactivity relationship of the alkyne substituents, nucleosides containing a oxopropynyl (oxypropargyl) unit (propargyl alcohol, 9a, \(R = \text{CH}_2\text{O}H\)), alkyl disubstituted \(9b/9c, R = \text{C}(\text{OH})(\text{Me})_2/\text{C}(\text{OH})(\text{Me})\text{Et}\), aryl monosubstituted \(9d/9e, R = \text{CH}(\text{OH})\text{Ph}/\text{C}(\text{OH})\text{Ph}_2\), as well as the one-carbon-extended homologue (9f, \(R = \text{CH}_2\text{CH}_2\text{O}H\)), methyl ether (9g, \(R = \text{CH}_2\text{O}Me\)), and acetate (9h, \(R = \text{CH}_2\text{O}Ac\)) groups, were obtained in 92–94% yield by using literature procedures (Table 1). Numerous 2'-deoxy-5-alkynyluridines have

### Table 1. Preparation of 2'-deoxy-5-alkynyluridines 9a–h and conversion into hexacarbonyl dicobalt derivatives 10a–h.

<table>
<thead>
<tr>
<th>R</th>
<th>Alkylnucleoside 9</th>
<th>Yield [%]</th>
<th>Cobalt complex 10</th>
<th>Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{CH}_2\text{OH})</td>
<td>9a</td>
<td>50</td>
<td>10a</td>
<td>58</td>
</tr>
<tr>
<td>(\text{C}(\text{OH})\text{Me}_2)</td>
<td>9b</td>
<td>41</td>
<td>10b</td>
<td>31</td>
</tr>
<tr>
<td>(\text{C}(\text{OH})\text{Me}\text{Et})</td>
<td>9c</td>
<td>56</td>
<td>10c</td>
<td>64</td>
</tr>
<tr>
<td>(\text{CH}(\text{OH})\text{Ph})</td>
<td>9d</td>
<td>46</td>
<td>10d</td>
<td>71</td>
</tr>
<tr>
<td>(\text{CH}(\text{OH})\text{Ph}_2)</td>
<td>9e</td>
<td>92</td>
<td>10e</td>
<td>62</td>
</tr>
<tr>
<td>(\text{CH}_2\text{CH}_2\text{OH})</td>
<td>9f</td>
<td>54</td>
<td>10f</td>
<td>54</td>
</tr>
<tr>
<td>(\text{CH}_2\text{O}Me)</td>
<td>9g</td>
<td>69</td>
<td>10g</td>
<td>83</td>
</tr>
<tr>
<td>(\text{CH}_2\text{O}Ac)</td>
<td>9h</td>
<td>66</td>
<td>10h</td>
<td>83</td>
</tr>
</tbody>
</table>
been synthesized because of their interesting biological activity. However compounds 9a–f and 9h, to our knowledge, have not yet been reported. The structures of alkynyl nucleosides 9 were confirmed by 1H NMR and 13C NMR spectroscopy. The high-resolution mass spectra of 9a–h exhibit m/z signals of [M+H]⁺ as molecular ions.

The conversion of alkynyl nucleosides 9 into 10, which are the corresponding dicobalt hexacarbonyl complexes of the 2'-deoxy-5-alkynyluridines, was accomplished at room temperature [Co2(CO)8, 22 °C, 1 h], and these compounds were obtained in 83–31 % yield after silica gel column chromatography (Scheme 1, Table 1).

The structures of new nucleosides 10a–h were confirmed by NMR and IR spectroscopy and HRMS. In most cases, the 1H NMR spectra exhibit one signal in the region of H6, as expected (δ = 8.35–8.11 ppm). However, two signals are observed for compounds 10c and 10d (δ = 8.30/8.27 and 8.17/8.11 ppm, respectively), presumably due to the epimers at the stereocenter located at the propargyl carbon atom. It can be assumed that most molecules, due to steric reasons, would exist in solution in the conformation that resembles the one observed in the crystal structure of 10f. This includes the planar assembly of C6–C5–C7–C8–C9, leading to potential hydrogen-bonding engagement of the propargyl and H-O5' hydroxy groups, which would lead to restricted rotation across the C8–C9 bond. Under such circumstances, H6 would be positioned gauche to the hydroxy group and one of the substituents of the stereocenter, which would lead to non-equivalent environments for each of the propargyl (C9) epimers. The H6 signals for 10c/10d are separated by 21.4/30.9 Hz, respectively, in line with the anticipated lower magnetic impact of the alkyl group versus the phenyl group. The variable-temperature 1H NMR spectra show significant line broadening at 60 °C, but incomplete coalescence (CDC13; higher temperature leads to decomposition). The reformation of well-separated signals is observed upon returning to 20 °C. In the 13C NMR spectrum, nonequivalency of the cobalt carbonyl signals (CoCO) is observed only for 10d (δ = 199.45 and 198.93 ppm). The IR spectra exhibit characteristic bands for the alkynyl [Co(CO)]1 group [ν around 2092 (m), 2052 (s), and 1606 cm⁻¹ (vs)]. Selected representative compounds as well as their alkynyl counterparts (i.e., compounds 9a/10a, 9g/10g, and 9h/10h) gave acceptable elemental analyses. The high-resolution mass spectra exhibit m/z signal of ions [M−OH]⁻ for 10a–f and 10g and [M−OAc]⁻ for 10h, which are presumably formed due to a dehydration or deacylation reaction during acquisition; the ability of the dicobalt hexacarbonyl moiety to stabilize a positive charge at an adjacent carbon atom is well known. Nucleoside 10f containing a homopropargyl group, which lacks an option to form a well-stabilized carbocation, shows the m/z [M+H]⁺ signal for the molecular ion.

Efforts to obtain diffraction-quality crystals were so far only successful in the case of 10f by diffusion of pentane into a THF solution (−20 °C) under a dry nitrogen atmosphere. X-ray crystallography confirms the structure of dicobalt hexacarbonyl 2'-deoxy-5-(4-hydroxybut-1-yn-1-yl)uridine (10f). Although nucleosides are often resistant to crystallization, intramolecular hydrogen bonding O10−H-O5' forms a 14-membered ring that rigidifies the structure and presumably facilitates formation of the X-ray-quality crystals (Figure 2). Coordination of the alkyne to the dicobalt changes the position of the CH3CH2OH group relative to the pyrimidine base through the C–C=C–C planar unit of the cobalt complex with angles of 142–143°. The R group is directed towards the ribose unit and is long enough to create a contact of O10 with the hydrogen atom of the 5'-hydroxy group O10−H−O5' 2.809(7) Å [calcd 2.756 Å].

In the crystalline form, the hydrogen atoms of the remaining hydroxy groups (H-O3' and CH3CH2OH) are each stabilized by a molecule of THF (O3'−O17 2.791 Å, calcd 2.754 Å; O18−O10 2.750 Å; O18A−O10 2.703 Å, calcd 2.693 Å). The C2 carbonyl group of 10f adopts an anti orientation towards the ribose ring: the glycosidic bond torsion angle χ (O4'−C1'−N1−C2) is 108.3(6)°. The Co–Co bond is perpendicular to the uracil plane, and the dicobalt carbonyl unit is located syn to the ribose ring.

2.1. Computational Studies

DFT calculations were conducted on 10f in the gas phase to optimize the ground-state structure. The energies were acquired by using PBE0/6-31G*. Selected calculated metric parameters for the geometry-optimized structure were compared to the experimental results (Figure 2). The largest difference between the experimental and calculated bond lengths was 0.07 Å (Co1–Co2), whereas the bond angles were in good agreement. Despite slight differences, the calculated structure is quite close to the experimental structure (even reproducing hydrogen bonds), allowing electronic properties to be confidently extracted. The calculated Mulliken charge value for Co1 and Co2 in 10f is −0.08, consistent with neutrality. Figure 3 il-
The structure of new nucleoside 11 was confirmed by $^1$H NMR, $^{13}$C NMR, $^{31}$P NMR, and IR spectroscopy and HRMS. The $^{31}$P NMR spectrum features a characteristic resonance at $\delta = 41.05$ ppm in [D$_2$]DMSO ($\delta = 40.84$ ppm in CDCl$_3$), and this clearly differs from the spectrum of Co$_2$(CO)$_6$(dppm).$^{[52]}$ The IR spectrum exhibits the characteristic $\nu_{CO}$ pattern at $\tilde{\nu} = 2022$, 1990, and 1961 cm$^{-1}$.$^{[53]}$ Similar to the high-resolution mass spectrum of 10h, an m/z signal for [M−OAc]$^-{}$ is observed.

2.3. Inhibition of Cell Proliferation

The cytotoxic properties of compounds 9a–h, 10a–h, and 11 were tested for their activity in HeLa (human cervix carcinoma), K562 (chronic myelogenous leukemia), and HUVEC (human umbilical vein endothelial cells) cells. As the control (100% viability in the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay), cells treated with DMSO (1%) were used. The viability of cells was determined at six different drug concentrations: $2 \times 10^{-5}$, $1 \times 10^{-5}$, $5 \times 10^{-5}$, $1 \times 10^{-5}$, $1 \times 10^{-4}$, and $1 \times 10^{-3}$ mM. As the control of the whole experiment, staurosporine (1 µM) was used.

All cobalt compounds displayed significant antiproliferative effects with median inhibitory concentration (IC$_{50}$) values reaching 20(±5.1) µM (for 10e in HeLa cells) and 16(±3.5) µM (for 11 in K562 cells). Thus, the potency of the more active target compounds is well within the range of established anticancer drugs such as cisplatin, 20(±6.0) and 40(±7.0) µM in HeLa and K562 cells, respectively (Table 2).

The structure–reactivity relationship seems not to be straightforward. The lead compound for cytotoxic hexacarbonyl dicobalt complexes was diphenyl-substituted 10e (IC$_{50}$ values in the range of 18 to 20 µM in this assay). HeLa cells were less sensitive towards the structure of metalnucleosides than K562 cells (20–80 vs. 18–30 µM, respectively). Cobalt nucleosides 10c, 10d, and 10f showed selectivity towards K562 cells and were less active in HeLa cells. This selectivity almost disappeared for derivative 10e, as good antiproliferative effects could be noted in both cells.

For corresponding cobalt carbonyl species 10h and its dppm homologue 11, the antiproliferative activity was slightly increased for the latter (IC$_{50}$ values from 30 to 25 µM and from 22 to 16 µM); however, toxicity against HUVEC cells also increased. Interestingly, dppm derivative 11, containing fewer carbonyl groups than 10h, was more active in both tumor cell cultures investigated (Table 2).

In regard to the alkyne precursors, preliminary results obtained for noncoordinated alkylnitrilides 9a–h were all above >100 µM; thus, no further screening was attempted. Clearly, coordination of the alkynes to Co$_2$(CO)$_6$ has a strong influence on the biological activity of the respective alkyne compounds. In general, the coordination process led to a significant increase in the cytotoxic potency for all substituents at the non-nucleoside side of the alkyne.

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2.2. Synthesis of Co$_2$(CO)$_6$(dppm) Complex

We were intrigued to investigate the effect of coordinating a phosphine ligand to the cobalt complex. Phosphine ligands have been employed in complexes screened for anticancer properties.$^{[39,40]}$ The affinity of the 1,1-bis(diphenylphosphino)alkane (dppm) ligand towards cobalt carbonyls is well known,$^{[39]}$ and to our knowledge, no biological studies have been reported so far for (alkyne)Co$_2$(CO)$_6$(dppm)-connected compounds. Dicobalt hexacarbonyl alkyne complexes are known to react with dppm; however, we elected first to coordinate dppm to the cobalt carbonyl ligand and, subsequently, to react the product with a free alkyne nucleoside. Accordingly, acyl-containing nucleoside 9h was combined with Co$_2$(CO)$_6$(dppm), which was prepared from dicobalt octacarbonyl and dppm in toluene following a known procedure.$^{[52]}$ Workup gave nucleoside 11 in 25% yield (Scheme 2). Although the presence of dppm in the molecule could enhance the crystallinity, efforts to obtain an X-ray-quality crystal of 11 have not been successful thus far.

Figure 3. Plots of molecular orbitals: HOMO−1, HOMO, LUMO, and LUMO+1 for 10f. Orbital energies [eV] are indicated.

Scheme 2. Synthesis of dppm–dicobalt tetracarbonyl nucleoside 11 from alkyne 9h.
Growing evidence suggests that cancer cells and oxidative stress in the presence of cytotoxic compounds 10a–h and 11, as well as representative free-aldehyde corresponding nucleoside 9c, were determined in K562 (chronic myelogenous leukemia) cells by using DCFDA (2,7-dichlorofluorescein diacetate) dye assay. After staining, the cells were incubated for 4 h with the test compounds at concentrations of 5, 10, 20, 50, 100, and 200 μM. Cells treated with 50 and 100 μM H₂O₂ served as positive controls. All cobalt compounds showed a significant increase in the level of reactive oxygen species, which is confirmed by the DCF (2,7-dichlorofluorescein) fluorescence intensity values shown in Table 3.

The DCF fluorescence intensity in the presence of the tested cobalt compounds (1.1 ± 0.07 to 5.2 ± 0.06-fold change in relation to control cells) was comparable to the value for the positive control (i.e. H₂O₂, 3.7 ± 0.10- to 6.1 ± 0.17-fold change in relation to control cells), which is one of the reactive forms of oxygen. The DCF fluorescence intensity increased for all of the tested cobalt compounds upon increasing the concentration of the cobalt complexes. Studies showed that compound 11 (1.4 ± 0.10- to 5.2 ± 0.06-fold change in relation to control cells) was the most potent inducer of oxidative stress in K562 cells.

Confirmation that one of the major mechanisms of action of cobalt nucleoside derivatives involved overproduction of ROS was sought. Therefore, the effect of representative compound 10a in the presence of a generalized intracellular ROS inhibitor was investigated. HeLa and K562 cancer cells were incubated in the presence of N-acetyl-L-cysteine (NAC). Pretreatment of HeLa and K562 cells (30 min) with NAC solutions (1 or 2 mM) enhanced their viability by 20–51 and 10–56%, respectively, compared to treatment with 10a alone (Figure 4). Thus, the role of ROS induction in the potency of the cobalt complexes was confirmed.

Also, non-cobalt nucleoside derivative 9c was studied, and this compound did not increase the level of reactive oxygen species (0.9 ± 0.03-fold change in relation to control cells). This result confirms that the cobalt atom present in the structure is responsible for the pro-oxidative properties of the cobalt nucleosides. In conclusion, the results show that the mechanism of action of most antiproliferative cobalt compounds in leukemia cells (K562) may be related to the induction of oxidative stress.

### 2.4. Oxidative Stress

Uncontrolled production of reactive oxygen species (ROS) in cells results in oxidative stress that impairs cellular functions and contributes to the development of cancer, chronic disease, and toxicity. Growing evidence suggests that cancer cells exhibit increased intrinsic ROS stress, due in part to oncogenic stimulation, increased metabolic activity, and mitochondrial malfunction. Given that the mitochondrial respiratory chain (electron-transport complexes) is a major source of ROS generation in cells, the vulnerability of mitochondrial DNA to ROS-mediated damage appears to be a mechanism to amplify ROS stress in cancer cells. As this state of oxidative stress makes cancer cells vulnerable to agents that further augment ROS levels, the use of pro-oxidant agents is emerging as an exciting strategy to target tumor cells selectively. Accumulation of peroxides and free radicals leads to death of cancer cells, for example, by apoptosis. This biochemical aspect can be exploited to develop novel therapeutic drugs to target cancer cells preferentially and selectively through ROS-mediated mechanisms. The contribution of reactive oxygen species to the antitumor activity of many chemotherapeutic agents commonly used in treatment has already been documented.

### 3. Conclusions

Novel 5-oxopropynyl-substituted 2'-deoxyuridines containing alkyl and aryl substituents at the propargyl carbon atom and hydroxy, methoxy, and acetoxy groups were synthesized and converted into their dicobalt hexacarbonyl derivatives. X-ray crystallography confirmed the structure of the cobalt nucleoside containing the homopropargyl alcohol (but-3-yn-1-ol) unit (see compound 10f) and determined the conformation and hydrogen bonding present in the solid state. The acetoxy cobalt derivative was converted into the corresponding complex containing a bidentate 1,1-bis(diphenylphosphino)-

### Table 2. Cytotoxic activity (effect of the substituents) of cobalt nucleosides 10a–h and 11 for the proliferation of the HeLa, K562, and HUVEC cell lines after incubating for 48 h.

<table>
<thead>
<tr>
<th>Compound</th>
<th>HeLa IC₅₀ [μM]</th>
<th>K562 IC₅₀ [μM]</th>
<th>HUVEC IC₅₀ [μM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a</td>
<td>34 ± 3.4</td>
<td>28 ± 4.3</td>
<td>40 ± 7.6</td>
</tr>
<tr>
<td>10a - NAC (1 mM)</td>
<td>140 ± 8.4</td>
<td>90 ± 7.5</td>
<td></td>
</tr>
<tr>
<td>10a - NAC (2 mM)</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>10b</td>
<td>28 ± 4.5</td>
<td>18 ± 3.6</td>
<td>20 ± 2.0</td>
</tr>
<tr>
<td>10c</td>
<td>80 ± 6.1</td>
<td>29 ± 4.7</td>
<td>40 ± 4.8</td>
</tr>
<tr>
<td>10d</td>
<td>80 ± 6.5</td>
<td>25 ± 3.6</td>
<td>31 ± 3.1</td>
</tr>
<tr>
<td>10e</td>
<td>20 ± 5.1</td>
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<td>80 ± 5.7</td>
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<td>10g</td>
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<td>28 ± 4.8</td>
<td>20 ± 3.8</td>
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<td>10h</td>
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<td>11</td>
<td>25 ± 3.2</td>
<td>16 ± 3.5</td>
<td>7 ± 5.2</td>
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<tr>
<td>cisplatin</td>
<td>20 ± 6.0</td>
<td>40 ± 7.0</td>
<td>30 ± 6.5</td>
</tr>
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</table>

(a) Results were obtained in two separate experiments, each n = 6.
methane (dppm) ligand. It was demonstrated that the presence of the cobalt carboxyl was essential to achieve a cytotoxic effect, as the alkynyl precursor did not exhibit pronounced activity against HeLa and K562 human cancer cells in vitro, with higher efficacy seen in the last one. Similar to the results obtained for other cobalt carboxyl complexes, the activity depended on the presence of the cobalt carboxyl moiety, suggesting that metal carboxyls are useful functional groups for modifying or inducing biological activity.

### Experimental Section

#### General Instrumentation

All NMR spectroscopy measurements were performed with a Bruker Avance III 500 spectrometer operating at 500.13 MHz for $^1$H, 125.75 MHz for $^{13}$C, and 202.45 MHz for $^{31}$P at 22 °C. Mass spectra were recorded with an Agilent 6520 Q-TOF LC-MS (HRMS). FTIR spectra were recorded with a Thermo Scientific Nicolet 6700 ATR, and Bruker Alpha-P ATR spectrometers. All products were stored in a refrigerator (4 °C).

#### Syntheses

**General Procedure for the Synthesis of 2'-Deoxy-5-alkynyluridines 9a–h**

A round-bottomed flask was charged with 2'-deoxy-5iodouridine (I-dU; 2.09 g, 5.90 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (0.690 g, 0.597 mmol), CuI (0.120 g, 0.628 mmol), DMF (15 mL), Et$_3$N (1.8 mL, 12 mmol), and the respective terminal alkyne. The mixture was stirred at 40 °C for 22 h (oil bath). The solvent was removed (by oil pump vacuum), and the residue was dissolved in chloroform (10 mL) and kept in the freezer (−4 °C) for 12 h. The precipitate was filtered off and washed with cold chloroform (x 3 mL). The mother liquor was concentrated and subjected to column chromatography (230–400 mesh silica gel, 0–12% MeOH/CHCl$_3$). The product was dried by oil pump vacuum for 2 h to give 9a–h.

2'-Deoxy-5-(3-hydroxyprop-1-yn-1-yl)uridine (9a): From 2-propyn-1-ol (propargyl alcohol, 0.86 mL, 14.8 mmol). Yield: 0.830 g, 2.94 mmol (50%). $R_f=0.25$ (CHCl$_3$/MeOH 7:1). $^1$H NMR (CD$_3$ODMSO): $\delta=$11.57 (s, 1 H, N-H), 8.14 (s, 1 H, H-6), 6.07 (t, $J=6.5$ Hz, 1 H, H-1), 5.26 (t, $J=6.1$ Hz, 1 H, OH-3), 5.21 (d, $J=4.51$ Hz, 1 H, OH), 5.08 (t, $J=5.15$ Hz, 1 H, OH-5'), 4.20 (s, 2 H, CH$_2$), 4.19 (s, 1 H, H-3'), 3.77–3.74 (m, 1 H, H-4'), 3.60–3.55 (m, 1 H, H-5), 3.54–3.49 (m, 1 H, H-5'), 2.10–2.06 ppm (m, 2 H, H-2', H-2'); $^{13}$C NMR (CD$_3$ODMSO): $\delta=$163.10, 150.91, 144.99, 99.69, 93.97, 89.06, 86.15, 77.80, 71.67, 62.44, 50.96, 40.38 ppm; IR (KBr): $\nu=3401$ (br m), 2924 (m), 1726 (s), 1667 (s), 1630, 1501, 900, 780, 716, 62.44, 50.96, 40.38 ppm; HRMS (ESI-TOF): m/z: calculated for C$_{12}$H$_9$N$_4$O$_2$: 283.0825 [M+H$^+$]; found: 283.0827; elemental analysis calculated (%) for C$_{12}$H$_9$N$_4$O$_2$: 51.07, H 5.00, N 9.93; found: C 51.18, H 5.49, N 9.79.

2'-Deoxy-5-(3-hydroxy-3-methylbut-1-yn-1-yl)uridine (9b): From 2-methyl-3-butyne-2-ol (1.43 mL, 14.8 mmol). Yield: 0.750 g, 2.42 mmol (41%). $R_f=0.27$ (CHCl$_3$/MeOH 8:1). $^1$H NMR (CD$_3$ODMSO): $\delta=11.54$ (s, 1 H, N-H), 8.10 (s, 1 H, H-6), 6.07 (t, $J=6.9$ Hz, 1 H, H-1),

### Table 3. Oxidative stress of cobalt nucleosides 10a–h and 9c (bar plot for 100 μM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>DCF fluorescence intensity$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 μM</td>
</tr>
<tr>
<td>DMSO + DCFDA</td>
<td>1 ± 0.0$^a$</td>
</tr>
<tr>
<td>$H_2O_2$</td>
<td>0.9 ± 0.03</td>
</tr>
<tr>
<td>9a</td>
<td>12.0 ± 0.03</td>
</tr>
<tr>
<td>10a</td>
<td>12.0 ± 0.03</td>
</tr>
<tr>
<td>9b</td>
<td>12.0 ± 0.03</td>
</tr>
<tr>
<td>10b</td>
<td>12.0 ± 0.03</td>
</tr>
<tr>
<td>10c</td>
<td>12.0 ± 0.03</td>
</tr>
<tr>
<td>10d</td>
<td>12.0 ± 0.03</td>
</tr>
<tr>
<td>10e</td>
<td>12.0 ± 0.03</td>
</tr>
<tr>
<td>10f</td>
<td>12.0 ± 0.03</td>
</tr>
<tr>
<td>10g</td>
<td>12.0 ± 0.03</td>
</tr>
<tr>
<td>10h</td>
<td>12.0 ± 0.03</td>
</tr>
<tr>
<td>11</td>
<td>1.4 ± 0.10</td>
</tr>
</tbody>
</table>

$^a$ Change normalized to control sample. $^b$ Control, concentration 1% DMSO, 20 μM DCFDA. $^c$ Positive control, concentration 50 μM. $^d$ Positive control, concentration 100 μM.

**Figure 4.** Survival rate of HeLa (top) and K562 (bottom) cells after incubating with compounds 10a and 10a + NAC (1 or 2 mM) for 48 h.
2′-Deoxy-5-(3-hydroxy-propyl-1-yn-1-yl)uridine (9d): From 1-phenyl-2-propyn-1-ol (1.80 mL, 14.8 mmol). Yield: 0.972 g, 2.72 mmol (66%). R<sub>f</sub> = 0.59 (CHCl<sub>3</sub>/MeOH 8:1). 1<sup>H</sup> NMR (DMSO): δ = 11.61 (s, 1 H, N-H), 8.13 (s, 1 H, H-6), 6.10 (t, J = 6.6 Hz, 1 H, H-1), 5.27–5.52 (m, 2 H, CH<sub>2</sub>), 0.93 (br s, 1 H, OH), 3.58 (s, 1 H, OMe). 13<sup>C</sup> NMR (DMSO): δ = 161.57, 149.51, 143.17, 98.53, 97.47, 87.59, 86.49, 79.23, 74.29, 70.13, 67.22, 60.94, 36.47, 29.31, 9.10 ppm; IR (KBr): ν = 3345 (brm), 2973 (m), 1668 (s), 1541 (s), 1278 (m), 1088 cm<sup>−1</sup> (m); HRMS (ESI-TOF): m/z: calcd for C<sub>17</sub>H<sub>11</sub>N<sub>5</sub>O<sub>4</sub>: 341.1312 [M+H]<sup>+</sup>; found: 341.1313.

2′-Deoxy-5-(3-hydroxy-3,3-diphenyl-prop-1-yn-1-yl)uridine (9e): From 1,1-diphenyl-2-propyn-1-ol (3.10 g, 14.8 mmol). Yield: 2.36 g, 5.43 mmol (92%). R<sub>f</sub> = 0.23 (CHCl<sub>3</sub>/MeOH 9:1). 1<sup>H</sup> NMR (DMSO): δ = 11.68 (s, 1 H, N-H), 8.29 (s, 1 H, H-6), 7.63–7.59 (m, 4 H, Ph), 7.33–7.28 (m, 4 H, Ph), 7.23–7.19 (m, 2 H, Ph), 6.85 (s, 1 H, OH), 6.14 (t, J = 6.7 Hz, 1 H, H-1), 5.26 (brs, 1 H, OH-3), 5.12 (brs, 1 H, OH-5), 4.26 (brs, 1 H, H-3), 3.83–3.80 (m, 1 H, H-4), 3.65–3.61 (m, 1 H, H-5), 3.60–3.56 (1 H, H-5), 2.18–2.13 ppm (m, 2 H, H-2′); 13<sup>C</sup> NMR (DMSO): δ = 160.72, 149.51, 143.16, 143.47, 128.01, 120.07, 125.77, 98.12, 96.11, 87.72, 84.92, 79.26, 74.77, 70.25, 63.13, 60.16 ppm; IR (KBr): ν = 3345 (brm), 2973 (m), 1668 (s), 1541 (s), 1278 (m), 1088 cm<sup>−1</sup> (m); HRMS (ESI-TOF): m/z: calcd for C<sub>29</sub>H<sub>21</sub>N<sub>5</sub>O<sub>4</sub>: 431.1445 [M+OH]<sup>+</sup>; found: 431.1447.

General Procedure for the Synthesis of Dichobalt Hexacarbonyl 2′-Deoxy-5-Salkynyluridinoids 10a–h

Under a nitrogen atmosphere, a round-bottomed flask was charged with Co<sub>2</sub>(CO)<sub>6</sub> (0.690 g, 2.02 mmol), 9a–h (1.69 mmol), and THF (18 mL). The mixture was stirred at room temperature (22 °C) for 1 h. The solvent was removed by rotary evaporation. Column chromatography (230–400 mesh silica gel, 0–5% MeOH/CHCl<sub>3</sub>) gave 10a–h as a red-brown compound.

Dichobalt hexacarbonyl 2′-deoxy-5-(3-hydroxy-propyl-1-yn-1-yl)uridine (10a): From compound 9a (0.477 g, 1.69 mmol). Yield: 0.557 g, 0.98 mmol (58%). R<sub>f</sub> = 0.75 (CHCl<sub>3</sub>/MeOH 7:1). 1<sup>H</sup> NMR (DMSO): δ = 11.60 (s, 1 H, N-H), 8.18 (s, 1 H, H-6), 6.17 (brs, 1 H, H-1), 5.53 (brs, 1 H, OH-3), 5.24 (brs, 1 H, OH-5), 5.04 (brs, 1 H, OH), 4.73 (brs, 2 H, CH<sub>2</sub>), 4.23 (brs, 1 H, H-3), 3.84 (brs, 1 H, H-5), 3.59–3.44 (m, 2 H, H-5′), 2.16 (brs, 1 H, H-2′), 2.02 ppm (brs, 2 H, CH<sub>2</sub>); 13<sup>C</sup> NMR (DMSO): δ = 200.25, 161.27, 150.21, 139.69, 111.64, 103.36, 88.49, 85.52, 82.63, 79.63, 71.49, 62.52, 62.08, 40.83, 40.20 ppm; IR (KBr): ν = 3421 (brm), 2927 (w), 2094 (s), 2054 (s), 2026 (s), 1693 (m), 1456 (m), 1276 (m), 1095 cm<sup>−1</sup> (m); HRMS (ESI-TOF): m/z: calcd for C<sub>30</sub>H<sub>17</sub>Co<sub>2</sub>N<sub>5</sub>O<sub>4</sub>·C<sub>6</sub>H<sub>5</sub>O<sub>2</sub>: 550.9178 [M − OH]<sup>−</sup>; found: 550.9173; elemental analysis calcd (%) for C<sub>30</sub>H<sub>17</sub>Co<sub>2</sub>N<sub>5</sub>O<sub>4</sub>: 35.80 H, 24.8 N, 9.33; found: C 37.27, H 2.52, N 5.13.

Dichobalt hexacarbonyl 2′-deoxy-5-(3-hydroxy-3-methyl-butyl-1-yn-1-yl)uridine (10b): From compound 9b (0.524 g, 1.69 mmol). Yield: 0.312 g, 0.52 mmol (31%). R<sub>f</sub> = 0.53 (CHCl<sub>3</sub>/MeOH 8:1). 1<sup>H</sup> NMR (DMSO): δ = 11.79 (s, 1 H, N-H), 8.27 (s, 1 H, H-6), 6.20–6.15 (m, 1 H, H-1), 5.74 (brs, 1 H, OH), 5.25 (brs, 1 H, OH-3), 5.02 (brs, 1 H, OH-5), 4.23 (brs, 1 H, H-3), 3.84 (brs, 1 H, H-5), 3.58–3.45 (m, 2 H, H-5′), 2.21–2.14 (m, 1 H, H-2′), 2.02–1.94 (m, 1 H, H-2′), 1.46 ppm (s, 6 H, CH<sub>3</sub>); 13<sup>C</sup> NMR (DMSO): δ = 199.88, 161.55, 149.68, 140.12, 111.23, 110.50, 88.08, 85.02, 83.38, 79.24, 71.78.
Dicobalt hexacarbonyl 2-deoxy-5-(3-methoxyprop-1-yn-1-yl)uridine (10c): From compound 9g (0.500 g, 1.69 mmol). Yield: 0.812 g, 1.39 mmol (83%). Rf = 0.09 (CHCl/MeOH 7:1). 1H NMR ([D6]DMSO): δ = 11.59 (s, 1H, N-3), 8.19 (s, 1H, H-6), 5.24 (brs, 1H, OH-3), 5.06 (brs, 1H, OH-5), 4.67 (s, 2H, CH2), 4.24 (brs, 1H, H-3), 3.84 (brs, 1H, H-1), 3.63–3.46 (m, 2H, H-5, H-5'), 3.39 (s, 3H, OCH2), 2.15 (brs, 1H, H-2), 2.03 ppm (brs, 1H, H-2'); 13C NMR ([D6]DMSO): δ = 199.60, 160.73, 149.83, 139.42, 111.04, 97.01, 88.12, 85.13, 82.54, 76.12, 70.19, 66.18, 58.41 40.51 ppm; IR (KBr): ν = 3425 (brm), 2933 (w), 2094 (s), 2053 (s), 2026 (s), 1690 (m), 1453 (m), 1275 (m), 1093 cm⁻¹ (m); HRMS (ESI-TOF): m/z: calc for C21H19Co4N3O13, 928.9440 [M+H]+; found: 928.9441; elemental analysis calc (%) for C21H19Co4N3O13: 582.20 (20): C 39.20, H 2.77, N 4.81; found: C 39.87, H 3.08, N 4.98.

Dicobalt hexacarbonyl 2-deoxy-5-(3-acetylxyprop-1-yn-1-yl)uridine (10h): From compound 9h (0.548 g, 1.69 mmol). Yield: 0.856 g, 1.40 mmol (83%). Rf = 0.79 (CHCl/MeOH 7:1). 1H NMR ([D6]DMSO): δ = 11.63 (s, 1H, NH), 8.22 (s, 1H, H-6), 6.17 (t, J = 6.7 Hz, 1H, H-1), 5.47–5.53 (m, 2H, CH3), 5.24 (brs, 1H, OH-3), 5.09–5.04 (m, 1H, OH-5), 4.24 (brs, 1H, H-3'), 3.84 (brs, 1H, H-4'), 3.59–3.53 (m, 1H, H-5'), 3.32–3.48 (m, 1H, H-2'), 2.08–2.04 (m, 1H, H-2'); 13C NMR ([D6]DMSO): δ = 199.22, 170.12, 160.77, 149.79, 139.83, 110.92, 94.91, 88.19, 85.30, 82.43, 79.24, 71.09, 64.92, 61.62, 20.33 ppm; IR (KBr): ν = 3423 (brm), 2936 (w), 2095 (s), 2056 (s), 1698 (m), 1454 (m), 1273 (m), 1096 cm⁻¹ (m); HRMS (ESI-TOF): m/z: calc for C21H19Co4N3O13, 928.9440 [M+H]+; found: 928.9438; elemental analysis calc (%) for C21H19Co4N3O13: 582.20. C 39.20, H 2.77, N 4.81; found: C 39.17, H 2.43, N 5.11.

Bis[diphenylphosphino]methane dicobalt tetracarbonyl 2-deoxy-5-(3-acetoxylprop-1-yn-1-yl)uridine (11): Under a nitrogen atmosphere, a 50 mL flask was charged with Co4(CO)8 (0.500, 1.46 mmol), toluene (20 mL), and 1,1-bis(diphenylphosphino)methane (dppm; 0.561 g, 1.46 mmol). The mixture was stirred at room temperature for 3 h. The solvent was removed by rotary evaporation. Column chromatography (230–400 mesh silica gel, 0–2% MeOH/CHCl3) gave Co4(CO)8(dppm)2 (0.842 g, 1.26 mmol, 86%). A 50 mL flask was charged with Co4(CO)8(dppm)2 (0.402 g, 0.600 mmol), toulene (20 mL), and THF (12 mL). The solution was stirred at room temperature (22°C) for 24 h. The solvent was removed by rotary evaporation. Column chromatography (230–400 mesh silica gel, 0–10% MeOH/CHCl3) gave compound 11 (0.141 g, 0.150 mmol, 50%). Rf = 0.83 (CHCl3/MeOH 9:1). 1H NMR ([D6]DMSO): δ = 11.42 (s, 1H, NH), 7.78 (brs, 1H, H-6), 7.52–7.09 (m, 10H, 4Ph), 6.26–6.17 (m, 1H, H-1), 5.29 (brs, 2H, CH2), 4.91 (brs, 1H, OH-3), 4.23 (brs, 1H, OH-5), 3.9–3.84 (m, 2H, CH3), 3.81 (brs, 1H, H-3), 3.49 (brs, 1H, H-4), 3.43 (m, 2H, H-5, H-5'), 3.33 (brs, 2H, H-5, H-5'), 2.23–2.16 (m, 1H, H-2'), 2.04–1.96 (m, 1H, H-2'); 13C NMR ([D6]DMSO): δ = 205.96, 203.42, 201.20, 200.76, 169.55, 160.23, 149.70, 137.20, 130.71, 136.85, 135.85, 131.46, 131.04, 129.0, 128.92, 128.07, 127.87, 116.21, 96.99, 87.40, 84.16, 80.00, 70.94, 67.08, 61.74, 32.97, 23.11, 20.07 ppm; 31P NMR ([D6]DMSO): δ = -40.05 ppm; HRMS (ESI-TOF): m/z: calc for C31H16Co3N6P3O6: 879.0477 [M-OAc]+; found: 879.0476.
Cells and Cytotoxicity Assay

HeLa (human cervix carcinoma) and K562 (chronic myelogenous leukemia) cells were cultured in RPMI 1640 medium supplemented with antibiotics and 10% fetal calf serum (HeLa, K562) under a 5% CO₂/95% air atmosphere. A total of 7 × 10⁴ cells were seeded on each well of a 96-well plate (Nunc). After 24 h, cells were exposed to the test compounds for an additional 48 h. Stock solutions of test compounds were freshly prepared in DMSO. The final concentrations of the compounds tested in the cell cultures were: 2 × 10⁻¹, 1 × 10⁻¹, 5 × 10⁻², 1 × 10⁻², 1 × 10⁻³, and 1 × 10⁻⁴ M. The concentration of DMSO in the cell culture medium was 1%.

Human umbilical vein endothelial cells (HUVEC) were acquired from Life Technologies and were cultured in Medium 200 with 20% serum growth supplement (Life Technologies) according to the manufacturer’s instructions. A total of 10 × 10⁴ cells were seeded on each well of a 96-well plate (Nunc) in the presence of antibiotics (100 μL⁻¹ penicillin and 100 μg mL⁻¹ streptomycin).

The cytotoxicity of each compound was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma] assay, as previously described. Briefly, after 24 or 48 h of incubation with the drug, the cells were treated with the MTT agent and incubation was continued for 2 h. MTT-formazan crystals were dissolved in 20% sodium dodecyl sulfate (SDS) and 50% DMF at pH 4.7, and the absorbance was read at λ = 570 and 650 nm with an ELISA-PLATE READER (FLUOstar Omega). As control (100% viability), cells grown in the presence of only vehicle (1% DMSO) were used.

In a separate assay, the HeLa and K562 cells were pretreated with 1 or 2 mM N-acetyl-L-cysteine (NAC), a ROS inhibitor, for 30 min before completing the above steps, following the previously described procedure.

The IC₅₀ values (the concentration of test compound required to reduce the cell survival fraction to 50% of the control) were calculated from dose–response curves and were used as a measure of cellular sensitivity to a given treatment.

Intracellular ROS Measurement in Living Cells

Intracellular ROS levels were assessed with a 2,7-dichlorofluorescein diacetate (DCFDA) dye assay. DCFDA (Sigma–Aldrich) is a cell-permeant reagent, a fluorogenic dye that measures the activity of hydroxyl, peroxyl, and other ROS within the cell. After diffusion into the cell, DCFDA was deacetylated by cellular esterases to a non-fluorescent compound, which was later oxidized by the ROS into 2,7-dichlorofluorescein (DCF). DCF is a highly fluorescent compound that can be detected by fluorescence spectroscopy with maximum excitation and emission bands at λ = 485 and 520 nm, respectively.

K562 (chronic myelogenous leukemia) cells were cultured in RPMI 1640 medium supplemented with antibiotics and 10% fetal calf serum under a 5% CO₂/95% air atmosphere. Next, 10 × 10⁴ cells per well were stained with 20 μM DCFDA in the culture media and were then incubated for 30 min at 37°C. After staining, the cells were centrifuged and suspended in complete medium without phenol red, and 10 × 10⁴ cells were seeded on each well of a 96-well plate (PerkinElmer). Next, the cells were incubated for 4 h with the test compounds at concentrations of 5, 10, 20, 50, 100, and 200 μM. Stock solutions of the test compounds were freshly prepared in DMSO. The concentration of DMSO in the cell culture medium was 1%. Cells treated with 50 and 100 μM H₂O₂ served as positive controls. After incubation, fluorescence in each well was measured (excitation at λ = 485 nm, emission measured at λ = 520 nm) by using a microplate reader FLUOstar Omega (BMG-Labtech, Germany). The increase in the fluorescence intensity of DCF was a sign of an increase in ROS levels. For normalization of the data, the DCF fluorescence intensity level in the control cells (exposed to 1% DMSO and 20 μM DCFDA) was taken as 1.0.

Computational Details

Quantum-chemical calculations providing molecular orbitals (HOMO–LUMO), energy-minimized molecular geometries, and vibrational spectra of 10 f were obtained by using density functional theory (DFT) as implemented in the Gaussian 09 (Revision C.01) program package. We utilized the PBE0 hybrid functional and 6-31G* basis set. Complete ground-state geometry optimization was afforded without symmetry constraints. Only default convergence criteria were used during geometry optimization. Initial atomic coordinates were imported from the crystal structure. Optimized structures in the singlet state were indicated to be local minima (no imaginary frequencies). Selected theoretical and experimental metric parameters are provided in Figure 2. Molecular orbitals were acquired by using Avogadro (an open-source molecular builder and visualization tool, Version 1.1.0).

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Keywords: alkynes · anti proliferative agents · antitumor agents · cobalt · nucleosides
