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Structural Modifications of the Antiinflammatory Oxicam Scaffold and Preparation of Anticancer Organometallic Compounds

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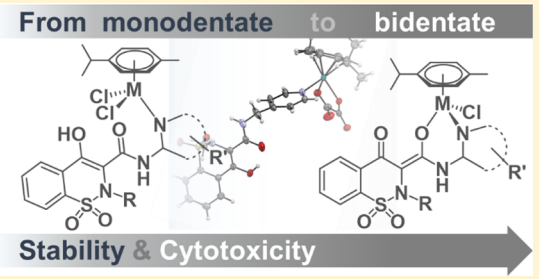
Supporting Information

ABSTRACT: Nonsteroidal antiinflammatory drugs (NSAIDs) have chemopreventive effects in several cancer types, and the oxicam-based NSAIDs meloxicam and piroxicam exhibit potential to treat cancer. We prepared a series of novel oxicams and coordinated them to Ru^{II}(cym)Cl and Os^{II}(cym)Cl moieties (η^6-*p*-cymene = cym). The oxicam ligands acted either as monodentate N-donors or bidentate N,O-chelators, depending upon the ligand structure as well as reaction conditions such as the pH value and solvent used in the reaction. The cytotoxic activity of the complexes toward carcinoma cells was investigated. The isoxazolyl motif-containing ligand **1** and its complexes with Ru^{II}(cym)Cl **1a** and the Os analogue **1b** proved to have anticancer activity with IC_{50} values in a range similar to that observed for the Ru^{III} investigational drug IT-139, and in general the Os compounds were equally or even slightly more potent than the Ru derivatives. Since meloxicam is known as a selective inhibitor of COX-2, molecular docking studies were carried out to understand the possible interactions of the compounds with COX-2, where the organic ligands gave higher scores than their organometallic counterparts.



INTRODUCTION

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely used for the treatment of a range of acute and chronic inflammatory diseases. Some NSAIDs, such as aspirin, diclofenac, naproxen, and ibuprofen, are available over the counter for short-term use as analgesic, antipyretic, and antiinflammatory agents.[1−3] Some of these NSAIDs have shown significant advantages in terms of prevention and inhibition of various cancers. Recent studies have demonstrated the tumor-preventive effects of aspirin.[4] Prolonged use of aspirin has been suggested to reduce the risk of colon, gastrointestinal, lung, ovarian, and breast cancer.[5−8] The mode of action of these NSAIDs is associated with the inhibition of the enzymes cyclooxygenase-1 (COX-1) and -2 (COX-2). The isoform COX-1 is present in most tissues and is responsible for the synthesis of prostaglandins, which is important for normal cell function. On the other hand, COX-2 is an inducible isoform and is primarily produced in response to inflammatory mediators or growth factors. Most NSAIDs nonspecifically inhibit COX-1 over COX-2, which results in adverse effects associated with the use of these drugs.[1,3,9] Moreover, these carboxylate-containing NSAIDs undergo facile elimination by glucuronide and/or sulfate conjugation. This causes short plasma half-lives and hence affects the long-term treatment of diseases such as chronic arthritis.[10]

These implications prompted research on the design of antiinflammatory drugs which are devoid of a carboxylic acid group and more selectively bind to COX-2. Lombardino et al. developed the oxicams as non-carboxylic acid-containing antiinflammatory agents,[11−13] and prominent examples of oxicams are piroxicam, meloxicam, isoxicam, lornoxicam, and sulfoxicam. Some of them are highly effective and have longer plasma half-lives. These oxicams are widely used drugs for the treatment of a variety of inflammatory and rheumatic diseases in humans.

In recent years, a variety of antiinflammatory drugs have been coordinated to metal centers to achieve synergistic effects. Such strategies often resulted in enhanced biological activity in comparison to the parent drugs. For example, the Pt[14] and Co[2] complexes of acetylsalicylic acid derivatives demonstrated promising antiproliferative properties. Dyson and co-workers recently reported organo-Ru and -Os complexes bearing modified indomethacin and diclofenac. Some of the complexes showed enhanced antiproliferative properties and better tumor selectivity in comparison to the original drugs.[15] We and others have demonstrated that antiinflammatory oxicams can act as versatile ligands.[16,17] We carried out coupling of these bioactive ligands with Ru(cym) and Os(cym) chlorido moieties to develop new bioorganometallics, and their activity against different cancer cell lines

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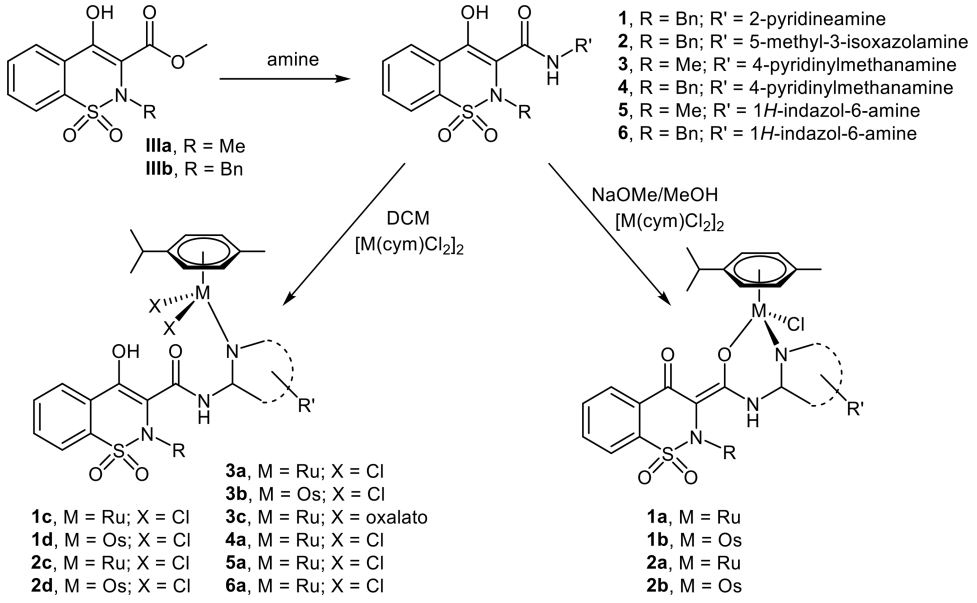
Organometallics XXXX, XXX, XXX–XXX

Scheme 1. Synthetic Route to Novel Oxicams with Different Substituents in Positions 2 and 3 and Their Organo-Ru and -Os Compounds[a]



[a]When the ligands are coordinated bidentately to Ru or Os centers, the latter are labeled **a** and **b**, while in complexes where the ligands are coordinated monodentately Ru complexes are denoted as **a** and **c** and the Os derivatives as **b** and **d**.

was established.[17] These compounds exhibited low to moderate anticancer activity, which in some cases was dependent on the lipophilicity of the oxicam ligand.

The oxicams comprise a 1,2-benzothiazine 1,1-dioxide core and varying aromatic heterocyclic substituents at the 3-carboxamide position: e.g., 2-pyridyl in piroxicam, 2-thiazolyl in meloxicam, and 3-(5-methyl)-isoxazolyl in isoxicam. The presence of these heteroaryl substituents resulted in enhanced antiinflammatory activity[18,19] and binding affinity of the drugs to the active site of the target enzyme.[13,20] Keeping this in mind, we aimed to modulate the pharmacological properties of the oxicam scaffold through the modification of its backbone as well as the amide moiety.[17] The synthesized ligands derived from the antiinflammatory drugs piroxicam and isoxicam were then used to prepare their organo-Ru and -Os complexes. The chemical and biological properties of these oxicams and their organometallic compounds were evaluated with regard to their stability and their tumor-inhibiting potency as well as potential target interaction.

RESULTS AND DISCUSSION

Analogues of the anti inflammatory drugs piroxicam, meloxicam, and isoxicam were prepared for coordination to Ru and Os. The precursor (1,1,3-trioxo-1,3-dihydro-1λ^6-benzo[*d*]isothiazol-2-yl)-acetic acid methyl ester (**I**) was prepared in excellent yield from commercially available sodium saccharin.[21] In a Gabriel–Colman-type ring expansion the five-membered isothiazole ring was converted to a six-membered thiazine ring to give methyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide (**II**).[22] The latter was subjected to methyl or benzyl alkylation to prepare compounds **IIIa**,**b**. They were treated with the heterocycle amines 2-pyridineamine, 5-methyl-3-isoxazolamine, 4-pyridinylmethanamine, and 1*H*-indazol-6-amine in refluxing xylene to give the desired ligands **1**–**6** (Scheme 1). The formation of these compounds was confirmed by NMR spectroscopy and ESI-MS. In the ^{1}H NMR spectra of **1**–**6**, the peaks assigned to the protons of the pyridyl (**1**, **3**, **4**) and indazole rings (**5** and **6**) were observed in the ranges of 8.5–7.1 and 8.5–7.3 ppm, respectively. The signals of the methyl protons in **2**, **3**, and **5** appeared in the aliphatic region at 2.48, 2.88, and 2.89 ppm, respectively. The methylene protons of the pyridyl moieties in **3** and **4** were identified at 4.52 and 4.50 ppm, while the methylene protons of the benzyl group in position 2 of the thiazine rings in **1**, **2**, **4**, and **6** were diastereotopic and were observed as two doublets in the ranges of 4.50–4.67 and 4.32–4.44 ppm, with geminal coupling constants of 14 Hz.

The ESI-MS results confirmed the formation of the oxicam derivatives with the m/z values of the pseudomolecular ion peaks corresponding to $[M + Na]^+$ for **1**, **2**, and **4**–**6** and $[M + H]^+$ for **1** and **3**.

Single crystals of **3** suitable for X-ray diffraction analysis were grown by slow evaporation of a solution in hot methanol. Crystal structure data and selected bond lengths are given in Tables S1 and S2. Intramolecular hydrogen bonds were formed between the carbonyl oxygen atoms and the hydroxyl group in position 4, with an O2H···O1 distance of 1.778 Å. Intermolecular H bonds were present between the amidate hydrogen and the oxygen atom of the sulfonyl group (NH···O4S 2.181 Å; Figure 1).

The oxicams **1**–**6** were converted to organometallic Ru and Os complexes. The coordination mode of ditopic oxicams can be controlled as monodentate or bidentate by the reaction conditions such as the pH value and solvent.[17] When **1**–**6** were reacted with $[M(cym)Cl_2]_2$ dimers in DCM without deprotonating the acidic proton of the OH in position 4 of the benzothiazine, all heterocyclic ligands coordinated exclusively monodentately through their N-donors to the Ru and Os centers and gave complexes **1c**,**d**, **2c**,**d**, **3a**–**c**, and **4a**–**6a**. Activating the OH in position[4] by deprotonation with sodium methoxide in methanol resulted in bidentate coordination to Ru and Os to yield **1a**,**b** and **2a**,**b**.

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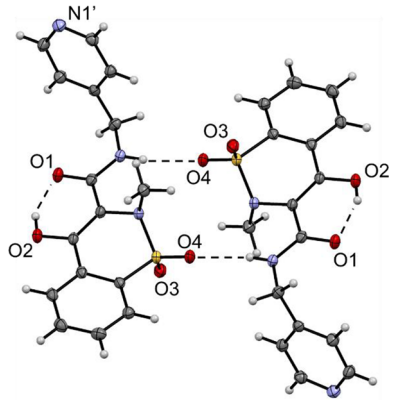

Figure 1. Molecular structure of **3** drawn at the 50% probability level. Inter- and intramolecular H bonds are indicated as dashed lines.

The compounds were characterized by different analytical methods. The infrared spectra of the complexes were compared with those of the ligands. For complexes with monodentately bound oxicams, the hydroxyl groups resulted in broad bands in the range 3100−2700 cm^{-1}. Such bands were absent in the spectra recorded for **1a**,**b** and **2a**,**b**, as the hydroxyl group was deprotonated and its oxygen atom was coordinated to the metal center. The C═N stretching frequency in **1a**,**b** and **2a**,**b** shifted from 1576 to 1569−1559 cm^{-1}, indicating the involvement of this group in coordination.

The ^{1}H NMR spectra of **1a**,**b** and **2a**,**b** did not contain signals for the OH group due to the involvement of the O atom in coordination to the metal center. However, the OH group appeared in the range of 11.8−11.2 ppm in complexes with monodentately coordinated oxicams. The NH signals were observed to shift downfield from 9.5−8.7 to 11.8−11.2 ppm in the monodentate complexes **1c**,**d** and **2c**,**d** in comparison to the respective ligands. No NH signals were found for **1a**,**b** and **2a**,**b**, possibly due to H/D exchange in $CDCl_3$. In comparison to the ligands **1** and **2**, a considerable downfield shift was observed for the H-6′ proton signal next to the pyridyl nitrogen due to the coordination of the pyridyl N atom to the metal center. The formation of the six-membered ring with the metal ion through the amidate oxygen and isoxazole nitrogen in **2a**,**b** stabilizes the molecule and also induces a shift of the H-4′ proton signal. In the osmium complexes **1b**,**d** and **2b**,**d** the cymene aromatic protons appeared in the range of 6.6−5.7 ppm while for the Ru counterparts these protons resonated slightly upfield (6.2−5.2 ppm). In complexes with an *N*-benzyl group, the methylene protons were diastereotopic and the signals split into two doublets.

In the monodentate complexes **3a**−**c** and **4a**, two signals were observed for H-2′/H-6′ (8.8−8.2 ppm) and H-3′/H-5′ (7.3−7.0 ppm) and were significantly shifted downfield in comparison to the spectra of **3** and **4**. Similarly in **5b** and **6a**, H-1′ and H-3′ were directly influenced by coordination of the metal ion and showed a considerable downfield shift in comparison to **5** and **6**. Compound **5a** showed very limited solubility in solvents other than DMSO, in which it may however undergo ligand exchange reactions, as often observed for monodentate N-donor ligands coordinated to Ru(arene) moieties.

In the $^{13}C\{^{1}H\}$ NMR spectra of the complexes, considerable shifts were observed for carbon atoms close to the N-donor atoms coordinated to the metal centers. This caused, for example, slight downfield shifts for the signals assigned to C-2′ and C-6′ in **3a**−**c** and **4a**. In the case of **1a**,**b** and **2a**,**b**, considerable downfield shifts were observed for C-4 (154−149 ppm) and C-11 (176−170 ppm) while C-2′ was less affected (169−165 ppm). These factors support the involvement of the pyridyl nitrogen and carbonyl oxygen atoms in the complex formation. The ESI-mass spectra of all complexes gave base peaks with m/z values attributable to $[M - X]^{+}$ ions. The observed m/z values were in close agreement with the theoretical values and confirm the structures proposed. The low solubility of **5a** prevented us from recording a $^{13}C\{^{1}H\}$ NMR spectrum, and in some cases the quaternary carbon atoms were not detected due to low solubility.

Single crystals of **2b**, **3c**, and **4a** were analyzed by X-ray diffraction and demonstrated pseudo-octahedral geometry at the metal center with a piano-stool configuration characteristic of these half-sandwich organometallics. In **2b**, oxicam **2** coordinated to Os as an anionic N,O-chelator, forming a six-membered ring through the amidate oxygen and endocyclic nitrogen atoms of the isoxazole moiety (Figure 2). In the molecular structures of **3c** and **4a** the oxicams were exclusively bound as neutral monodentate ligands (Figures 3 and 4), coordinated to the metal centers through their pyridyl nitrogen atoms.

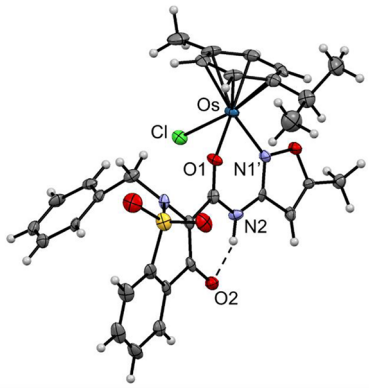

Figure 2. Molecular structure of one of the two enantiomers of **2b** drawn at the 50% probability level with the oxicam acting as a bidentate ligand and intramolecular H bonding shown between the amide NH and the carbonyl O2 atom.

The M−$cym_{centroid}$ distances were 1.653, 1.664, and 1.660 Å in **2b**, **3c**, and **4a**, respectively, and these and other bond lengths around the metal center were comparable to those reported earlier for related complexes.[23,24] The M−N bond lengths were slightly shorter at 2.052(7) and 2.105(2) Å for **2b** and **4a** with its bi- or monodentate ligands coordinated to the Os centers, respectively, in comparison to that for the Ru compound **3c** at 2.123(3) Å. The remaining coordination sites at the metal center were completed by chlorido as well as O donors of either oxicam in the case of **2b** or oxalato for **3c**. In contrast to **3c** and **4a**, in the structure of **2b** the O1−C11 and O2−C4 bond lengths show double-bond character while the connecting carbon backbone has more single-bond character, suggesting delocalization of the electron density (Supporting Information), as observed for related structures.[17]

Intramolecular hydrogen bonding was observed in the molecular structures between the carbonyl oxygen atom O2 and the amidate hydrogen with a O2···HN2 distance of 1.848 Å for **2b**. In the case of **3c** and **4a**, hydrogen bonds were observed between the amidate carbonyl O1 and O2−H at 1.774 and

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Organometallics XXXX, XXX, XXX−XXX

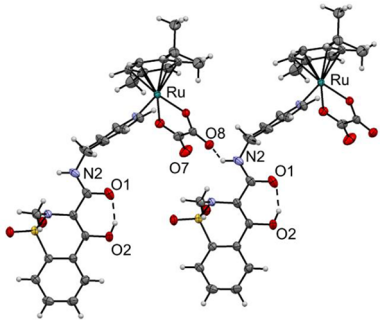

Figure 3. Molecular structure of **3c** drawn at the 50% probability level. The neutral oxicam coordinated to the metal center in monodentate fashion. The intramolecular H bonding is shown between the amide carbonyl oxygen atom O1 and the enolic OH group, while the amide proton interacts with the oxalato ligand.

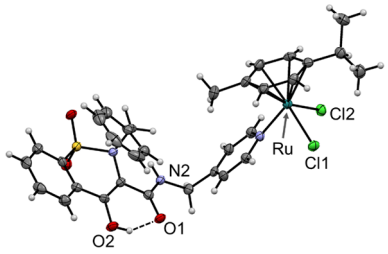

Figure 4. Molecular structure of **4a** drawn at the 50% probability level. The neutral oxicam coordinated to Ru in a monodentate fashion. The intramolecular H bonding is shown between the amide carbonyl oxygen atom O1 and the enolic O2–H group, while the amide proton N2–H interacts with the chlorido ligand Cl1 (not shown).

1.807 Å, respectively. Intermolecular hydrogen bonds were also formed by the amidate hydrogen N2–H with an oxygen atom of the oxalato coligand (**3c**) or the chlorido leaving group (**4a**) with distances of 2.161 and 2.382 Å, respectively. Similar observations were made for related complexes.[17]

The heterocyclic thiazine ring was found to be nonplanar with regard to the benzene ring. The nitrogen atom of thiazine was significantly out of plane, and the torsion angles (C10–C9–SO_2–N1) were 36.64, 37.35, 40.05, and 34.96° for **3**, **2b**, **3c** and **4a**, respectively. These torsion angles are in a range similar to that observed for other oxicam complexes.[25,26] In **2b** and **4a**, the benzyl substituent at the ring nitrogen formed π-stacking interactions with the oxicam phenyl ring and the shortest C···C distances were observed at 3.443 and 3.353 Å, respectively.

Stability in DMSO and Aqueous Solution. Due to limited solubility in aqueous solution, compounds tested in biological experiments are usually first dissolved in DMSO and then diluted with cell culture media to reach final concentrations of ≤1% DMSO. However, DMSO can act as a ligand and, therefore, the stability of the complexes **1a–d**, **2a–d**, and **3a–c** was evaluated in DMSO-d_6 by ^{1}H NMR spectroscopy over a period of 96 h. The ^{1}H NMR spectra of compounds **1c,d**, **2c,d**, and **3a,c** revealed an immediate exchange of the monodentately coordinated oxicam ligands with DMSO, as observed for related complexes with monodentate nitrogen donor ligands (Figure S1).[23,24,27] However, **1a,b** and **2a,b** bearing the oxicams as anionic N,O-chelators were stable in DMSO for 24 h. After 24 h, coordination of DMSO was observed along with cleavage of the cym ligand.[17,25,28–30] This effect was significantly suppressed in DMSO-d_6/D_2O (5/95), where predominantly chlorido/aqua ligand exchange reactions occurred during the 72 h incubation period. These findings indicate that these reactions would not influence the outcome of biological experiments. Furthermore, replacing the chlorido leaving groups with oxalato as in **3c** resulted in high stability of the metal complex in comparison to the chlorido analogues (for a comparison of the ^{1}H NMR spectra for **3a** and **3c** in DMSO after 2 and 24 h, respectively, see Figure S1). The replacement of labile ligands with stronger, bidentate ligands is another strategy to overcome the degradation of organometallic compounds in aqueous environments.[25]

Anticancer Activity. There is emerging evidence that NSAIDs such as aspirin and meloxicam can play a role in the prevention and inhibition of tumors.[4–8] The coordination of NSAIDs to bioactive metal ions has resulted in promising anticancer effects.[2,14] Therefore, the antiproliferative potential of the novel oxicams and their organometallic compounds was tested in human colorectal carcinoma (HCT116), non-small-cell lung carcinoma (NCI-H460), cervical carcinoma (SiHa), and human colon adenocarcinoma (SW480) cell lines using the sulforhodamine B (SRB) assay (Table 1). The new

Table 1. In Vitro Anticancer Activity (Mean IC_{50} Values ± Standard Deviations) of New Oxicam Derivatives 1–3 and Their Organometallic Compounds in Comparison to Investigational Ru Drug Candidates in Human Colorectal (HCT116), Non-Small-Cell Lung (NCI-H460), Colon (SW480),and Cervical (SiHa) Carcinoma Cell Lines (Exposure Time 72 h)

	IC_{50} (μM)			
compound	NCI-H460	SiHA	HCT116	SW480
1	85 ± 5	93 ± 6	90 ± 2	124 ± 2
2	105 ± 11	155 ± 7	142 ± 7	53 ± 1
3	>300	>300	>300	>300
1a	79 ± 19	67 ± 7	70 ± 4	81 ± 4
1b	61 ± 6	69 ± 9	64 ± 5	63 ± 3
1c	>150	>150	>150	>150
1d	>150	>150	>150	>150
2a	102 ± 6	101 ± 2	89 ± 5	97 ± 5
2b	93 ± 14	93 ± 9	98 ± 22	92 ± 7
2c	>200	>200	>200	>200
2d	>200	>200	>200	>200
3a	>225	>225	>225	>225
3b	>225	>225	>225	>225
3c	>270	>270	>270	>270
$[Ru(cym)Cl_2]_2$	441 ± 46[a]	394 ± 70[a]	433 ± 28[a]	346 ± 48
RAPTA-C	>300	>300	>300	>300
NAMI-A	>300	>300	>300	>300
NKP-1339 (IT-139)	92 ± 27	102 ± 7	100 ± 7	84 ± 15

[a]Taken from ref 26.

oxicams **1** and **2**, which are analogous to low-cytotoxicity piroxicam[31] and its derivative isoxicam, respectively, were moderately active against all these cell lines. The IC_{50} values of **1** were in a similar range in all of the cell lines, while **2** was more, though still moderately, potent against SW480 cells. The organo-Ru and -Os compounds **1a,b** were equally or slightly more cytotoxic in comparison to their ligand **1** and **2a,b**. A comparison of the effect of the metal ion reveals that the Os complexes (**1b** and **2b**) were equally or slightly more active in comparison to their Ru counterparts (**1a** and **2a**). All of the complexes in which the oxicams were featured as monodentate ligands, i.e., **1c,d**, **2c,d**, and **3a–c**, were not active. For at least

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Organometallics XXXX, XXX, XXX–XXX

the compounds with the ligand coordinated monodentately, this is likely a result of the low stability due to fast ligand exchange in aqueous DMSO solution. Because of low solubility, oxicams **4**−**6** and their respective organo-Ru complexes **4a**−**6a** were not tested.

Overall, the *N*-benzyl-substituted oxicam **1** analogous to piroxicam and its metal complexes **1a**,**b** showed moderate cytotoxicity which is in a range similar to that observed for other investigational Ru drug candidates such as the ruthenium(III) complexes NAMI-A and NKP-1339 (IT-139). Low cytotoxic activity is often observed for organo-Ru^{II} compounds. An excellent example is RAPTA-C, which is not cytotoxic against primary tumors but displayed remarkable antimetastatic as well as antiangiogenic properties in vivo.[32,33] Similarly, NSAIDs have low toxicity and therefore it is not surprising that especially the complexes that are unstable under the conditions used would result in low antiproliferative activity.

Molecular Modeling. To explain the findings from the cytotoxicity assays in cancer cell lines and to estimate the likelihood of binding of active ligands **1**−**3** and their Ru/Os complexes **1a**,**b**, **2a**,**b**, and **3a** to COX-2 as the target for oxicams, docking studies were conducted with a crystal structure of COX-2 with cocrystallized meloxicam.[20,34] Meloxicam was docked into the binding pocket as a positive control, and a very good fit with the crystallized structure was found. The predicted scores for oxicams **1**−**3** were similar to or higher than those of the known drug meloxicam, suggesting possible binding to COX-2 (Table S3). All of the structures were found inserted deep into the lipophilic pocket and showed overlap with the configuration of meloxicam, with the **3** derivative, however, slightly shifted away from the binding site in comparison to the oxicam scaffold of the other ligands. The best predicted binding modes and hydrogen bond interactions of **1**−**3** (Figure S2) closely resembled that of cocrystallized meloxicam.[20]

As Gold Score (GS) is the only scoring function able to treat metal complexes,[25] it was used in the docking of the Ru and Os compounds. For this purpose, the GS parameter file was modified to include the parameters of Ru and Os which are not included in the GOLD database.[35] The docked configuration of the active derivative **1a** into the binding site is shown in Figure 5. Due to steric constraints, **1a** did not fit into the binding pocket of COX-2 but occupied a cleft exposed to the water environment (Figure 5A). Compound **1a** was found to be in lipophilic contact with Lys83, Pro86, and Val89 through its isopropyl group and benzyl moieties, respectively (Figure 5B). When **1b** and **2a**,**b** were docked, similar binding poses and interactions were observed. However, complex **3a** extends significantly in one dimension and therefore was able to reach deep into the binding pocket (Figure 5C). Notably, all of the metal complexes gave lower GS scores in comparison to their ligands (Table S3). As the cytotoxic activity of **1a** is higher than that of the corresponding ligand **1**, there may be other factors that account for the antiproliferative potency.

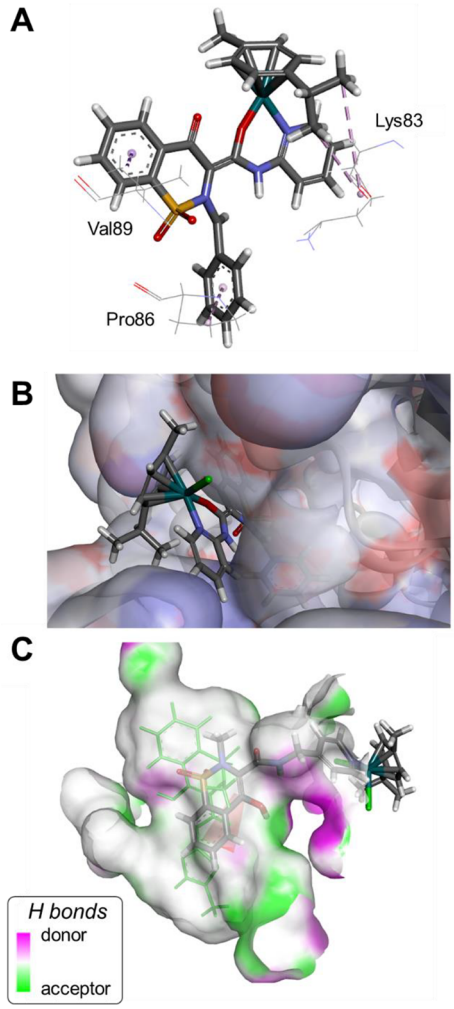

Figure 5. Docked configurations of **1a** and **3a** in the binding site of COX-2 (PDB ID: 4M11). (A) Lipophilic contacts are depicted as purple dotted lines between **1a** and the amino acids Lys83, Pro86, and Val89. (B) Compound **1a** is shown in the binding pocket with the protein surface rendered. It occupies a cleft exposed to the water environment. Blue depicts hydrophilic areas on the surface, while hydrophobic areas are shown in brown/white. (C) Complex **3a** in the binding pocket with the protein surface rendered overlaid with the cocrystallized meloxicam in green.

CONCLUSIONS

The coordination of antiinflammatory drugs to bioactive metal ions has given rise to compounds with tumor-inhibiting properties, thus opening the possibility to design multitargeted anticancer drugs. Inspired by these studies, we prepared novel anti inflammatory oxicams which were utilized to obtain organo-Ru and -Os complexes. The oxicams **1** and **2** exhibited either monodentate or bidentate coordination modes depending on the reaction conditions, such as pH or nature of solvent, while **3**−**6** coordinated as monodentate ligands to the metal centers. Organo-Ru and -Os complexes **1c**,**d**, **2c**,**d**, and **3a**−**6a** bearing oxicams coordinated in a monodentate fashion turned out to be less stable and immediately underwent ligand exchange reactions with DMSO. In contrast, the organometallics **1a**,**b** and **2a**,**b** with the oxicam ligands bound as anionic bidentate chelators were stable in DMSO for at least 24 h and for an even longer time in aqueous solutions.

Oxicams **1** and **2** were moderately cytotoxic against cancer cells. Interestingly, **2** was most potent against human colon adenocarcinoma SW480 cells. Coordination of **1** to Ru(cym) and Os(cym) led to the most cytotoxic organometallics of this series with IC_{50} values in the range of 61−81 μM for **1a**,**b** in different cancer cells. The Os complexes showed equal or slightly better cytotoxicity profiles in comparison to their Ru counterparts.

Since oxicams are known inhibitors of COX-2, the interactions of selected oxicams and their organo-Ru and -Os

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Organometallics XXXX, XXX, XXX−XXX

complexes were studied by molecular modeling. Oxicams **1**−**3** demonstrated binding interactions similar to those observed for the known drug meloxicam, while organometallic compounds gave lower scores than the oxicams alone. As the binding pocket of COX-2 lies deep in the enzyme and is also size-restricted, this may limit the access of the bulky organo-Ru and -Os compounds and other modes of action than COX-2 inhibition may play a role in inducing anticancer activity.

Overall, the mode of coordination and the nature of the metal ion and ligand have clear effects on the cytotoxicity of the compounds and their interactions with COX-2. The organo-Os complexes showed anticancer activity which is similar to or better than that of the investigational drug NKP-1339 (IT-139), which is in clinical trials.

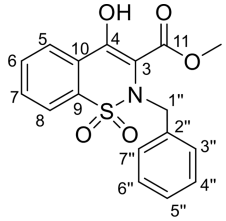
EXPERIMENTAL SECTION

Materials and Methods. All the reactions were carried out under inert conditions. All of the reagents and chemicals were of analytical grade and were used as received from commercial suppliers. Chloroacetic acid, sodium metal, and benzyl chloride were purchased from Sharlu, and 2-aminopyridine, 5-amino-3-methylisoxazole, 4-pyridinylmethanamine, 1*H*-indazol-6-amine, and OsO_4 were purchased from Sigma-Aldrich. $RuCl_3{\cdot}xH_2O$ was purchased from Precious Metals Online. (1,1,3-Trioxo-1,3-dihydro-1λ^6-benzo[*d*]-isothiazol-2-yl)acetic acid methyl ester (**I**),[21] methyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide (**II**),[22] methyl 4-hydroxy-2-methyl-2*H*-benzo[*e*][1,2]thiazine-3-carboxylate 1,1-dioxide (**IIIa**),[36] bis[dichlorido(cym)ruthenium(II)],[37] and bis[dichlorido-(cym)osmium(II)][38] were synthesized according to literature procedures.

High-resolution mass spectra were recorded on a 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} NMR chemical shifts are reported vs $SiMe_4$ and were determined by reference to the residual 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. Elemental analyses were carried out on an Exeter Analytical Inc. CE-440 Elemental Analyzer.

The X-ray diffraction data of crystals of **3**, **2b**, **3c**, and **4a** were collected on a Bruker Smart APEX II diffractometer with graphite-monochromated Mo Kα radiation (λ_{Mo} = 0.71073 Å) at 100 K (see Table S1 for the measurement parameters). Data reduction was carried out using the SAINT program.[39] Semiempirical absorption corrections were applied on the basis of equivalent reflections using SADABS.[40] The structure solution and refinements were performed with the SHELXS-97 and SHELXL-2013 program packages.[41]

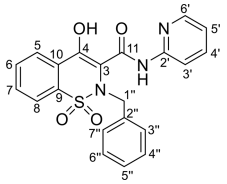
Synthesis of Compound IIIb.



Benzyl chloride (0.45 mL, 3.92 mmol) was added to a solution of methyl 4-hydroxy-2*H*-benzo[*e*][1,2]thiazine-3-carboxylate 1,1-dioxide (**II**; 1 g, 3.92 mmol) and sodium hydroxide (0.47 g, 11.75 mmol) in methanol (35 mL).[21] The reaction mixture was allowed to react at room temperature for 12 h under a nitrogen atmosphere. After completion of the reaction, the reaction mixture was acidified with 10% HCl. This yielded **IIIb** as a white precipitate, which was filtered and dried at room temperature. Yield: 59% (0.81 g, white), FTIR (KBr, cm^{-1}): 2958 (CH), 1645 (C═O), 1440 (CH def, 1348, 1174 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 11.77 (s, 1H, OH), 7.79−7.72 (m, 2H, H-8, H-5), 7.69−7.64 (m, 2H, H-6, H-7), 7.06−7.02 (m, 1H, H-5″), 6.99−6.96 (m, 2H, H-4′′, H-6′′), 6.91−6.89 (m, 2H, H-3′′, H-7′′), 4.61 (s, 2H, H-1′′), 3.93 (s, 3H, H-Me) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 168.5 (C-11), 158.7 (C-4), 137.3 (C-2″), 132.9 (C-6), 132.7 (C-7), 132.5 (C-9), 129.2 (C-4′′, C-6′′), 127.9 (C-10), 127.6 (C-3′′, C-7′′), 127.4 (C-5′′), 126.0 (C-5), 122.5 (C-8), 106.7 (C-3), 54.3 (C-1′′), 53.0 (C-Me) ppm.

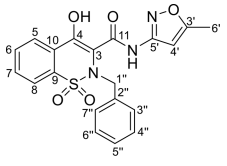
General Procedure for the Synthesis of Oxicam Derivatives 1−6. A suspension of **IIIa** or **IIIb** (1 equiv) and a heterocyclic amine (1.05 equiv) was subjected to reflux in xylene (150 mL) for 24−72 h in the presence of molecular sieves (4 Å). After completion of the reaction, xylene was removed, which yielded a solid product. This was washed with *n*-hexane and recrystallized in methanol to isolate the oxicam-based ligands **1**−**6** in a manner similar to that reported for related compounds.[19]

*2-Benzyl-N-(pyridin-2-yl)-4-hydroxy-2H-benzo[e][1,2]thiazine-3-carboxamide 1,1-Dioxide (**1**).*



Compound **1** was synthesized by following the general procedure using **IIIb** (3.00 g, 8.7 mmol) and 2-aminopyridine (0.82 g, 8.7 mmol). Yield: 56% (2.00 g, off-white). Mp: 157−158 °C. MS (ESI$^+$): *m*/*z* 430.0826 [M + Na]$^+$, calcd 430.0832; *m*/*z* 408.1006 [M + H]$^+$, calcd 408.1018; calcd 406.0867. Anal. Found: C, 61.85; H, 4.38; N, 10.22; S, 7.99. Calcd for $C_{21}H_{17}N_3O_4S$: C, 61.90; H, 4.21; N, 10.31; S, 7.87. FT-IR (KBr, cm^{-1}): $\tilde{\nu}$ 3381, 3309 (NH), 3050−2700 (OH), 1622 (C═O), 1576 (C═N), 1438 (CH def, 1346, 1160 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.32 (s, 1H, OH), 8.70 (s, 1 H, NH), 8.34 (dd, $^3J_{(H6',H5')}$ = 5 Hz, $^4J_{(H6',H4')}$ = 1 Hz, H-6′), 8.16 (d, $^3J_{(H3',H4)}$ = 7 Hz, 1H, H-3′), 7.77−7.71 (m, 2H, H-8, H-4′), 7.67 (dd, $^3J_{(H5,H6)}$ = 8 Hz, $^4J_{(H5,H7)}$ = 1 Hz, 1H, H-5), 7.57 (td, $^3J_{(H6,H7)/(H6,H5)}$ = 8 Hz, $^4J_{(H6,H8)}$ = 1 Hz, 1H, H-6), 7.52 (td, $^3J_{(H7,H6)/(H7,H8)}$ = 8 Hz, $^4J_{(H7,H5)}$ = 1 Hz,1H, H-7), 7.11−7.09 (m, 1H, H-5′), 7.06−7.04 (m, 2H, H-4″, H-6′′), 6.99−6.95 (m, 3H, H-3′′, H-5′′, H-7′′), 4.64 (d, $^2J_{(H1''A,H1''B)}$ = 14 Hz, 1H, H-1″A), 4.44 (d, $^2J_{(H1''B,H1''B)}$ = 14 Hz, 1H, H-1″B) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 166.9 (C-11), 159.2 (C-4), 150.2 (C-2′), 140.0 (C-6′), 137.6 (C-2″), 134.8 (C-7), 134.5 (C-4′), 133.6 (C-6), 132.3 (C-9), 131.9 (C-10), 129.7 (C-4′′, C-6′′), 127.8 (C-5′′), 127.4 (C-3′′, C-7′′), 126.1 (C-5), 122.4 (C-8), 119.8 (C-5′), 116.4 (C-3′), 113.8 (C-3), 54.8 (C-1′′) ppm.

*2-Benzyl-N-(5-methylisoxazol-3-yl)-4-hydroxy-2H-benzo[e][1,2]-thiazine-3-carboxamide 1,1-Dioxide (**2**).*

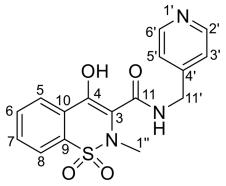


Compound **2** was synthesized by following the general procedure using **IIIb** (3.00 g, 8.67 mmol) and 5-methylisoxazol-3-amine

DOI: 10.1021/acs.organomet.8b00751
Organometallics XXXX, XXX, XXX−XXX

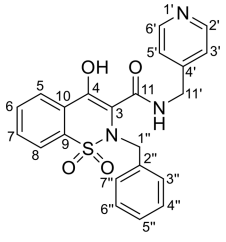
(0.85 g, 8.67 mmol). Yield: 74% (2.66 g, off-white). Mp: 193−195 °C. MS (ESI$^+$): m/z 434.0774 [M + Na]$^+$, calcd 434.0781. Anal. Found: C, 58.65; H, 4.22; N, 10.03; S, 7.99. Calcd for $C_{20}H_{17}N_3O_5S$: C, 58.38; H, 4.16; N, 10.21; S, 7.79. FT-IR (KBr, cm^{-1}): $\tilde{\nu}$ 3153 (NH), 3063−2850 (OH), 1642 (C═O), 1606 (C═N), 1541 (NH def), 1424 (CH def), 1341, 1174 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.18 (s, 1H, OH), 9.25 (s, 1H, H-NH), 7.77 (dd, $^3J_{(H8,H7)}$ = 8 Hz, $^4J_{(H8,H5)}$ = 1 Hz, 1H, H-8), 7.67 (dd, $^3J_{(H5,H6)}$ = 8 Hz, $^4J_{(H5,H7)}$ = 1 Hz,1H, H-5), 7.58 (td, $^3J_{(H7,H8)/(H7,H6)}$ = 8 Hz, $^4J_{(H7,H5)}$ = 1 Hz, H-7), 7.52 (td, $^3J_{(H6,H7)/(H6,H5)}$ = 8 Hz, $^4J_{(H6,H8)}$ = 1 Hz, H-6), 7.04−7.00 (m, 5H, H-3″, H-4′′, H-5′′, H-6′′, H-7′′), 6.68 (d, $^4J_{(H4',H6')}$ = 1 Hz, 1H, H-4′), 4.60 (d, $^2J_{(H1''A,H1''B)}$ = 14 Hz, 1H, H-1″A), 4.42 (d, $^2J_{(H1''A,H1''B)}$ = 14 Hz, 1H, H-1″B), 2.48 (s, 3H, H-6′) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 170.5 (C-3′), 167.1 (C-11), 161.1 (C-4), 157.0 (C-5′), 137.5 (C-2″), 132.5 (C-6), 132.3 (C-7), 131.1 (C-9), 130.3 (C-4′′, C-6′′), 128.7 (C-5′′), 128.4 (C-10), 128.1 (C-3′′, C-7′′), 126.5 (C-5), 123.8 (C-8), 107.8 (C-3), 96.5 (C-4′), 57.2 (C-1′′), 12.8 (C-6′) ppm.

*2-Methyl-4-hydroxy-1,1-dioxo-1,2-dihydro-1λ^6-benzo[e][1,2]-thiazine-3-carboxylic Acid (Pyridin-4-ylmethyl)amide (**3**).*



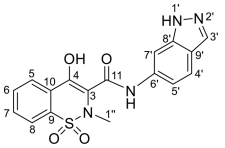
Compound **3** was synthesized by following the general procedure using **IIIa** (0.60 g, 3.72 mmol) and 4-pyridylmethylamine (0.60 g, 5.58 mmol). Yield: 58% (0.70 g, off-white). Mp: 196−197 °C. MS (ESI$^+$): m/z 346.0850 [M + H]$^+$, calcd 346.0862. Anal. Found: C, 55.89; H, 4.48; N, 12.17; S, 9.13. Calcd for $C_{16}H_{15}N_3O_4S$: C, 55.64; H, 4.38; N, 12.17; S, 9.28. ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.39 (s, 1H, OH), 8.58−8.57 (m, 2H, H-2′, H-6′), 8.05−8.03 (dd, $^3J_{(H8,H7)}$ = 8 Hz, $^4J_{(H8,H5)}$ = 1 Hz 1H, H-8), 7.85−7.83 (dd, $^3J_{(H5,H6)}$ = 7 Hz, $^4J_{(H5,H7)}$ = 1 Hz 1H, H-5), 7.78−7.74 (td, $^3J_{(H6,H5)/(H6,H7)}$ = 8 Hz, $^4J_{(H6,H8)}$ = 1 Hz, 1H, H-6), 7.73−7.69 (td, $^3J_{(H7,H8)/(H7,H6)}$ = 8 Hz, $^4J_{(H7,H5)}$ = 1 Hz, 1H, H-7), 7.39 (t, $^3J_{(HNH,H11')}$ = 6 Hz, 1H, H-NH), 7.23 (d, $^3J_{(H7,H8)/(H7,H6)}$ = 5 Hz, 2H, H-3′, H-5′), 4.52 (d, 2H, H-11′), 2.88 (s, 3H, H-1″) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 169.0 (C-11), 157.8 (C-4), 150.6 (C-2′, C-6′), 146.8 (C-4′), 133.5 (C-7), 132.6 (C-6), 128.8 (C-9), 128.8 (C-10), 127.0 (C-5), 125.0 (C-8), 122.5 (C-3′, C-5′), 111.5 (C-3), 42.4 (C-1″), 40.1 (C-11′) ppm.

*2-Benzyl-4-hydroxy-N-(pyridin-4-ylmethyl)-2H-benzo[e][1,2]-thiazine-3-carboxamide 1,1-Dioxide (**4**).*



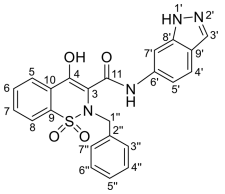
Compound **4** was synthesized by following the general procedure using **IIIb** (3.00 g, 8.7 mmol) and 4-aminomethylpyridine (0.94 g, 8.7 mmol). Yield: 27% (1.00 g, off-white). MS (ESI$^+$): m/z 444.0978 [M + Na]$^+$, calcd 444.0988. Anal. Found: C, 52.63; H, 3.75; N, 8.20; S, 6.01. Calcd for $C_{22}H_{19}N_3O_4S{\cdot}1.25CH_2Cl_2$: C, 52.92; H, 4.11; N, 7.96; S, 6.08. FTIR (KBr, cm^{-1}): 3450, 3330 (NH), 3100−2850 (OH), 1606 (C═O), 1596 (C═N), 1416 (CH def), 1341, 1171 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 14.13 (s, 1H, OH), 9.45 (t, $^3J_{(HNH,H11')}$ = 6 Hz, 1H, H-NH), 8.56−8.55 (m, 2H, H-2′, H-6′), 7.73−7.70 (dd, $^3J_{(H8,H7)}$ = 8 Hz, $^3J_{(H8,H6)}$ = 1 Hz, 1H, H-8), 7.68−7.64 (td, $^3J_{(H6,H7)/(H6,H5)}$ = 8 Hz, $^3J_{(H6,H8)}$ = 1 Hz, 1H, H-6), 7.60−7.56 (td, $^3J_{(H7,H8)/(H7,H5)}$ = 8 Hz, $^3J_{(H7,H5)}$ = 1 Hz, 1H, H-7), 7.49−7.47 (dd, $^3J_{(H5,H6)}$ = 8 Hz, $^3J_{(H5,H7)}$ = 1 Hz, 1H, H-5), 7.34−7.32 (m, 2H, H-5′, H-3′), 7.03−6.99 (m, 1H, H-5″), 6.94−6.90 (m, 2H, H-4′′, H-6′′), 6.82−6.80 (m, 2H, H-3′′, H-7′′), 4.55 (s, 2H, H-1′′), 4.50 (m, 2H, H-11′) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 169.1 (C-11), 159.3 (C-4), 150.29 (C-2′, C-6′), 146.3 (C-4′), 136.8 (C-2″), 132.6 (C-7), 132.0 (C-6), 132.2 (C-9), 130 (C-4′′, C-6′′), 128.8 (C-10), 128.7 (C-5′′), 128.3 (C-3′′, C-7′′), 126.5 (C-5), 123.8 (C-8), 122.4 (C-3′, C-5′), 108.4 (C-3), 57.3 (C-1′′), 42.2 (C-11′) ppm.

*4-Hydroxy-N-(1H-indazol-6-yl)-2-methyl-2H-benzo[e][1,2]-thiazine-3-carboxamide 1,1-Dioxide (**5**).*



Compound **5** was synthesized by following the general procedure using **IIIa** (3.00 g, 11.14 mmol) and 6-aminoindazole (1.49 g, 11.14 mmol). Yield: 65% (2.10 g, off-white). MS (ESI$^+$): m/z 393.0628 [M + Na]$^+$, calcd 393.0633. Anal. Found: C, 55.42; H, 3.72; N, 15.21; S, 8.45. Calcd for $C_{17}H_{14}N_4O_4S$: 55.13; H, 3.81; N, 15.13; S, 8.66. FTIR (KBr, cm^{-1}): 3399, 3268 (NH), 3100−2850 (OH), 1607 (C═O), 1594 (C═N), 1468 (CH def), 1335, 1173 (SO_2). ^{1}H NMR (400.13 MHz, DMSO-d_6, 25 °C): δ 14.08 (s, 1H, OH), 13.07 (s, 1H, H1′), 10.39 (s, 1H, NH), 8.09 (s, 1H, H-3′), 8.06−8.04 (m, 2H, H-4′, H-7′), 7.93−7.86 (m, 3H, H-8, H-6, H-7), 7.76−7.74 (d, $^3J_{(H5,H6)}$ = 9 Hz,1H, H-5), 7.51−7.48 (dd, $^3J_{(H5',H4')}$ = 9 Hz, $^4J_{(H5',H7')}$ = 2 Hz, 1H, H-6′), 2.89 (s, 3H, H-1″) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, DMSO-d_6, 25 °C): δ 167.3 (C-11), 157.3 (C-4), 140.0 (C-8′), 135.2 (C-5′), 134.3 (C-9), 133.6 (C-3′), 133.5 (C-7), 133.1 (C-6), 127.9 (C-10), 126.3 (C-5), 124.2 (C-8), 120.4 (C-4′), 120.2 (C-9′), 116.4 (C-5′), 111.7 (C-3), 102.6 (C-7′), 39.5 (C-1″) ppm.

*2-Benzyl-4-hydroxy-N-(1H-indazol-6-yl)-2H-benzo[e][1,2]-thiazine-3-carboxamide 1,1-Dioxide (**6**).*



Compound **6** was synthesized by following the general procedure using **IIIb** (3.00 g, 8.7 mmol) and 6-aminoindazole (1.16 g, 8.7 mmol). Yield: 77% (2.80 g, off-white). MS (ESI$^+$): m/z 469.0938 [M + Na]$^+$, calcd 469.0941. Anal. Found: C, 61.57; H, 4.22; N, 12.23; S, 6.87. Calcd for $C_{23}H_{18}N_4O_4S$: C, 61.87; H, 4.06; N, 12.55; S, 7.18. FTIR (KBr, cm^{-1}): 3350 (NH), 3050−2800 (OH), 1636 (C═O), 1592 (C═N), 1455 (CH def), 1334, 1168 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.51 (s, 1H, OH), 10.20 (s, 1H, H-1′), 8.11 (s, 1H, H-3′), 8.02−8.0 (m, 2H, NH, H-4′), 7.8.6−7.82 (m, 2H, H-8,

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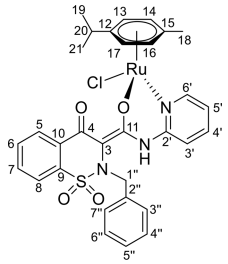
Organometallics XXXX, XXX, XXX−XXX

H-5), 7.66−7.61 (m, 3H, H-7′, H-6, H-7), 7.22−7.20 (m, 2H, H-4″, H-6′′), 7.15−7.12 (m, 3H, H-3′′, H-5′′, H-7′′), 6.68−6.66 (dd, $^{3}J_{(H5',H4')}$ = 9 Hz, $^{4}J_{(H5',H7')}$ = 2, 1H, H-5′), 4.67 (d, $^{2}J_{(H12a,H12b)}$ = 14 Hz, 1H, H-1″), 4.32 (d, $^{2}J_{(H1''A,H1''B)}$ = 14 Hz, 1H, H-1″) ppm. $^{13}C\{^{1}H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ = 167.2 (C-11), 159.7 (C-4), 140.5 (C-8′), 136.7 (C-13), 135.3 (C-6′), 135.1 (C-3′), 132.9 (C-7), 132.7 (C-9), 132.2 (C-6), 130 (C-4″, C-6′′), 128.9 (C-5′′), 128.8 (C-10), 128.7 (C-3′′, C-7′′), 126.7 (C-5), 124.1 (C-8), 121.4 (C-4′), 120.7 (C-9′), 115.3 (C-5′), 109.5 (C-3), 100.6 (C-7′), 57.98 (C-1′′) ppm.

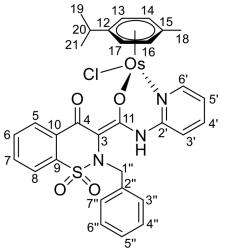
General Procedure for the Synthesis of Complexes 1a,b and 2a,b. Sodium methoxide was added to a suspension of ligand **1** or **2** in methanol (30 mL) under inert conditions to give a light yellow solution. After 1/2 h of stirring at room temperature, $[M(cym)Cl_2]_2$ was added and the reaction mixture was stirred for a further 4 h. After completion of the reaction, the solvent was evaporated. The reaction mixture was dissolved in DCM and the solution filtered to remove excess inorganic salts. The filtrate was concentrated to obtain a precipitate, which was collected by filtration and washed with diethyl ether to isolate the product.[25]

*Chlorido(2-benzyl-4-oxido-N-(pyridin-2-yl)-2H-benzo[e][1,2]-thiazine-3-carboxamide 1,1-dioxide)(cym)ruthenium(II) (**1a**).*



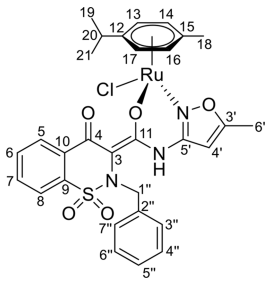
Complex **1a** was synthesized by following the general procedure using **1** (122 mg, 0.30 mmol), sodium methoxide (19 mg, 0.36 mmol), and $[Ru(cym)Cl_2]_2$ (92 mg, 0.15 mmol). Yield: 75% (150 mg, orange crystals), MS (ESI^+): m/z 700.0626 $[M + Na]^+$, calcd 700.0587; m/z 642.1017 $[M - Cl]^+$, calcd 642.1001. Anal. Found: C, 54.93; H, 4.81; N, 5.86; S, 4.38. Calcd for $C_{31}H_{30}ClN_3O_4RuS$: C, 54.98; H, 4.47; N, 6.21; S, 4.74. FT-IR (KBr, cm^{-1}): $\tilde{\nu}$ 3150 (NH), 1611 (C═O), 1569 (C═N), 1503 (NH def), 1466 (CH def), 1328, 1170 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 8.55 (dd, $^{3}J_{(H6',H5')}$ = 6 Hz, $^{4}J_{(H6',H4')}$ =1 Hz, 1H, H-6′), 7.74 (td, $^{3}J_{(H4',H5')/(H4',H3')}$ = 9 Hz, $^{4}J_{(H4',H6')}$ = 2 Hz, 1H, H-4′), 7.65−7.61 (m, 2H, H-8, H-5), 7.41 (dtd, $^{3}J_{(H6,H7)/(H-6,H5)}$ = $^{3}J_{(H7,H8)/(H7,H6)}$ = 8 Hz, $^{3}J_{(H6,H8)/(H7,H6)}$ = 8 Hz, H-6, H-7), 7.09−7.01 (m, 2H, H-3′, H-5′), 6.95−6.89 (m, 3H, H-4″, H-5′′, H-6′′), 6.86−6.82 (m, 2H, H-3′′, H-7′′), 5.78−5.74 (m, 2H, H-13, H-17), 5.42 (d, $^{3}J_{(H14,H13)/(H16,H17)}$ = 6 Hz, 1H, H-14/H-16), 5.22 (d, $^{3}J_{(H14,H13)/(H-16,H17)}$ = 6 Hz, 1H, H-14/H-16), 4.91 (d, $^{2}J_{(H1''A,H1''B)}$ = 14 Hz, 1H, H-1″), 4.41 (d, $^{2}J_{(H1''A,H1''B)}$ = 14 Hz, 1H, H-1″), 3.11−3.08 (m, 1H, H-20), 2.05 (s, 3H, H-18), 1.40 (d, $^{3}J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 3H, H-19/H-21), 1.36 (d, $^{3}J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 3H, H-19/H-21) ppm. $^{13}C\{^{1}H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 175.7 (C-11), 168.7 (C-2′), 153.78 (C-6′), 151.0 (C-4), 140.0 (C-4′), 139.7 (C-2″), 134.5 (C-9), 133.8 (C-10), 131.8 (C-6), 130.9 (C-7), 130.34 (C-3′′, C-7′′), 127.6 (C-4′′, C-6′′), 127.2 (C-5), 122.5 (C-8), 119.8 (C-3′), 115.4 (C-5′), 104.8 (C-3), 104.1 (C-12), 98.0 (C-15), 85.3 (C-13/C-17), 83.8 (C-13/C-17), 81.6 (C-14/C-16), 79.6 (C-14/C-16), 66.2 (C-1′′), 31.2 (C-20), 22.2 (C-19/C-21), 22.1 (C-19/C-21), 18.7 (C-18) ppm.

*Chlorido(2-benzyl-4-oxido-N-(pyridin-2-yl)-2H-benzo[e][1,2]-thiazine-3-carboxamide 1,1-dioxide)(cym)osmium(II) (**1b**).*



Complex **1b** was synthesized by following the general procedure using **1** (41 mg, 0.10 mmol), sodium methoxide (7 mg, 0.12 mmol), and $[Os(cym)Cl_2]_2$ (39 mg, 0.10 mmol). Yield: 62% (50 mg, pale yellow solid), MS (ESI^+): m/z 732.1568 $[M - Cl]^+$, calcd 732.1572. Anal. Found: C, 48.71; H, 3.79; N, 5.61; S, 4.38. Calcd for $C_{31}H_{30}ClN_3O_4OsS$: C, 48.59; H, 3.95; N, 5.48; S, 4.18. FT-IR (KBr, cm^{-1}): $\tilde{\nu}$ 3150 (NH), 1613 (C═O), 1568 (C═N), 1530 (NH def), 1466 (CH def), 1330, 1171 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 8.55 (dd, $^{3}J_{(H6',H5')}$ = 7 Hz, $^{4}J_{(H6',H4')}$ = 1 Hz, 1H, H-6′), 7.71 (td, $^{3}J_{(H4',H5')/(H4',H3')}$ = 8 Hz, $^{4}J_{(H4',H6')}$ = 2 Hz, 1H, H-4′), 7.65 (dd, $^{3}J_{(H8,H7)}$ = 8 Hz, $^{4}J_{(H8,H6)}$ = 2 Hz, 1H, H-8), 7.62 (dd, $^{3}J_{(H5,H6)}$ = 8 Hz, $^{4}J_{(H5,H7)}$ = 2 Hz, 1H, H-5), 7.44 (td, $^{3}J_{(H6,H7)/(H6,H5)}$ = 7 Hz, $^{4}J_{(H6,H4)}$ = 1 Hz, 1H, H-6), 7.39 (td, $^{3}J_{(H7,H8)/(H7,H6)}$ = 7 Hz, $^{4}J_{(H7,H5)}$ = 1 Hz, 1H, H-7), 7.09−7.01 (m, 7H, H-3′, H-5′, H-3″, H-4′′, H-5′′, H-6′′, H-7′′), 6.22 (d, $^{3}J_{(H13,H14)/(H17,H16)}$ = 6 Hz, 1H, H-13/H-17), 6.13 (d, $^{3}J_{(H13,H14)/(H17,H16)}$ = 6 Hz, 1H, H-13/H-17), 5.89 (d, $^{3}J_{(H14,H13)/(H16,H17)}$ = 6 Hz, 1H, H-14/H-16), 5.72 (d, $^{3}J_{(H14,H13)/(H16,H17)}$ = 6 Hz, 1H, H-14/H-16), 4.89 (d, $^{2}J_{(H1''A,H1''B)}$ = 15 Hz, 1H, H-1″), 4.40 (d, $^{2}J_{(H1''A,H1''B)}$ = 15 Hz, 1H, H-1″), 2.97−2.90 (m, 1H, H-20), 2.08 (s, 3H, H-18), 1.37 (d, $^{3}J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 3H, H-19/H-21), 1.35 (d, $^{3}J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 3H, H-19/H-21) ppm. $^{13}C\{^{1}H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 153.9 (C-6′), 149.7 (C-4), 139.9 (C-4′), 137.8 (C-2″), 134.1 (C-9), 133.7 (C-10), 131.6 (C-6), 130.8 (C-7), 130.2 (C-3′′/C-7′′), 130.1 (C-3′′/C-7′′), 127.44 (C-4′′/ C-6′′), 127.1 (C-4′′/ C-6′′), 126.9 (C-5), 122.4 (C-8), 119.7 (C-3′), 115.2 (C-5′), 103.8 (C-3), 94.0 (C-12), 91.1 (C-15), 76.5 (C-13/C-17), 75.1 (C-13/C-17), 72.3 (C-14/C-16), 70.1 (C-14/C-16), 55.5 (C-1′′), 31.5 (C-20), 23.5 (C-19/C-21), 22.1 (C-19/C-21), 18.8 (C-18) ppm.

*Chlorido(2-benzyl-N-(5-methylisoxazol-3-yl)-4-oxido-2H-benzo[e]-[1,2]thiazine-3-carboxamide 1,1-dioxide)(cym)ruthenium(II) (**2a**).*

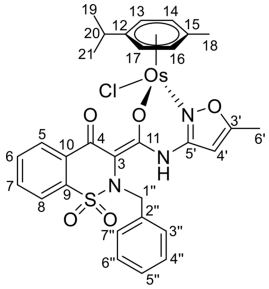


Complex **2a** was synthesized by following the general procedure using **2** (123 mg, 0.30 mmol), sodium methoxide (19 mg, 0.36 mmol), and $[Ru(cym)Cl_2]_2$ (92 mg, 0.15 mmol). Yield: 73% (148 mg, yellow powder). MS (ESI^+): m/z 646.0946 $[M - Cl]^+$, calcd 646.0949. Anal. Found: C, 52.91; H, 4.33; N, 6.12; S, 4.69. Calcd for $C_{30}H_{30}ClN_3O_5RuS$: C, 52.90; H, 4.44; N, 6.17; S, 4.71. FT-IR (KBr, cm^{-1}): $\tilde{\nu}$ 3110

DOI: 10.1021/acs.organomet.8b00751
Organometallics XXXX, XXX, XXX−XXX

(NH), 1590 (C═O, C═N), 1540 (NH def), 1428 (CH def), 1323, 1169 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 7.63 (dd, $^3J_{(H8,H7)}$ = 8 Hz, $^4J_{(H8,H5)}$ = 1 Hz, 1H, H-8), 7.56 (dd, $^3J_{(H5,H6)}$ = 8 Hz, $^4J_{(H5,H7)}$ = 1 Hz, 1H, H-5), 7.40 (td, $^3J_{(H6,H7)/(H6,H5)}$ = 8 Hz, $^4J_{(H6,H8)}$ = 1 Hz, H-6), 7.35 (td, $^3J_{(H7,H8)/(H7,H6)}$ = 8 Hz, $^4J_{(H7,H5)}$ = 1 Hz, H-7), 7.09−7.07 (m, 2H, H-4″, H-6′′), 6.92−6.85 (m, 3H, H-5′′, H-3′′, H-7′′), 5.88 (s, 1H, H-4′), 5.64 (d, $^3J_{(H13,H14)/(H17,H16)}$ = 5 Hz,1H, H-13/H-17), 5.59 (d, $^3J_{(H13,H14)/(H16,H17)}$ = 5 Hz,1H, H-13/H-17), 5.46 (d, $^3J_{(H14,H13)/(H17,H16)}$ = 6 Hz, 2H, H-14, H-16), 4.85 (d, $^2J_{(H1''A,H1''B)}$ = 15 Hz, 1H, H-1″), 4.47 (d, $^2J_{(H1''A,H1''B)}$ = 15 Hz, 1H, H-1″), 3.05−2.98 (m, 1H, H-20), 2.49 (s, 3H, H-6′), 2.32 (s, 3H, H-18), 1.37 (d, $^3J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 3H, H-19/H-21), 1.36 (d, $^3J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 3H, H-19/H-21) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 171.9 (C-3′), 171.1 (C-11), 165.5 (C-5′), 153.4 (C-4), 139.4 (C-2″), 133.8 (C-9), 133.4 (C-10), 131.3 (C-6), 130.4 (C-7), 130.0 (C-4′′, C-6′′), 127.1 (C-3′′, C-7′′), 127.1 (C-5′′), 126.8 (C-5), 122.1 (C-8), 105.7 (C-3), 103.5 (C-12), 98.0 (C-15), 97.4 (C-4′), 83.0 (C-13/C-17), 82.2 (C-13/C-17), 82.0 (C-14/C-16), 81.1 (C-14/C-16), 54.5 (C-1′′), 31.0 (C-20), 22.5 (C-19/C-21), 22.2 (C-19/C-21), 18.4 (C-18), 12.8 (C-6′) ppm.

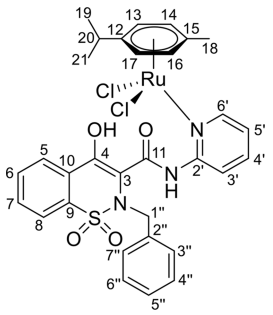
Chlorido(2-benzyl-N-(5-methylisoxazol-3-yl)-4-oxido-2H-benzo-[e][1,2]thiazine-3-carboxamide 1,1-dioxide)(cym)osmium(II) (**2b**).



Complex **2b** was synthesized following the general procedure using ligand **2** (123 mg, 0.30 mmol), sodium methoxide (19 mg, 0.36 mmol), and $[Os(cym)Cl_2]_2$ (118 mg, 0.15 mmol). Yield: 82% (190 mg, light yellow). MS (ESI$^+$): m/z 736.1528 $[M - Cl]^+$, calcd 736.1515. Anal. Found: C, 46.85; H, 3.94; N, 5.53; S, 4.27. Calcd for $C_{30}H_{30}ClN_3O_5OsS$: C, 46.78; H, 3.93; N, 5.45; S, 4.16. FT-IR (KBr, cm^{-1}): $\tilde{\nu}$ 3100 (NH), 1600 (C═O, C═N), 1566 (NH def), 1488 (CH def), 1327, 1170 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 7.63 (dd, $^3J_{(H8,H7)}$ = 8 Hz, $^4J_{(H8,H5)}$ = 1 Hz, 1H, H-8), 7.57 (dd, $^3J_{(H5,H6)}$ = 8 Hz, $^4J_{(H5,H7)}$ = 1 Hz, 1H, H-5), 7.42 (td, $^3J_{(H6,H7)/(H6,H5)}$ = 8 Hz, $^4J_{(H6,H8)}$ = 1 Hz, H-6), 7.35 (td, $^3J_{(H7,H8)/(H7,H6)}$ = 8 Hz, $^4J_{(H7,H5)}$ = 1 Hz, H-7), 7.11−7.09 (m, 2H, H-4″, H-5′′), 6.94−6.85 (m, 3H, H-5′′, H-3′′, H-7′′), 5.95−5.91 (m, 3H, H-4′, H-13, H-17), 5.75 (d, $^3J_{(H14,H13)/(H16,H17)}$ = 6 Hz, 1H, H-14/H-16), 5.72 (d, $^3J_{(H14,H13)/(H16,H17)}$ = 6 Hz, 1H, H-14/H-16), 4.85 (d, $^2J_{(H1''A,H1''B)}$ = 15 Hz, 1H, H-1″), 4.43 (d, $^2J_{(H1''A,H1''B)}$ = 15 Hz, 1H, H-1″), 2.91−2.84 (m, 1H, H-20), 2.48 (s, 3H, H-6′), 2.37 (s, 3H, H-18), 1.35 (d, $^3J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 3H, H-19/H-21), 1.33 (d, $^3J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 3H, H-19/H-21) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 171.7 (C-3′), 170.8 (C-11), 165.5 (C-5′), 151.5 (C-4), 139.6 (C-2″), 133.7 (C-9), 133.0 (C-10), 131.5 (C-6), 130.7 (C-7), 130.1 (C-4′′, C-6′′), 127.3 (C-3′′, C-7′′), 127.2 (C-5′′), 126.9 (C-5), 122.2 (C-8), 105.8 (C-3), 97.9 (C-4′), 93.9 (C-12), 89.5 (C-15), 74.8 (C-13/C-17), 73.6 (C-13/C-17), 73.3 (C-14/C-16), 71.9 (C-14/C-16), 54.5 (C-1′′), 31.5 (C-20), 23.1 (C-19/C-21), 22.6 (C-19/C-21), 18.7 (C-18), 12.8 (C-6′) ppm.

General Procedure for the Synthesis of Complexes 1c,d, 2c,d, 3a,b, 4a, 5a,b, and 6a. Oxicam **1**−**6** and $[M(cym)X_2]_2$ (M = Ru, Os) were dissolved in dichloromethane at room temperature. The reaction mixture was subjected to stirring for 4 h under anhydrous conditions. After completion of the reaction, the volume of the solvent was reduced to approximately 5 mL and diethyl ether was added to precipitate the product. The solid was collected by filtration, washed with ethyl ether, and dried under vacuum.

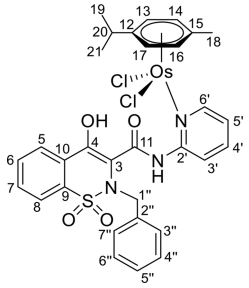
Dichlorido(2-benzyl-N-(pyridin-2-yl)-4-hydroxy-2H-benzo[e]-[1,2]thiazine-3-carboxamide 1,1-dioxide)(cym)ruthenium(II) (**1c**).



Complex **1c** was synthesized following the general procedure using **1** (122 mg, 0.30 mmol) and $[Ru(cym)Cl_2]_2$ (92 mg, 0.15 mmol). Yield: 61% (130 mg, orange crystals). ESI-MS: m/z 678.0784 $[M - Cl]^+$, calcd 678.0766. Anal. Found: C, 52.13; H, 4.47; N, 5.61; S, 4.26. Calcd for $C_{31}H_{31}Cl_2N_3O_4RuS$: C, 52.17; H, 4.38; N, 5.89; S, 4.49. FT-IR (KBr, cm^{-1}): $\tilde{\nu}$ 3465 (NH), 3100−2700 (OH), 1626 (C═O), 1559 (C═N), 1432 (CH def), 1340, 1174 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.50 (s, 1H, OH), 11.80 (s, 1H, NH), 9.24 (dd, $^3J_{(H6',H5')}$ = 6 Hz, $^4J_{(H6',H4')}$ = 1 Hz, 1H, H-6′), 8.01 (d, $^3J_{(H3',H4')}$ = 8 Hz, 1H, H-3′), 7.82 (td, $^3J_{(H4',H5')/(H4',H3')}$ = 8 Hz, $^4J_{(H4',H6')}$ = 1 Hz, 1H, H-4′), 7.78 (d, $^3J_{(H8,H7)}$ = 7 Hz, 1H, H-8), 7.41 (d, $^3J_{(H5,H6)}$ = 8 Hz, 1H, H-5), 7.57 (td, $^3J_{(H7,H8)/(H7,H6)}$ = 8 Hz, $^4J_{(H7,H5)}$ = 1 Hz, 1H, H-7), 7.49 (td, $^3J_{(H6,H7)/(H6,H5)}$ = 8 Hz, $^4J_{(H6,H4)}$ = 1 Hz, 1H, H-6), 7.18−7.15 (m, 1H, H-5′), 6.98−6.91 (m, 5H, H-4″, H-6′′, H-5′′, H-3′′, H-7′′), 6.15 (d, $^3J_{(H13,H14)/(H-17/H16)}$ = 6 Hz, 1H, H-13/H-17), 5.61 (d, $^3J_{(H13,H14)/(H-17/H16)}$ = 6 Hz, 1H, H-13/H-17), 5.51 (d, $^3J_{(H14,H13)/(H-16/H17)}$ = 6 Hz, 1H, H-14/H-16), 5.43 (d, $^3J_{(H14,H13)/(H-16/H17)}$ = 6 Hz, 1H, H-14/H-16), 5.01 (d, $^2J_{(H1''A,H1''B)}$ = 14 Hz, 1H, H-1″), 4.44 (d, $^2J_{(H1''A,H1''B)}$ = 14 Hz, 1H, H-1″), 2.95−2.88 (m, 1H, H-20), 1.86 (s, 3H, H-18), 1.36 (d, $^3J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 3H, H-19/H-21), 1.27 (d, $^3J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 3H, H-19/H-21) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 168.0 (C-11), 162.4 (C-4), 154.6 (C-6′), 153.5 (C-2′), 139.4 (C-4′), 138.0 (C-2″), 132.3 (C-6, C-7), 131.1 (C-9), 130.6 (C-4′′, C-6′′), 128.5 (C-10), 128.3 (C-5′′), 127.7 (C-3′′, C-7′′), 126.4 (C-8), 123.4 (C-5), 121.0 (C-5′), 119.9 (C-3′), 107.4 (C-3), 101.9 (C-12), 98.8 (C-15), 85.7 (C-13/C-17), 84.3 (C-13/C-17), 81.1 (C-14/C-16), 80.9 (C-14/C-16), 57.1 (C-1′′), 30.8 (C-20), 22.5 (C-19, C-21), 18.3 (C-18) ppm.

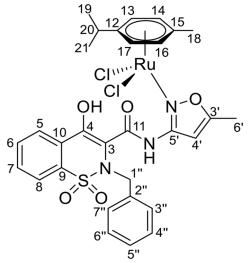
Dichlorido(2-benzyl-N-(pyridin-2-yl)-4-hydroxy-2H-benzo[e]-[1,2]thiazine-3-carboxamide 1,1-dioxide)(cym)osmium(II) (**1d**). Complex **1d** was synthesized following the general procedure using **1** (41 mg, 0.10 mmol) and $[Os(cym)Cl_2]_2$ (39 mg, 0.05 mmol). Yield: 77% (62 mg, greenish yellow precipitate). MS (ESI$^+$): m/z 768.1319 $[M - Cl]^+$, calcd 768.1322. Anal. Found: C, 46.61; H, 3.91; N, 5.12; S, 4.01. Calcd for $C_{31}H_{31}Cl_2N_3O_4OsS$: C, 46.38; H, 3.89; N, 5.23; S, 3.99. FT-IR (KBr, cm^{-1}): $\tilde{\nu}$ 3100 (NH), 2960−2750 (OH), 1630 (C═O), 1559 (C═N), 1431 (CH def), 1344, 1174 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.45 (s, 1H, OH), 11.66 (s, 1H, H-NH), 9.16 (dd, $^3J_{(H6',H5')}$ = 6 Hz, $^4J_{(H6',H4')}$ = 1 Hz, 1H, H-6′), 7.99 (d, $^3J_{(H3',H4')}$ = 8 Hz, 1H, H-3′), 7.79−7.73 (m, 2H, H-5, H-4′),

DOI: 10.1021/acs.organomet.8b00751
Organometallics XXXX, XXX, XXX−XXX



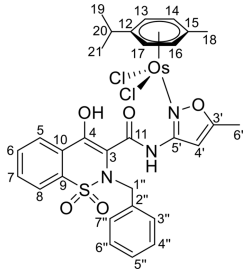
7.63 (dd, $^{3}J_{(H5,H6)}$ = 7 Hz, $^{4}J_{(H5,H7)}$ = 1 Hz, 1H, H-8), 7.58 (td, $^{3}J_{(H7,H8)/(H7,H6)}$ = 8 Hz, $^{4}J_{(H7,H5)}$ = 1 Hz, 1H, H-7), 7.50 (td, $^{3}J_{(H6,H5)/(H6,H7)}$ = 8 Hz, $^{4}J_{(H6,H4)}$ = 1 Hz, 1H, H-6), 7.17 (td, $^{3}J_{(H5′,H6′)/(H5′/H4′)}$ = 6 Hz, $^{3}J_{(H5′,H3′)}$ = 1 Hz, 1H, H-5′), 7.01−6.90 (m, 5H, H-4″, H-6′′, H-5′′, H-3′′, H-7′′), 6.57 (d, $^{3}J_{(H13,H14)/(H17,H16)}$ = 5 Hz, 1H, H-13/H-17), 6.03 (d, $^{3}J_{(H13,H14)/(H17,H16)}$ = 5 Hz, 1H, H-13/H-17), 5.91 (d, $^{3}J_{(H14,H13)/(H16,H17)}$ = 6 Hz, 2H, H-14, H-16), 4.99 (d, $^{2}J_{(H1″A,H1′′B)}$ = 14 Hz, 1H, H-1″), 4.42 (d, $^{2}J_{(H1″A,H1′′B)}$ = 14 Hz, 1H, H-1″), 2.85−2.75 (m, 1H, H-20), 1.87 (s, 3H, H-18), 1.35 (d, $^{3}J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 3H, H-19/H-21), 1.27 (d, $^{3}J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 3H, H-19/H-21) ppm. $^{13}C\{^{1}H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 167.8 (C-11), 162.4 (C-4), 154.3 (C-6′), 152.8 (C-2′), 139.5 (C-4′), 138.0 (C-13), 132.3 (C-6, C-7), 131.0 (C-9), 130.6 (C-4″, C-6′′), 128.5 (C-10), 128.4 (C-5′′), 127.7 (C-3′′, C-7′′), 126.4 (C-8), 123.5 (C-5), 120.9 (C-5′), 119.7 (C-3′), 107.4 (C-3), 92.7 (C-12), 90.8 (C-15), 77.9 (C-13, C-17), 72.5 (C-14/C-16), 72.0 (C-14/C-16), 57.1 (C-1′′), 31.2 (C-20), 22.8 (C-19/C-21), 22.7 (C-19/C-21), 18.4 (C-18) ppm.

*Dichlorido(2-benzyl-N-(5-methylisoxazol-3-yl)-4-hydroxy-2H-benzo[e][1,2]thiazine-3-carboxamide 1,1-dioxide)(cym)ruthenium-(II) (**2c**).*



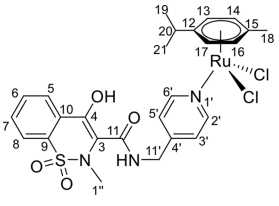
Complex **2c** was synthesized by following the general procedure using **2** (123 mg, 0.30 mmol) and $[Ru(cym)Cl_2]_2$ (92 mg, 0.15 mmol). Yield: 84% (199 mg, orange precipitate). MS (ESI^+): m/z 740.0295 $[M + Na]^+$, calcd 740.0302; m/z 682.0711 $[M - Cl]^+$, calcd 682.0716; m/z 646.0946 $[M - 2Cl - H]^+$, calcd 646.0949. Anal. Found: C, 50.35; H, 4.38; N, 5.77; S, 4.57. Calcd for $C_{30}H_{31}Cl_2N_3O_5RuS$: C, 50.21; H, 4.35; N, 5.86; S, 4.47. FT-IR (KBr, cm^{-1}): $\tilde{\nu}$ 3209 (NH), 3064−2700 (OH), 1639 (C═O), 1608 (C═N), 1543 (NH def), 1428 (CH def), 1341, 1174 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.05 (s, 1H, OH), 11.27 (s, 1H, H-NH), 7.68 (d, $^{3}J_{(H8,H7)}$ = 8 Hz, 1H, H-8), 7.49−45 (m, 2H, H-6, H-5), 7.60 (td, $^{3}J_{(H7,H8)/(H7,H6)}$ = 8 Hz, $^{4}J_{(H7,H5)}$ = 1 Hz, H-7), 7.04 (s, 1H, H-4′), 6.91−6.78 (m, 5H, H-5″, H-3′′, H-4′′, H-6′′, H-7′′), 5.71 (d, $^{3}J_{(H13,H14)/(H16,H17)}$ = 5 Hz, 1H, H-13/H-17), 5.62 (d, $^{3}J_{(H13,H14)/(H16,H17)}$ = 5 Hz, 1H, H-13/H-17), 5.47−5.46 (m, 2H, H-14, H-16), 5.03 (d, $^{2}J_{(H1″A,H1′′B)}$ = 15 Hz, 1H, H-1″), 4.65 (d, $^{2}J_{(H1″,H1′′B)}$ = 15 Hz, 1H, H-1″), 3.11−3.05 (m, 1H, H-20), 2.54 (s, 3H, H-6′), 2.29 (s, 3H, H-18), 1.34−1.31 (m, 6H, H-19/H-21) ppm. $^{13}C\{^{1}H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 171.0 (C-3′), 167.5 (C-11), 162.1 (C-4), 159.5 (C-5′), 139.1 (C-2″), 131.9 (C-6), 131.7 (C-7), 131.4 (C-9), 130.5 (C-4′′, C-6′′), 128.4 (C-10), 127.8 (C-5′′), 127.4 (C-3′′, C-7′′), 126.1 (C-5), 123.2 (C-8), 107.2 (C-3), 104.1 (C-12), 99.8 (C-4′), 99.7 (C-15), 83.7 (C-13/C-17), 83.6 (C-13/C-17), 82.0 (C-14/C-16), 81.3 (C-14/C-16), 57.3 (C-1′′), 30.9 (C-20), 22.4 (C-19/C-21), 22.3 (C-19/C-21), 19.0 (C-18), 13.0 (C-6′) ppm.

*Dichlorido(2-benzyl-N-(5-methylisoxazol-3-yl)-4-hydroxy-2H-benzo[e][1,2]thiazine-3-carboxamide 1,1-dioxide)(cym)osmium(II) (**2d**).*



Complex **2d** was synthesized by following the general procedure using **2** (123 mg, 0.30 mmol) and $[Os(cym)Cl_2]_2$ (119 mg, 0.15 mmol). Yield: 87% (210 mg, light yellow). MS (ESI^+): m/z 830.0845 $[M + Na]^+$, calcd 830.0848; m/z 772.1228 $[M - Cl]^+$, calcd 772.1228; m/z 646.0946 $[M - 2Cl - H]^+$, calcd 646.0949. Anal. Found: C, 44.51; H, 3.92; N, 5.35; S, 3.85. Calcd for $C_{30}H_{31}Cl_2N_3O_5OsS$: C, 44.66; H, 3.87; N, 5.21; S, 3.97. FT-IR (KBr, cm^{-1}): $\tilde{\nu}$ 3199 (NH), 3050−2700 (OH), 1642 (C═O), 1606 (C═N), 1541 (NH def), 1485 (CH def), 1341, 1174 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.05 (s, 1H, OH), 11.48 (s, 1H, H-NH), 7.67 (d, $^{3}J_{(H8,H7)}$ = 7 Hz, $^{4}J_{(H8,H5)}$ = 1 Hz, 1H, H-8), 7.49−7.43 (m, 2H, H-6, H-5), 7.36 (td, $^{3}J_{(H7,H8)/(H7,H6)}$ = 8 Hz, $^{4}J_{(H7,H5)}$ = 1 Hz, H-7), 7.07 (s, 1H, H-4′), 6.90−6.79 (m, 5H, H-5″, H-3′′, H-4′′, H-6′′, H-7′′), 6.01 (d, $^{3}J_{(H13,H14)/(H17,H16)}$ = 5 Hz, 1H, H-13/H-17), 5.99 (d, $^{3}J_{(H13,H14)/(H17,H16)}$ = 5 Hz, 1H, H-13/H-17), 5.85−5.81 (m, 2H, H-14, H-16), 5.05 (d, $^{2}J_{(H1″A,H1′′B)}$ = 15 Hz, 1H, H-1″), 4.73 (d, $^{2}J_{(H1″A,H1′′B)}$ = 15 Hz, 1H, H-1″), 2.95−2.88 (m, 1H, H-20), 2.54 (s, 3H, H-6′), 2.31 (s, 3H, H-18), 1.32−1.29 (m, 6H, H-19/H-21) ppm. $^{13}C\{^{1}H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 170.2 (C-3′), 167.5 (C-11), 162.2 (C-4), 158.6 (C-5′), 139.1 (C-2″), 132.0 (C-6), 132.0 (C-7), 131.3 (C-9), 130.6 (C-4′′, C-6′′), 128.5 (C-10), 127.9 (C-5′′), 127.3 (C-3′′, C-7′′), 126.2 (C-5), 123.2 (C-8), 107.3 (C-3), 100.1 (C-4′), 94.7 (C-12), 91.6 (C-15), 76.1 (C-13/C-17), 75.8 (C-13/C-17), 73.2 (C-14/C-16), 72.6 (C-14/C-16), 57.6 (C-1′′), 31.1 (C-20), 22.8 (C-19/C-21), 22.5 (C-19/C-21), 18.9 (C-18), 12.8 (C-6′) ppm.

*Dichlorido[2-methyl-N-(pyridin-4-ylmethyl)-4-hydroxy-2H-benzo[e][1,2]thiazine-3-carboxamide 1,1-dioxide](cym)ruthenium-(II) (**3a**).*



Compound **3a** was synthesized by following the general procedure using **3** (138 mg, 0.40 mmol) and $[Ru(cym)Cl_2]_2$ (123 mg, 0.20 mmol). Yield: 65% (168 mg, orange powder). mp 222−225 °C. MS (ESI^+): m/z 580.0842 $[M - 2Cl - H]^+$, calcd 580.0844. Anal. Found: C, 47.98; H, 4.58; N, 6.45; S, 4.62, Calcd for $C_{26}H_{29}Cl_2N_3O_4RuS{\cdot}0.75H_2O$: C, 47.93; H,

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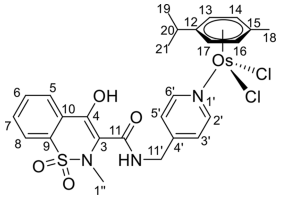
Organometallics XXXX, XXX, XXX−XXX

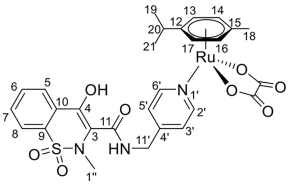
4.49; N, 6.45; S, 4.92. ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.33 (brs, 1H, O−H), 8.87 (d, $^3J_{(H2',H3')/(H6',H5')}$ = 5 Hz, 2H, H-2′, H-6′), 8.04−8.02 (dd, $^3J_{(H8,H7)}$ = 8 Hz, $^4J_{(H8,H6)}$ = 2 Hz, 1H, H-8), 7.91−7.89 (dd, $^3J_{(H5,H6)}$ = 7 Hz, $^4J_{(H5,H7)}$ = 2 Hz, 1H, H-5), 7.78−7.74 (td, $^3J_{(H6,H7)/(H6,H5)}$ = 9 Hz, $^4J_{(H6,H8)}$ = 1 Hz, 1H, H-6), 7.74−7.70 (td, $^3J_{(H7,H8)/(H7,H6)}$ = 9 Hz, $^4J_{(H5,H7)}$ = 1 Hz, 1H, H-7), 7.21−7.19 (m, 2H, H-3′, H-5′), 5.43 (d, $^3J_{(H13,H14)/(H17,H16)}$ = 6 Hz, 2H, H-13, H-17), 5.21 (d, $^3J_{(H13,H14)/(H16,H17)}$ = 6 Hz, 2H, H-14, H-16), 4.50 (brs, 2H, H-11′), 3.03−2.95 (m, 1H, H-20), 2.94 (s, 3H, H-1″), 2.08 (s, 3H, H-18), 1.31 (d, $^3J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 6H, H-19, H-21) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 169.1 (C-11), 157.5 (C-4), 154.3 (C-2′, C-6′), 150.3 (C-4′), 135.1 (C-9), 133.1 (C-6), 132.4 (C-7), 129.1 (C-10), 126.8 (C-5), 124.9 (C-8), 123.9 (C-3′, C-5′), 111.8 (C-3), 104.0 (C-15), 97.5 (C-12), 83.2 (C-14, C-16), 82.5 (C-13, C-17), 41.8 (C-11′), 40.5 (C-1″), 31.0 (C-20), 22.6 (C-19, C-21), 18.5 (C-18) ppm.

*Dichlorido[2-methyl-N-(pyridin-4-ylmethyl)-4-hydroxy-2H-benzo[e][1,2]thiazine-3-carboxamide 1,1-dioxide](cym)osmium(II) (**3b**).*



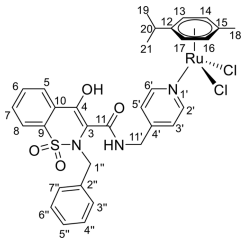
Compound **3b** was synthesized by following the general procedure using **3** (138 mg, 0.40 mmol) and $[Os(cym)Cl_2]_2$ (158.2 mg, 0.20 mmol). Yield: 74% (220 mg, yellow powder). Mp: 220−228 °C. MS (ESI$^+$): m/z 670.1424 $[M - 2Cl - H]^+$, calcd 670.1415. Anal. Found: C, 42.17; H, 3.92; N, 5.68; S, 4.28, Calcd for $C_{26}H_{29}Cl_2N_3O_4SOs$: C, 42.16; H, 3.95; N, 5.67; S, 4.33. ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.42 (s, 1H, OH), 8.72 (d, $^3J_{(H2',H3')/(H6',H5')}$ = 7 Hz, 2H, H-2′, H-6′), 8.01−7.99 (dd, $^3J_{(H7,H8)}$ = 7 Hz, $^4J_{(H6,H8)}$ = 2 Hz, 1H, H-8), 7.89−7.87 (dd, $^3J_{(H5,H6)}$ = 7 Hz, $^4J_{(H5,H7)}$ = 2 Hz,1H, H-5), 7.84 (t, $^3J_{(H10',H11')}$ = 7 Hz, 1H, NH), 7.76−7.72 (td, $^3J_{(H6,H7)/(H6,H5)}$ = 9 Hz, $^4J_{(H6,H8)}$ = 2 Hz, 1H, H-6), 7.72−7.68 (td, $^3J_{(H7,H8)/(H7,H6)}$ = 9 Hz, $^4J_{(H7,H5)}$ = 1 Hz, 1H, H-7), 7.17 (d, $^3J_{(H3',H2')/(H5',H6')}$ = 6 Hz, 2H, H-3′, H-5′), 5.85 (d, $^3J_{(H13,H14)/(H17,H16)}$ = 6 Hz, 2H, H-13, H-17), 5.57 (d, $^3J_{(H14,H13)/(H16,H17)}$ = 6 Hz, 2H, H-14, H-16), 4.52 (brs, 2H, H-11′), 2.93 (s, 3H, H-1″), 2.85−2.80 (m, 1H, H-20), 2.03 (s, 3H, H-18), 1.30 (d, $^3J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 6H, H-19, H-21) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 169.1 (C-11), 157.6 (C-4), 154.1 (C-2′, C-6′), 150.2 (C-4′), 135.0 (C-9), 133.2 (C-6), 132.5 (C-7), 129.0 (C-10), 126.9 (C-5), 124.9 (C-8), 123.9 (C-3′, C-5′), 111.7 (C-3), 94.4 (C-15), 88.9 (C-12), 75.2 (C-14, C-16), 73.4 (C-13, C-17), 41.6 (C-11′), 40.4 (C-1″), 31.2 (C-20), 22.9 (C-19, C-21), 18.5 (C-18) ppm.

*Oxalato[2-methyl-N-(pyridin-4-ylmethyl)-4-hydroxy-2H-benzo-[e][1,2]thiazine-3-carboxamide 1,1-dioxide](cym)ruthenium(II) (**3c**).* Silver oxalate (291 mg, 0.96 mmol) and $[Ru(cym)Cl_2]_2$ (245 mg, 0.40 mmol) were added to 20 mL of deionized H_2O, and the mixture was stirred for 24 h in the dark. The reaction mixture was filtered through Celite under suction to yield water-soluble, red $[Ru(cym)(oxalato)(H_2O)]$. The water was removed, and the compound was dissolved in dry methanol (15 mL). Compound **3** (276 mg, 0.80 mmol) was dissolved in DCM (5 mL) and added to the methanol solution of the organoruthenium compound. The



reaction mixture was stirred for 20 h at room temperature, and the volume of the solvent was reduced under vacuum. Precipitation was induced by adding diethyl ether. The product was filtered, washed with diethyl ether, and dried under vacuum. Bright yellow crystals suitable for X-ray diffraction analysis were obtained by slow diffusion of diethyl ether into solution of compound in methanol. Yield: 76% (405 mg, bright yellow powder). Mp: 183−194 °C. MS (ESI$^+$): m/z 692.0616 $[M + Na]^+$, calcd 692.0617. Anal. Found: C, 47.75; H, 4.30; N, 6.00; S, 4.61. Calcd for $C_{28}H_{29}N_3O_8RuS{\cdot}0.5CH_2Cl_2$: C, 48.13; H, 4.25; N, 5.91; S, 4.51. ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.7 (brs, 1H, OH), 9.07 (t, $^3J_{(H10',H11')}$ = 7 Hz,1H, NH), 8.23 (d, $^3J_{(H2',H3')/(H6',H5')}$ = 6 Hz, 2H, H-2′, H-6′), 8.04 (dd, $^3J_{(H8,H7)}$ = 7 Hz, $^4J_{(H8,H6)}$ = 1 Hz, 1H, H-8), 7.91 (dd, $^3J_{(H5,H6)}$ = 7 Hz, $^4J_{(H5,H7)}$ = 2 Hz,1H, H-5), 7.75 (td, $^3J_{(H6,H7)/(H6,H5)}$ = 8 Hz, $^4J(H6,H8)$ = 2 Hz, 1H, H-6), 7.71 (td, $^3J_{(H7,H8)/(H7,H6)}$ = 9 Hz, $^3J_{(H7,H8)/(H7,H6)}$ = 8 Hz, $^4J_{(H7,H5)}$ = 2 Hz, 1H, H-7), 7.24 (d, $^3J_{(H3',H2')/(H5',H6')}$ = 6 Hz, 2H, H-3′, H-5′), 5.56 (d, $^3J_{(H13,H14)/(H17,H18)}$ = 6 Hz, 2H, H-13, H-17), 5.42 (d, $^3J_{(H14,H13)/(H16,H17)}$ = 6 Hz, 2H, H-14, H-16), 2.94−2.86 (m, 4H, H-1″, H-20), 2.18 (s, 3H, H-18), 2.16 (s, 2H, H-11′), 1.33 (d, $^3J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 6H, H-19, H-21) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 169.1 (C-11), 157.4 (C-4), 152.0 (C-2′, C-6′), 151.8 (C-4′), 134.8 (C-9), 132.8 (C-10), 132.0 (C-8), 128.9 (C-5), 126.5 (C-6), 124.4 (C-7), 124.1 (C-3′, C-5′), 111.5 (C-3), 102.1 (C-13, C-17), 96.7 (C-14, C-16), 81.9 (C-12), 81.2 (C-15), 41.5 (C-11′), 39.8 (C-1″), 30.9 (C-20), 22.5 (C-19, C-21), 17.9 (C-18) ppm.

*Dichlorido(2-benzyl-4-hydroxy-N-(pyridin-4-ylmethyl)-2H-benzo[e][1,2]thiazine-3-carboxamide 1,1-dioxide)(cym)ruthenium-(II) (**4a**).*

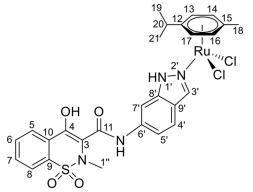


Compound **4a** was synthesized by following the general procedure using **4** (169 mg, 0.40 mmol) and $[Ru(cym)Cl_2]_2$ (123 mg, 0.20 mmol). Yield: 68% (198 mg, yellow powder). MS (ESI$^+$): m/z 750.0507 $[M + Na]^+$, calcd 750.0510. Anal. Found: C, 45.35; H, 4.00; N, 4.50; S, 3.34. Calcd for $C_{32}H_{33}Cl_2N_3O_4RuS{\cdot}2CH_2Cl_2$: C, 45.50; H, 4.16; N, 4.68; S, 3.57. FTIR (KBr, cm^{-1}): 3298, 3188 (NH), 1650 (C═O), 1577 (C═N), 1455 (NH def), 1333, 1180 (SO_2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.52 (s, 1H, OH), 8.78−8.77 (d, $^3J_{(H2',H\text{-}3')/(H6',H\text{-}5')}$ = 6 Hz, 2H, H-2′, H-6′), 7.84 (s, 1H, NH), 7.72−7.70 (dd, $^3J_{(H8,H7)}$ = 8 Hz, $^4J_{(H8,H6)}$ = 1 Hz, 1H, H-8), 7.60−7.58 (dd, $^3J_{(H5,H6)}$ = 8 Hz, $^4J_{(H5,H7)}$ = 1 Hz, 1H, H-5), 7.54−7.50 (td, $^3J_{(H6,H5)/(H6,H7)}$ = 8 Hz, $^4J_{(H6,H8)}$ = 1 Hz, 1H, H-6), 7.48−7.44 (td, $^3J_{(H7,H8)/(H7,H6)}$ = 8 Hz, $^4J_{(H7,H5)}$ = 1 Hz, 1H, H-7), 7.11−7.09 (d, $^3J_{(H5',H6')/(H3',H2')}$ = 6 Hz, 2H, H-3′, H-5′), 7.04−6.94 (m, 5H, H-4″, H-6′′, H-3′′, H-7′′, H-5′′), 5.49−5.45 (m, 2H, H-13, H-17), 5.22−5.21 (m, 2H, H-14, H-16), 4.70−4.24 (m, 4H, H-1′′, H-11′), 3.03−2.96 (m, 1H, H-20), 2.04 (s, 3H, H-18), 1.33−1.31 (m, 6H, H-19, H-21) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$, 25 °C): δ

DOI: 10.1021/acs.organomet.8b00751
Organometallics XXXX, XXX, XXX−XXX

169.0 (C-11), 159.3 (C-4), 154.45 (C-2′, C-6′), 149.9 (C-4′), 137.5 (C-2″), 132.2 (C-7), 131.7 (C-9), 131.5 (C-6), 130.2 (C-4″, C-6″), 128.9 (C-10), 128.4 (C-5″), 127.8 (C-3″, C-7″), 126.2 (C-5), 123.7 (C-3′, C-5′), 123.3 (C-8), 107.8 (C-3), 103.8 (C-1), 97.3 (C-15), 83.0 (C-13, C-17), 82.2 (C-14, C-16), 56.9 (C-1″), 41.5 (C-11′), 30.8 (C-18), 22.4 (C-20), 22.3 (C-19), 18.3 (C-21) ppm.

*Dichlorido(4-hydroxy-N-(1H-indazol-6-yl)-2-methyl-2H-benzo-[e][1,2]thiazine-3-carboxamide 1,1-dioxide)(cym)ruthenium(II) (**5a**).*



Compound **5a** was synthesized by following the general procedure using **5** (148 mg, 0.40 mmol) and $[Ru(cym)Cl_2]_2$ (123 mg, 0.20 mmol). Yield: 34% (92 mg, yellow powder). MS (ESI^+): *m*/*z* 641.0551 $[M - Cl]^+$, calcd 641.0563. Anal. Found: C, 44.01; H, 3.93; N, 7.31; S, 4.01. Calcd for $C_{27}H_{28}Cl_2N_4O_4RuS{\cdot}CH_2Cl_2$: C, 44.16; H, 3.97; N, 7.36; S, 4.21. FTIR (KBr, cm^{-1}): 3337, 3253 (NH), 3100−2750 (OH), 1617 (C═O), 1585 (C═N), 1443 (CH def), 1330, 1180 (SO_2). ^1H NMR (400.13 MHz, DMSO-d_6, 25 °C): 14.09 (s, 1H, OH), 13.07 (s, 1H, H-1′), 10.40 (s, 1H, NH), 8.10−8.06 (m, 3H, H-3′, H-4′, H-7′), 7.97−7.89 (m, 3H, H-8, H-6, H-7), 7.76 (d, $^3J_{(H5,H6)}$ = 9 Hz,1H, H-5), 7.51−7.49 (dd, $^3J_{(H5',H4')}$ = 9 Hz, $^4J_{(H5',H7')}$ = 1 Hz,1H, H-5′), 5.83 (d, $^3J_{(H14,H13)/(H16,H17)}$ = 6 Hz, 2H, H-14, H-16), 5.79 (d, $^3J_{(H13,H14)/(H17,H16)}$ = 6 Hz, 2H, H-13, H-17), 2.91 (s, 3H, H-1″), 2.89−2.82 (m, 1H, H-20), 2.10 (s, 3H, H-18), 1.21 (d, $^3J_{(H19,H20)/(H21,H20)}$ = 7 Hz, 6H, H-19, H-21) ppm.

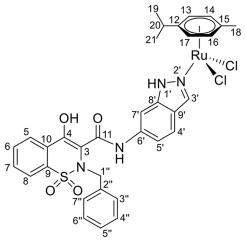
*Dichlorido(2-benzyl-4-hydroxy-N-(1H-indazol-6-yl)-2H-benzo[e]-[1,2]thiazine-3-carboxamide 1,1-dioxide)(cym)ruthenium(II) (**6a**).* Compound **6a** was synthesized by following the general procedure using **6** (134 mg, 0.30 mmol) and $[Ru(cym)Cl_2]_2$ (123 mg, 0.20 mmol). Yield: 45% (113 mg, pale yellow powder). MS (ESI^+): *m*/*z* 717.0869 $[M - Cl]^+$, calcd 717.0876. Anal. Found: C, 52.93; H, 4.25; N, 7.41; S, 4.31. Calcd for $C_{33}H_{32}Cl_2N_4O_4RuS$: C, 52.66; H, 4.29; N, 7.44; S, 4.26. FTIR (KBr, cm^{-1}): 3347, 3257 (NH), 1610 (C═O), 1587 (C═N), 1447 (CH def), 1333, 1160 (SO_2). ^1H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ 13.49 (s, 1H, OH), 11.58 (s, 1H, H-1′), 8.39 (s, 1H, H-3′), 8.25 (s, 1H, NH), 7.85−7.79 (m, 2H, H-8, H-5), 7.64−7.59 (m, 3H, H-7′, H-6, H-7), 7.36−7.33 (d, $^3J_{(H4',H5')}$ = 9 Hz, 1H, H-4′), 7.17−7.14 (m, 2H, H-4″, H-6″), 7.07−7.05 (m, 3H, H-5″, H-3″, H-7″), 6.89−6.87 (dd, $^3J_{(H5',H4')}$ = 9 Hz, $^4J_{(H5',H7')}$ = 1 Hz, 1H, H-5′), 5.69−5.66 (m, 2H, H-14, H-16), 5.48−5.47 (m, 2H, H-13, H-17), 4.61−4.46 (m, 2H, H-1″), 3.07−3.0 (m, 1H, H-20), 2.21 (s, 3H, H-18), 1.32−1.26 (m, 6H, H-19, H-21) ppm. $^{13}C\{^1H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ 167.2 (C-11), 160.0 (C-4), 141.1 (C-8′), 140.7 (C-3′), 136.9 (C-2″), 136.8 (C-6′), 132.8 (C-7), 132.2 (C-9), 132.1 (C-6), 130.2 (C-4″, C-6″), 128.8 (C-5″), 128.7 (C-10), 128.4 (C-3″, C-7″), 126.3 (C-5), 123.9 (C-8), 120.6 (C-9′), 120.5 (C-4′), 116.3 (C-5′), 108.9 (C-3), 104.2 (C-15), 99.9 (C-7′), 98.7 (C-12), 83.6 (C-14, C-16), 82.6 (C-13, C-17), 57.8 (C-1″), 31.5 (C-20), 22.6 (C-19, C-21), 19.2 (C-18) ppm.

Stability in DMSO and Aqueous Solution. For stability studies in DMSO-d_6, **1a−d**, **2a−d**, and **3a−c** (1−2 mg/mL) were dissolved in DMSO-d_6 and ^1H NMR spectra were recorded after 0.5, 6, 24, 48, and 72 h at room temperature on a Bruker Avance AV 300 spectrometer at a 300.13 (^1H) MHz operating frequency. For hydrolytic stability assaying, **1a−d**, **2a−d**, and **3a−c** (1−2 mg/mL) were dissolved in DMSO-d_6/D_2O (10/90) and ^1H NMR spectra were measured after 0.5, 6, 24, 48, and 72 h at room temperature.

Sulforhodamine B Cytotoxicity Assay. HCT116 and NCI-H460 cells were supplied by ATCC, while SiHa cells were supplied by Dr. David Cowan, Ontario Cancer Institute, Canada. Cells were grown in αMEM (Life Technologies) supplemented with 5% fetal calf serum (Moregate Biotech) at 37 °C in a humidified incubator with 5% CO_2. Cells were seeded at 750 (HCT116, NCI-H460) and 4000 (SiHa) cells/well in 96-well plates and left to settle for 24 h. Compounds were added to the plates in a series of 3-fold dilutions in 0.5% DMSO at the highest concentration for 72 h before the assay was terminated by addition of 10% trichloroacetic acid (Merck Millipore) at 4 °C for 1 h. Cells were stained with 0.4% sulforhodamine B (Sigma-Aldrich) in 1% acetic acid for 30 min in the dark at room temperature and then washed with 1% acetic acid to remove unbound dye. The stain was dissolved in unbuffered Tris base (10 mM; Serva) for 30 min on a plate shaker in the dark and quantitated on a BioTek EL808 microplate reader at an absorbance of 490 nm with a reference wavelength of 450 nm to determine the percentage of cell-growth inhibition by determining the absorbance of each sample relative to a negative (no inhibitor) and a no-growth control (day 0).

IC_{50} values were calculated with SigmaPlot 12.5 (Systat Software Inc.) using a three-parameter logistic sigmoidal dose−response curve between the calculated growth inhibition and the compound concentration. The presented IC_{50} values are the mean of at least 3 independent experiments, where 10 concentrations were tested in duplicate for each compound.

Molecular Modeling. The compounds were docked to the crystal structure of COX-2 (PDB ID 4M11, resolution 2.45 Å),[20] which was obtained from the Protein Data Bank (PDB).[42,43] The program Scigress Ultra version FJ 2.6[44] was used to prepare the crystal structure for docking. Hydrogen atoms were added, and the cocrystallized meloxicam was removed as well as crystallographic



water molecules. The Scigress software suite was also used to build the inhibitors and the MM2[45] force field to optimize the structures. The center of the binding pocket was defned as the position of the hydroxyl oxygen atom in meloxicam (*x* = 67.830, *y* = 15.016, *z* = 24.355)[25] with 20 Å radius. Fifty docking runs were allowed for each ligand with defult search efficiency (100%). The basic amino acids L-lysine and L-arginine were defned as protonated. Furthermore, L-aspartic and L-glutamic acids were assumed to be deprotonated. The GoldScore (GS),[34] ChemScore (CS),[46,47] Chem Piecewise Linear Potential (ChemPLP),[48] and Astex Statistical Potential (ASP)[49] scoring functions were implemented to validate the predicted binding modes and relative energies of the ligands using the GOLD v5.4 software suite. The parameter file for GS was augmented for Ru and Os according to Sciortino et al.[35] Meloxicam was redocked into the binding pocket using the four scoring functions and correlated to the cocrystallized conformation. The root-mean-square deviation between the heavy atoms of the cocrystallized ligand and its docked counterparts was as follows: ChemPLP, 1.03 Å; ASP, 1.62 Å; CS, 1.74 Å; GS, 1.20 Å.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.organomet.8b00751.

NMR spectra, X-ray crystallographic data, and molecular modeling data (PDF)

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Organometallics XXXX, XXX, XXX−XXX

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CCDC 1872872−1872875 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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