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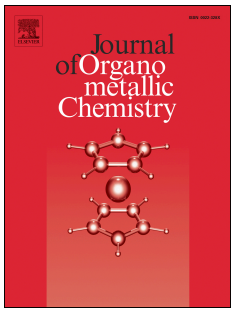

Aspirin-inspired organometallic compounds: Structural characterization and cytotoxicity

Adnan Ashraf, Muhammad Hanif, Mario Kubanik, Tilo Söhnel, Stephen M.F. Jamieson, Arindam Bhattacharyya, Christian G. Hartinger

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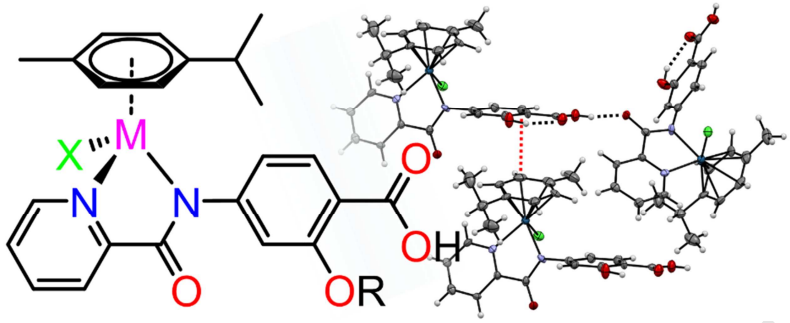

Synopsis

Inspired by the promising anticancer activity of aspirin–metal conjugates, aspirin-derived ligands were prepared and coordinated to organoruthenium and -osmium moieties. Most interestingly, crystallographic studies revealed a network of hydrogen bonding and π-stacking. Surprisingly, none of the prepared compounds showed antiproliferative activity.

Aspirin-inspired Organometallic Compounds: Structural Characterization and Cytotoxicity

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Abstract

We report here the preparation of 2-hydroxy-4-(picolinamido)benzoic acid and its aspirin analogue 2-acetoxy-4-(picolinamido)benzoic acid. Both ligands were used to synthesize organoruthenium and -osmium complexes. The compounds were characterized by NMR spectroscopy, electrospray ionization mass spectrometry and elemental analysis. The molecular structures of Ru and Os complexes of 2-hydroxy-4-(picolinamido)benzoic acid were determined by single crystal X-ray diffraction analysis. They showed a typical piano-stool configuration and the ligand coordinated as an *N*,*N*-bidentate chelator to the metal center. The cytotoxic potential of the selected compounds was evaluated in human colon (HCT116, SW480), non-small cell lung (NCI-H460) and cervical (SiHa) carcinoma cells. Surprisingly none of the compounds exhibited antiproliferative activity given the fact that other metal complexes of aspirin derivatives have shown promising anticancer activity.

Keywords: anticancer activity, aspirin, osmium complexes, ruthenium complexes, NSAIDs.

Introduction

Although the application of platinum-based drugs has improved the overall outcome of cancer therapy, undesired side effects, a limited spectrum of treatable tumors and resistance are key limitations in their clinical use [1,2]. This led to the search for non-platinum metallotherapeutics [3-11], among which ruthenium complexes occupy a prominent place due to their relatively low general toxicity [4,5,9]. The ruthenium complexes KP1019 (indazolium trans-[tetrachloridobis(1*H*-indazole)ruthenate(III)])/KP1339 {sodium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)]} and NAMI-A {imidazolium *trans*-[tetrachlorido(dimethylsulfoxide)(imidazole)ruthenium(III)]} have been tested in clinical trials [5,12,13]. The discovery of the anticancer activity of RAPTA-C [Ru(cym)(PTA)Cl_2] (PTA = 1,3,5-triaza-7-phosphatricyclodecane; cym = η^6-*p*-cymene) and RAED-C [Ru(cym)(en)Cl]PF_6 (en = 1,2-ethylenediamine) fueled research into half-sandwich Ru^{II}(η^6-arene) complexes [8,14-17]. The presence of the arene moiety not only enhances the hydrophobic character of the complexes but also assists in stabilizing the ruthenium in 2+ oxidation state [14]. The osmium analogues of many recently reported organoruthenium compounds were also investigated on their suitability as anticancer agents. Os complexes are however still relatively less well explored compared to their Ru congeners. Interestingly, it is hard to predict the biological effect when replacing a Ru center in a complex with Os. In some cases, the Ru complexes are more potent than the Os analogues, while in others the substitution does not impact the antiproliferative activity or the osmium complexes are found to be more cytotoxic than their ruthenium counterparts [10].

Picolinic acid and its derivatives have been used as ligands for the preparation of anticancer active organometallic complexes with Ru, Os, Rh and Ir centers [18-22]. Picolinato Os complexes were found to be active against ovarian human carcinoma, while both Ru and Os picolinamide compounds inhibited cell proliferation of colon, ovarian, and cisplatin-resistant ovarian human carcinoma cells [18,20]. Picolinamide derivatives can act as *N,O*- or *N,N*-coordinating bidentate ligands depending on the pH value of the solution (Figure 1) [18,22,23]. The coordination mode of the picolinamide ligands impacted aquation and cytotoxicity [18]. Furthermore, while Ru(cym) complexes of picolinamide in *N,N*-coordination mode selectively target the DNA base guanine [18], the analogous thiocarbamide derivatives, coordinating to a Ru(arene) moiety through an *S,N*-donor system, were shown to interact preferentially with proteins [24].

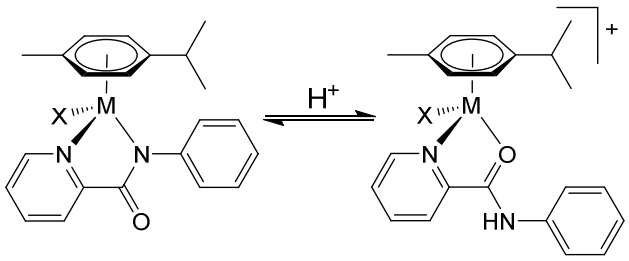
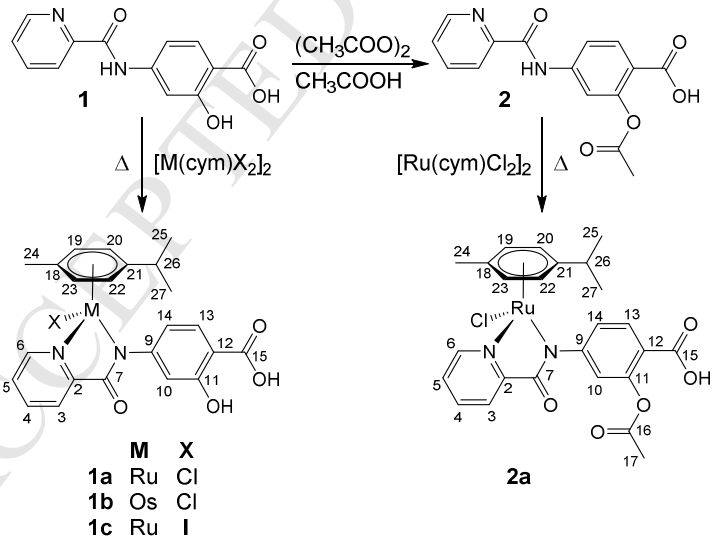


Figure 1. pH-dependent interconversion between *N,N*- and *N,O*-coordination modes in picolinamide Ru(cym) complexes.

Recent studies have shown that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) are effective for chemoprevention of cancer [25-27], while several metal complexes of NSAIDs have also showed promising anticancer activity [28-30]. Combining an organometallic moiety with bioactive ligands has opened up new avenues in the development of multi-targeted drugs [29,31-40]. With the aim of introducing aspririn-inspired properties into organometallic anticancer compounds, we synthesized salicylic acid-functionalized picolinamide and acetylated it. By using 4-aminosalicylic acid as the starting material, the key functional groups of aspirin remained unmodified. The prepared picolinamide derivatives were then coordinated to organo-Ru and –Os fragments with different halides and studied for the anticancer properties.

Results and Discussion

Aspirin is a well-known NSAID and recent reports highlighted its chemotherapeutic potential against cancer [25]. Prolonged and dose-dependent use of aspirin was found to reduce the risk of colon, gastrointestinal, lung and breast cancer [26,41,42]. In an attempt to exploit the advantageous biological properties of aspirin, 2-picolinamide was functionalized with 4-aminosalicylic acid to yield **1** (Scheme 1). The hydroxyl functional group of compound **1** was acetylated with acetic anhydride/acetic acid to obtain the aspirin analogue of 2-picolinamide **2**. Both **1** and **2** were converted into the respective half-sandwich Ru^{II} and Os^{II} organometallics by refluxing them with different dimeric $[M(cym)X_2]_2$ precursors. 2-Picolinamide ligands may behave as *N*,*N*- and *N*,*O*-bidentate chelators depending on the pH of the solution. When **1** was coordinated to the metal centers, only *N*,*N*-coordination was observed upon reflux in a dichloromethane and acetone mixture (9:1). In contrast, for **2** both *N*,*N*- and *N*,*O*-coordination modes were observed, the latter however to a minor degree and only when iodido complexes were prepared (data not shown). Attempts to push the equilibrium to either side showed that at pH < 2 the compounds start to decompose, as demonstrated by ^{1}H NMR spectroscopy.



Scheme 1. Preparation of the organoruthenium and -osmium compounds based on aspirin-inspired ligands derived from 4-picolinamide.

All complexes were characterized with 1D and 2D NMR spectroscopy, high resolution mass spectroscopy, microanalysis and X-ray crystallography. The acetylation of **1** resulted in a characteristic shift of the signals assigned to H-10 and H-14 (see Scheme 1 for the NMR

numbering scheme used) in the downfield region of the ^{1}H NMR spectra from 7.59 and 7.26 ppm to 7.81 and 7.74 ppm, respectively. An additional signal was observed at 2.31 ppm in **2** which was assigned to the methyl group. After complex formation the H-6 signal in all complexes was considerably moved downfield to chemical shifts > 9.2 ppm, compared to 8.69 ppm for the uncoordinated ligands **1** and **2**. The aromatic protons of cymene gave four doublets in case of *N,N* coordination mode while in case of *N,O* coordination only two doublets were observed.

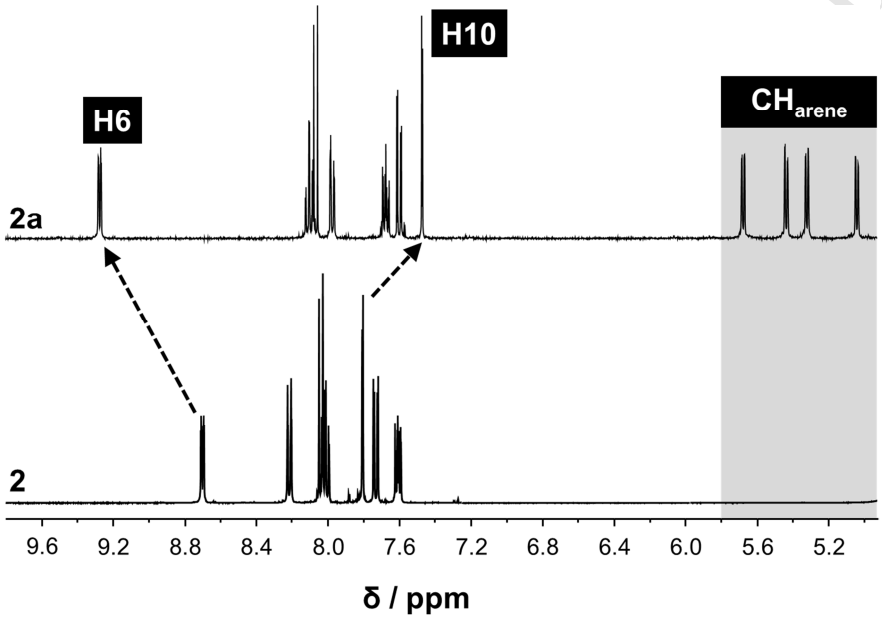


Figure 2. Comparison of the ^{1}H NMR spectra of ligand **2** and its Ru(cym) complex.

In the ^{13}C{^{1}H} NMR spectra of both **1** and **2**, the carbonyl carbon atoms C-15 were present in the most deshielded region at approximately δ = 171 ppm. After complexation to Ru or Os, the most significant shifts were observed for the amide carbonyl carbon atoms C-7 which appeared in the range 171–168 ppm in **1a**–**1c** and **2a**, as compared to 162 and 165 ppm for **1** and **2**, respectively. The signals assigned to C-2 and C-9, adjacent to the nitrogen atoms coordinated to the metal ions, were also shifted downfield. For the Ru complexes the aromatic carbon atoms of *p*-cymene appeared in the range 86–84 ppm while in case of the Os complex, these signals appeared around 76 ppm. In case of **2** and **2a**, an additional signal was present at δ = 21 ppm, which was assigned to the methyl carbon of the acetyl group.

The formation of both the ligands and the complexes was also confirmed by ESI-MS analysis in positive ion mode. The mass spectra featured the pseudomolecular $[M + Na]^{+}$ ions for the ligands and $[M - X]^{+}$ ions for the metal complexes. The *m/z* values obtained were very

similar to the theoretical values and the recorded isotopic pattern matched well the modeled counterparts.

Single crystals suitable for X-ray diffraction analysis of compounds **1a**–**1c** were obtained in methanol and diethyl ether by using slow diffusion method. The molecular structures of **1a**–**1c** showed pseudo-octahedral geometry around the metal center. Selected bond lengths are given in Table 1. In all cases, ligand **1** formed a five-membered ring with the metal centers showing *N*,*N* coordination through the nitrogen atoms of the pyridyl and carboxamide moieties. Compounds **1a** (Figure 3) and **1c** (Figure 4) crystallized in the chiral space group $P2_1$ and therefore only one of the two enantiomers with the Ru acting as the chiral center were identified. Furthermore, the molecular structures contained co-crystallized methanol which formed H bonds O5–H···O2 to the phenolic hydroxyl group. In contrast, **1b** crystallized in the non-chiral space group $P2_1/c$ and both enantiomers were detected.

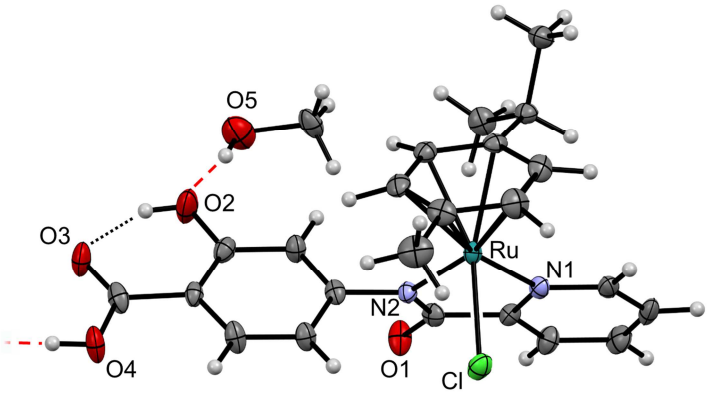


Figure 3. Molecular structure of **1a** drawn at 50% probability level, with the inter- (orange) and intramolecular (black) H bonds indicated as dashed lines. The H bond from the O1 atom to the next molecule of **1a** was omitted for clarity, as was one of the positions of the disordered MeOH found in the structure.

In all structures, both intramolecular and intermolecular hydrogen bonding was observed. Intramolecular H bonds O2–H···O3 involved the hydroxyl and carboxylic acid functional groups, while intermolecular hydrogen bonds were formed between the carboxamide oxygen O1 and the carboxylic acid hydroxyl group. These intermolecular H bonds extended to a one-dimensional network between molecules of the Ru complexes **1a** and **1c** and the *p*-cymene ligands interacted with the substituted phenyl groups by π stacking (3.312 and 3.274 Å for **1a** and **1c**, respectively).

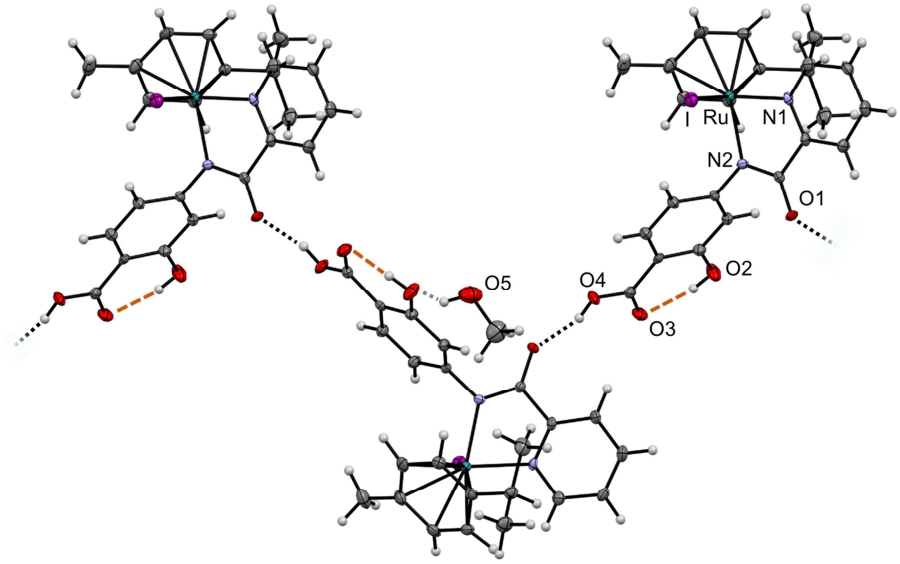


Figure 4. Molecular structure of **1c** forming a zigzag chain through intermolecular hydrogen bonding between the carboxylic acid group of one molecule and the carboxamide of another. The inter- and intramolecular H bonds are shown as orange and black dashed lines, respectively.

In case of the Os compound **1b** (Figure 5), hydrogen bonds were only detected between molecules of the same enantiomers and the two chains of enantiomers were connected through π stacking between the *p*-cymene ligands and the substituted phenyl groups of the same enantiomers (3.283 Å), while the two different enantiomers were also found to interact with each other through π stacking (3.380 Å).

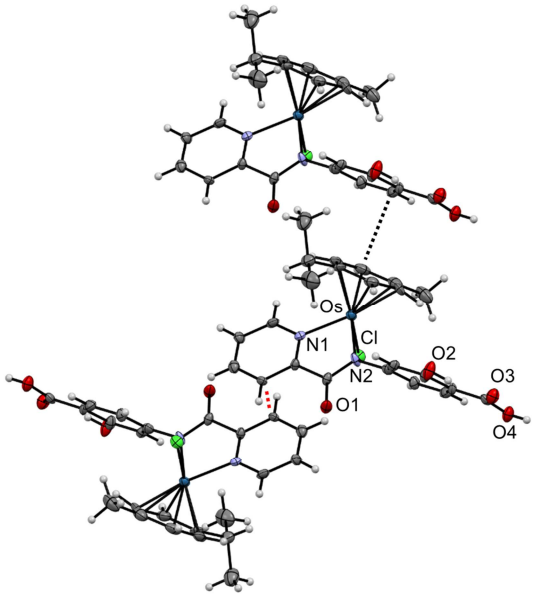


Figure 5. Molecular structure of the Os complex **1b** drawn at 50% probability level. The π-stacking interaction between the same enantiomers of **1b** are indicated by a black dashed line, while the π-stacking between the two enantiomers is shown as an orange dashed line between the pyridine moieties of the ligands.

As expected, the Ru–I bond distance in **1c** (2.7149(6) Å) was longer than the Ru–Cl bond in **1a** (2.4143(15) Å) which was similar to the Os–Cl bond length in **1b** (Table 1). The M–N distances were similar in all cases indicating similar electronic conditions around the metal center. This implies that in all cases the ligands were coordinated as carboxamidates to the metal centers after deprotonation of the amide group.

Table 1. Selected bond lengths [Å] observed in the molecular structures of **1a**, **1b** and **1c**.

Bond	**Bond length / Å**		
	1a	**1b**	**1c**
M–X	2.4143(15)	2.413(2)	2.7149(6)
M–N1	2.092(5)	2.098(7)	2.088(5)
M–N2	2.077(4)	2.087(7)	2.083(4)
C6–N1	1.356(7)	1.353(11)	1.345(8)
C1–N2	1.321(7)	1.325(11)	1.320(8)

The Ru and Os complexes as well as the ligands **1** and **2** were tested on their cytotoxicity against human colon HCT116 and SW480, non-small cell lung NCI-H460, and cervical SiHa carcinoma cells after 72 h incubation (Table 2). All tested compounds were inactive against these cell lines, as was aspirin in NCI-H460 and HCT116. In fact it was only possible to

determine IC_{50} values in all four cell lines for the closest aspirin derivative, *i.e.*, **2**, while for most of the other compounds the IC_{50} could not be reached due to limited solubility. This is surprising given the fact that coordination of an aspirin derivative functionalized with an alkyne to Co carbonyl complexes resulted in IC_{50} values in the low µM range in MCF-7 breast cancer cells [43] and the Pt derivative asplatin gave IC_{50} values around 1 µM in HeLa cervical and HepG2 liver cancer cells [30]. On the other hand aspirin was found inactive in these cell lines, as reported also by other groups for a variety of cell lines [44-46]. This may be related to aspirin exhibiting its anticancer activity by acting on the tumor microenvironment, *e.g.* through preventing prostaglandin E2-mediated evasion of T-cell dependent tumor control, rather than on individual cancer cells [47].

Table 2. IC_{50} values determined for the aspirin-inspired ligands and their Ru and Os complexes against human colon HCT116 and SW480, non-small cell lung NCI-H460, and cervical SiHa carcinoma cells after 72 h incubation.

Compound	**IC_{50} values / µM**			
	NCI-H460	**SiHa**	**HCT116**	**SW480**
aspirin	1569 ± 239	-	949 ± 108	-
1	> 426	325 ± 56	> 426	> 426
1a	> 1054	> 1054	> 280	> 1054
1b	> 280	> 280	> 280	> 280
2	399 ± 15	290 ± 24	323 ± 34	474 ± 47
2a	> 280	> 280	> 280	> 280

Conclusions

Picolinamides are an extensively studied class of ligands used for a wide variety of applications. By preparing 2-picolinamides functionalized with 4-aminosalicylic acid and subsequent acetylation we gained access to derivatives related to aspirin. The coordination of aspirin derivatives to metal centers has been reported to lead to metal complexes with anticancer activity. Therefore we used these ligands to prepare organo-Ru and -Os complexes. 2-Picolinamide can coordinate to metal centers either as an *N,N*- or *N,O*-bidentate ligand. While, *N,N*-coordination is thermodynamically favorable, the coordination mode is pH-dependent. In this case, the amide functional group underwent deprotonation and the ligands coordinated to the Ru^{II} and Os^{II} centers through their pyridyl and carboxamide

nitrogen atoms to obtain stable *N,N*-type of complexes. This was confirmed by single crystal X-ray diffraction analysis and ^{1}H NMR spectroscopy. In the molecular structures of the complexes a multitude of H bonds were detected leading to a 1D network that was cross-linked by π stacking interactions mostly between the phenolic ligands and the *p*-cymene moieties coordinated to the metal centers. The obtained metal complexes and their ligands were assayed on their antiproliferative activity against a series of different cancer cell lines but none of the compounds were significantly potent cell growth inhibitors.

Acknowledgments

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Experimental

Material and methods

All the reactions were carried out under inert conditions. All the chemicals were of analytical grade and used without further purification. Picolinic acid, α-terpinene, triethyl amine, 4-aminosalicylic acid, thionyl chloride, acetic acid, acetic anhydride and hydrazine hydrochloride were obtained from Sigma Aldrich. $RuCl_3 \cdot 3H_2O$ (40.4%) and OsO_4 were purchased from Precious Metals Online. Methanol, dichloromethane and dimethyl formamide were dried according to standard procedures. The dimers bis[dichlorido(η^6-*p*-cymene)ruthenium(II)] [48], bis[diiodido(η^6-*p*-cymene)ruthenium(II)] [49], bis[dichlorido(η^6-*p*-cymene)osmium(II)] [50] were synthesized by adapting reported procedures.

High-resolution mass spectra were recorded on the Bruker micro TOF-Q II electrospray ionization (ESI) mass spectrometer in positive ion mode. ^{1}H and ^{13}C{^{1}H} NMR spectra were recorded on Bruker DRX 400 MHz NMR spectrometers at ambient temperature at 400.13 (^{1}H) and 100.61 MHz (^{13}C{^{1}H}), and 2D NMR data were collected in a gradient-enhanced mode. ^{1}H and ^{13}C{^{1}H} chemical shifts are reported vs $SiMe_4$ and were determined by reference to the residual ^{1}H and ^{13}C{^{1}H} solvent peaks. All compounds were analyzed via multinuclear 2D (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, and HMBC) NMR spectroscopic experiments, allowing unambiguous assignments of the resonances.

The X-ray diffraction data of crystals of **1a−1c** were collected on a Bruker Smart APEX II diffractometer with graphite-monochromatized Mo Kα radiation (λ = 0.71073 Å) at 100 K. Data reduction was carried out using the SAINT program [51]. Semi-empirical absorption corrections were applied based on equivalent reflections using SADABS [52]. The structure solution and refinements were performed with the SHELXS-97 and SHELXL-2013 program packages [53].

Table 3. XRD data for the compound **1a**, **1b** and **1c**

Compound	**1a**	**1b**	**1c**
CCDC	1507689	1507690	1507691
Chemical Formula	$C_{23}H_{23}ClN_2O_4Ru \cdot CH_3OH$	$C_{23}H_{23}ClN_2O_4Os$	$C_{23}H_{23}IN_2O_4Ru \cdot CH_3OH$
M (g mol^{-1})	559.99	617.08	651.44
temperature (K)	100(2)	100(2)	100(2)
crystal size (mm)	0.34 × 0.10 × 0.08	0.24 × 0.10 × 0.05	0.38 × 0.12 × 0.08
crystal system	Monoclinic	Monoclinic	Monoclinic
space group	$P2_1$	$P2_1/c$	$P2_1$
a (Å)	7.9960(2)	8.7053(3)	8.4582(5)
b (Å)	16.6547(5)	16.7894(5)	16.6622(9)
c (Å)	8.8148(2)	15.5887(5)	8.7067(5)
β (º)	98.0820(10)	100.1845(15)	99.370(3)
V (A^3)	1162.22(5)	2242.49(13)	1210.68(12)
Z	2	4	2
D_c (mg m^{-3})	1.623	1.828	1.787
μ (mm^{-1})	0.828	5.838	1.959
F (000)	572	1200	644
Θ range (deg)	2.63 to 25.44	1.80 to 27.68	2.37 to 27.95
h range	-9 to 9	-10 to 10	-10 to 11
k range	-19 to 20	-21 to 20	-19 to 21
l range	-10 to 10	-20 to 17	-11 to 11
no. refls.	11819	17438	14746
no. parameters	313	285	302
R_{int}	0.0834	0.0570	0.0432
R_1 (obs.)	0.0289	0.0545	0.0324
wR_2 (all data)	0.0644	0.1126	0.0752
S	1.008	1.154	1.049

Syntheses

*2-Hydroxy-4-(picolinamido)benzoic acid **1***

A mixture of picolinic acid (0.96 g, 7.84 mmol) and thionyl chloride (1.15 mL, 15.60 mmol) was stirred in dry dichloromethane for 18 h at 40 °C under inert conditions. The solvent was removed under vaccuum, the residue was dissolved in dry DMF (10 mL) and triethyl amine (4.34 mL, 31.2 mmol) was added at 0 °C. After stirring for 5 min, 4-aminosalicylic acid (0.80 g, 5.22 mmol) was added and the reaction was stirred for 18 h at room temperature. The solvent was removed under vacuum and the residue was treated with water (200 mL) and HCl (35%; 3.3 mL), which resulted in the formation of off-white precipitates. The product was filtered, washed with water and dried.

Yield: 89% (1.20 g, off-white), MS (ESI^+): *m/z* = 281.0525 $[M + Na]^+$ (calcd. 281.0538). Anal. Found: C, 59.48; H, 3.96; N, 10.58. Calcd for $C_{13}H_{10}N_2O_4 \cdot 0.25H_2O$: C, 59.43; H, 4.03; N, 10.66. ^{1}H NMR (400.13 MHz, [d_4]MeOH, 25 °C): δ = 8.49 (d, ^3J = 8 Hz, 1H, H-6), 8.00 (d, ^3J = 8 Hz, 1H, H-3), 7.98 (t, ^3J = 8 Hz, 1H, H-4), 7.64 (d, ^3J = 9 Hz, 1H, H-13), 7.41-7.38 (m, 2H, H-5, H-10), 7.05 (dd, ^3J = 9 Hz, ^4J = 1 Hz, 1H, H-14) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, [d_4]MeOH, 25 °C): δ = 171.7 (C-15), 163.7 (C-11), 162.0 (C-7), 149.6 (C-2), 148.5 (C-6), 144.8 (C-9), 138.3 (C-4), 130.9 (C-13), 127.3 (C-5), 122.7 (C-3), 111.4 (C-10), 108.4 (C-12), 107.2 (C-14) ppm.

*2-Acetoxy-4-(picolinamido)benzoic acid **2***

2-Hydroxy-4-(picolinamido)benzoic acid (0.40 g, 1.55 mmol) was placed in a freshly prepared mixture of acetic acid (3 mL) and acetic anhydride (3 mL) in a 25 mL round bottom flask. The reaction mixture was heated to reflux for 0.5 h. After cooling down the reaction mixture was directly poured into 50 mL cold water and stirred vigorously to obtain the desired product. The precipitated product was filtered and washed with water to give rise of **2**.

Yield: 86% (0.40 g, off-white), MS (ESI^+): *m/z* = $[M + Na]^+$ Calcd. 323.0644, obs: 323.0636. Anal. Found: C, 58.25; H, 4.27; N, 8.25. Calcd for $C_{15}H_{12}N_2O_5 \cdot 0.5H_2O$: C, 58.25; H, 4.24; N, 9.06. ^{1}H NMR (400.13 MHz, [d_4]MeOD, 25 °C): δ = 8.71-8.69 (m, 1H, H-6), 8.23-8.20 (m, 1H, H-3), 8.04 (d, ^3J = 9 Hz, 1H, H-13), 7.98 (ddd, ^3J = 8 Hz, ^4J = 1 Hz, 1H, H-4), 7.81 (d, ^4J = 2 Hz, 1H, H-10), 7.73 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1H, H-14), 7.62-7.59 (m, 1H, H-5), 2.31 (s, 3H, H-17) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, [d_4]MeOH, 25 °C): δ = 171.4 (C-15), 167.2 (C-11), 164.8 (C-7), 153.2 (C-16), 150.7 (C-2), 149.7 (C-6), 144.5 (C-9), 139.1 (C-4), 133.8 (C-13), 128.3 (C-5), 123.6 (C-3), 120.1 (C-10), 118.0 (C-12), 115.7 (C-14), 21.1 (C-17) ppm.

General procedure for the synthesis of the Ru and Os complexes

A suspension of ligand (0.40 mmol) and $[M(cym)Cl_2]_2$ (0.20 mmol) in dichloromethane (20 mL) was subjected to reflux for 12 h to get an orange suspension. Then acetone was added (3 mL) to the reaction mixture and continued to reflux for a further 2 h. The solvent was evaporated completely under reduced pressure and the residue was washed with DCM and filtered to collect the orange precipitates.

Chlorido(η^6-p-cymene)[2-hydroxy-4-(picolinamido)benzoic acid]ruthenium(II) ***1a***

Complex **1a** was synthesized by following the general procedure using ligand **1** (103 mg, 0.40 mmol) and $[Ru(cym)Cl_2]_2$ (122 mg, 0.20 mmol).

Yield: 66% (141 mg). MS (ESI^+): *m/z* = $[M - Cl]^+$ Calcd. 493.0701, obs: 493.0739. Anal. Found: C, 51.89; H, 4.44; N, 5.23. Calcd for $C_{23}H_{23}ClN_2O_4Ru \cdot 0.25H_2O$: C, 51.88; H, 4.45; N, 5.26. ^{1}H NMR (400.13 MHz, [d_4]MeOH, 25 °C): δ = 9.27 (d, ^3J = 5 Hz, 1H, H-6), 8.10 (ddd, ^3J = 8 Hz, ^4J = 1 Hz, 1H, H-4), 7.96 (dd, ^3J = 8 Hz, ^4J = 1 Hz, 1H, H-3), 7.90 (d, ^3J = 9 Hz, 1H, H-13), 7.67 (ddd, ^3J = 7 Hz, ^4J = 1 Hz, 1H, H-5), 7.20-7.16 (m, 2H, H-10, H-14), 5.64 (d, ^3J = 6 Hz, 1H, H-20/H-22), 5.43 (d, ^3J = 6 Hz, 1H, H-20/H-22), 5.32 (d, ^3J = 6 Hz, 1H, H-21/H-23), 4.98 (d, ^3J = 6 Hz, 1H, H-21/H-23), 2.63-2.56 (m, 1H, H-26), 2.19 (s, 3H, H-24), 1.08 (d, ^3J = 7 Hz, 3H, H-25/H-27), 1.06 (d, ^3J = 7 Hz, 3H, H-25/H-27) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, [d_4]MeOH, 25 °C): δ = 173.2 (C-15), 168.6 (C-7), 163.5 (C-11), 159.7 (C-2) , 156.0 (C-9), 155.6 (C-6), 140.3 (C-4), 131.6 (C-13), 128.5 (C-5), 126.6 (C-3), 118.8 (C-10), 115.4 (C-12), 110.6 (C-14), 103.4 (C-21), 102.4 (C-18), 86.2 (C-20/C-22), 86.1 (C-20/C-22), 85.1 (C-19/C-23), 85.0 (C-19/C-23), 32.1 (C-26), 22.5 (C-25/C-27), 22.2 (C-25/C-27), 19.0 (C-24) ppm.

Chlorido(η^6-p-cymene)[2-hydroxy-4-(picolinamido)benzoic acid]osmium(II) ***1b***

Complex **1b** was synthesized by following the general procedure using ligand **1** (103 mg, 0.40 mmol) and $[Os(cym)Cl_2]_2$ (158 mg, 0.20 mmol).

Yield: 84% (214 mg). MS (ESI^+): *m/z* = $[M - Cl]^+$ Calcd. 583.1273, obs: 583.1270. Anal. Found: C, 43.41; H, 3.81; N, 4.42. Calcd for $C_{23}H_{23}ClN_2O_2Os \cdot H_2O$: C, 43.49; H, 3.97; N, 4.41. ^{1}H NMR (400.13 MHz, [d_4]MeOH, 25 °C): δ = 9.20 (d, ^3J = 6 Hz, 1H, H-6), 8.13 (ddd, ^3J = 8 Hz, ^4J = 6 Hz, 1H, H-4), 7.89 (dd, ^3J = 8 Hz, ^4J = 1 Hz, 1H, H-3), 7.79 (d, ^3J = 9 Hz, 1H, H-13), 7.67 (ddd, ^3J = 7 Hz, ^4J = 1 Hz, 1H, H-5), 7.12-7.10 (m, 2H, H-10, H-14), 5.96 (d, ^3J = 6 Hz, 1H, H-20/H-22), 5.68 (d, ^3J = 6 Hz, 1H, H-20/H-22), 5.58 (d, ^3J = 6 Hz, 1H, H-

21/H-23), 5.17 (d, ^{3}J = 6 Hz, 1H, H-21/H-23), 2.54-2.47 (m, 1H, H-26), 2.31 (s, 3H, H-24), 1.10-1.05 (m, 6H, H-25, H-27) ppm. $^{13}C\{^{1}H\}$ NMR (100.61 MHz, [d_4]MeOH, 25 °C): δ = 173.2 (C-15), 171.2 (C-7), 163.5 (C-11), 159.5 (C-2), 156.0 (C-6), 155.5 (C-9), 140.5 (C-4), 131.6 (C-13), 129.3 (C-5), 127.0 (C-3), 119.2 (C-10), 115.7 (C-12), 110.8 (C-14), 95.4 (C-21), 94.1 (C-18), 77.5 (C-20/C-22), 77.3 (C-20/C-22), 74.8 (C-19/C-23), 74.7 (C-19/C-23), 32.6 (C-26), 22.8 (C-25/C-27), 22.7 (C-25/C-27), 19.0 (C-24) ppm.

Iodido(η^6-p-cymene)[2-hydroxy-4-(picolinamido)benzoic acid]ruthenium(II) ***1c***

Complex **1c** was synthesized by following the general procedure using ligand **1** (77 mg, 0.30 mmol) and $[Ru(cym)I_2]_2$ (147 mg, 0.15 mmol).

Yield: 76% (141 mg). MS (ESI^+): *m/z* = $[M - I]^+$ Calcd. 493.0701, obs: 493.0712. Anal. Found: C, 43.45; H, 4.13; N, 4.32. Calcd for $C_{23}H_{24}IN_2O_4Ru$: C, 43.58; H, 4.05; N, 4.42. ^{1}H NMR (400.13 MHz, [d_4]MeOH, 25 °C): δ = 9.27 (d, ^{3}J = 5 Hz, 1H, H-6), 8.05 (td, ^{3}J = 8 Hz, ^{4}J = 1 Hz, 1H, H-4), 7.94 (dd, ^{3}J = 8 Hz, ^{4}J = 1 Hz, 1H, H-3), 7.89 (d, ^{3}J = 8 Hz, 1H, H-13), 7.61 (ddd, ^{3}J = 7 Hz, ^{4}J = 2 Hz, 1H, H-5), 7.29 (d, ^{4}J = 2 Hz, H-10), 7.25 (dd, ^{3}J = 8 Hz, ^{4}J = 2 Hz, 1H, H-14), 5.47 (d, ^{3}J = 6 Hz, 2H, H-20/H-22), 5.36 (d, ^{3}J = 6 Hz, 1H, H-21/H-23), 4.93 (d, ^{3}J = 6 Hz, 1H, H-21/H-23), 2.74-2.68 (m, 1H, H-26), 2.39 (s, 3H, H-24), 1.08 (d, ^{3}J = 7 Hz, 3H, H-25/H-27), 1.05 (d, ^{3}J = 7 Hz, 3H, H-25/H-27) ppm. $^{13}C\{^{1}H\}$ NMR (100.61 MHz, [d_4]MeOH, 25 °C): δ = 173.4 (C-15), 168.6 (C-7), 163.4 (C-11), 16.1 (C-2), 157.2 (C-6), 156.4 (C-9), 140.1 (C-4), 131.8 (C-13), 128.4 (C-5), 127.0 (C-3), 119.5 (C-10), 116.0 (C-12), 110.8 (C-14), 106.6 (C-21), 100.6 (C-18), 86.6 (C-20/C-22), 85.8 (C-20/C-22), 85.6 (C-19/C-23), 85.5 (C-19/C-23), 32.7 (C-24), 23.1 (C-26), 22.5 (C-25/27), 21.4 (C-25/27) ppm.

Chlorido(η^6-p-cymene)[2-acetoxy-4-(picolinamido)benzoic acid]ruthenium(II) ***2a***

Complex **2a** was synthesized by following the general procedure using **2** (120 mg, 0.40 mmol) and $[Ru(cym)Cl_2]_2$ (122 mg, 0.20 mmol).

Yield: 63% (141 mg). MS (ESI^+): *m/z* = $[M - Cl]^+$ Calcd. 535.0807, obs: 535.0814; $[M - Cl - CH_3CO]^+$ Calcd. 493.0701, obs: 493.0707. Anal. Found: C, 52.33; H, 4.61; N, 4.55. Calcd for $C_{25}H_{25}ClN_2O_5Ru$: C, 52.68; H, 4.42; N, 4.91. ^{1}H NMR (400.13 MHz, [d_4]MeOH, 25 °C): δ = 9.29 (dd, ^{3}J = 5 Hz, ^{4}J = 1 Hz, 1H, H-6), 8.10 (ddd, ^{3}J = 8 Hz, ^{4}J = 1 Hz, 1H, H-4), 8.07 (d, ^{3}J = 8 Hz, 1H, H-13), 7.97 (dd, ^{3}J = 8 Hz, ^{3}J = 1 Hz, 1H, H-3), 7.67 (ddd, ^{3}J = 6 Hz, ^{4}J = 1 Hz, 1H, H-5), 7.60 (dd, ^{3}J = 6 Hz, ^{4}J = 1 Hz, 1H, H-14), 7.47 (d, ^{4}J = 1 Hz, 1H, H-10), 5.67 (d, ^{3}J = 6 Hz, 1H, H-20/H-22), 5.43 (dd, ^{3}J = 6 Hz, ^{4}J = 1 Hz, 1H, H-20/H-22), 5.32 (dd, ^{3}J = 6 Hz, ^{4}J = 1 Hz, 1H, H-21/H-23), 5.04 (d, ^{3}J = 6 Hz, 1H, H-21/H-23), 2.58-2.51 (m, 1H, H-26),

2.34 (s, 3H, H-17), 2.18 (s, 3H, H-24), 1.06 (d, ^{3}J = 7 Hz, 6H, H-25, H-27) ppm. $^{13}C\{^{1}H\}$ NMR (100.61 MHz, [d_4]MeOH, 25 °C): δ = 171.9 (C-15), 168.9 (C-7), 167.5 (C-11), 158.5 (C-16), 156.0 (C-2), 155.9 (C-6), 152.6 (C-9), 14.5 (C-4), 132.9 (C-13), 128.7 (C-5), 123.7 (C-3), 125.3 (C-10), 123.2 (C-14), 120.2 (C-12), 103.5 (C-21), 103.0 (C-18), 86.5 (C-20/C-22), 86.3 (C-20/C-22), 85.1 (C-19/C-23), 84.9 (C-19/C-23), 32.2 (C-26), 22.5 (C-25/C-27), 22.2 (C-25/C-27), 21.1 (C-17), 18.9 (C-24) ppm.

Biological Studies

The antiproliferative activity of the compounds in HCT116, NCI-H460, SiHa and SW480 cells was determined using the sulforhodamine B assay as described previously [38,54].

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Highlights

- Preparation of aspirin-derived organometallic compounds
- Picolinamide-based ligands act as bidentate chelators to the metal center
- Structural characterization shows a network of hydrogen bonding and π stacking interactions
- Complexes were non-cytotoxic in human cancer cells