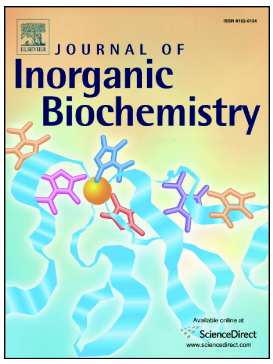

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Anticancer Ru(η^6-*p*-cymene) Complexes of 2-Pyridinecarbothioamides: A Structure–Activity Relationship Study

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ABSTRACT

Ru(II) and Os(II) complexes of 2-pyridinecarbothioamide ligands were introduced as orally administrable anticancer agents (S.M. Meier, M. Hanif, Z. Adhireksan, V. Pichler, M. Novak, E. Jirkovsky, M.A. Jakupec, V.B. Arion, C.A. Davey, B.K. Keppler, C.G. Hartinger, *Chem. Sci., 2013, 4, 1837–1846*). In order to identify structure-activity relationships, a series of *N*-phenyl substituted pyridine-2-carbothiamides (PCAs) were obtained by systematically varying the substituents at the phenyl ring. The PCAs were then converted to their corresponding $Ru^{II}(\eta^6$-p-cymene) complexes and characterized spectroscopically and by X-ray diffraction as well as in terms of stability in water and HCl. The cytotoxic activity of the PCA ligands and their respective organoruthenium compounds was evaluated in a panel of cell lines (HCT116, H460, SiHa and SW480). The lipophilic PCAs **1–4** showed cytotoxicity in the low micromolar range and **6** was the most potent compound of the series with an IC_{50} value of 1.1 μM against HCT116 colon cancer cells. These observations were correlated with calculated octanol/water partition coefficient (clog*P*) data and quantitative estimated druglikeness. A similar trend as for the PCAs was found in their Ru complexes, where the complexes with more lipophilic ligands proved to be more cytotoxic in all tested cell lines. In general, the PCAs and their organoruthenium derivatives demonstrated excellent drug-likeness and cytotoxicity with IC_{50} values in the low micromolar range, making them interesting candidates for further development as orally active anticancer agents.

Keywords

Anticancer Activity; Bioorganometallics; Organoruthenium Compounds; Oral Anticancer Agents; Pyridine-2-carbothiamide Ligands.

INTRODUCTION

Since the serendipitous discovery of cisplatin by Rosenberg [1] a variety of other metal complexes have been evaluated as anticancer agents with the aim to improve the activity and lessen the side effects [2-8]. Among the metal compounds, ruthenium compounds have the largest potential as anticancer drugs, as they are usually less toxic than cisplatin and hence better tolerated *in vivo* [3-5,9-12]. Ru is the main building block of the clinically evaluated anticancer agents imidazolium *trans*-[tetrachlorido(DMSO)(imidazole)ruthenate(III)] (NAMI-A), indazolium *trans*-[tetrachloridobis(1H-indazole)ruthenate(III)] (KP1019) and the sodium salt analogue of KP1019, *sodium* trans-[tetrachloridobis(1H-indazole)ruthenate(III)]) (NKP-1339) [13,14]. NAMI-A showed strong efficacy towards solid tumor metastases, but its clinical development was recently halted [15], whereas the indazole complexes KP1019 and NKP-1339 demonstrated excellent activity in several primary tumor models as well as in the clinic [13,14].

Organo-Ru compounds have extensively been investigated as catalysts but they were also found to have potential as tumor-inhibiting agents [2-4,8,16]. $[Ru(cym)(pta)Cl_2]$ (RAPTA-C; pta = 1,3,5-triaza-7-phosphaadamantane, cym = η^6-p-cymene), and $[Ru(\eta^6\text{-biphenyl})(1,2\text{-ethylenediamine})Cl]^+$ (RM175) [2,17-20] are considered the lead structures for anticancer-active half-sandwich Ru(arene) compounds. They feature different modes of action [21,22], with RM175 being active in cisplatin resistant *in vivo* models and RAPTA-C inhibiting metastases *in vivo* [2,3,9,23]. Diverse approaches have been explored to fine-tune the pharmacological properties of this class of compounds. These include mono- and dinuclear Ru(η^6-arene) complexes with monodentate *P*-, *N*- or *S*- donor ligands or bidentate *N,N-, O,O-,* or *N*,*S*-chelators, clusters, photoactive tetranuclear porphyrin derivatives, or hexanuclear cages [3,8,24-27]. It has clearly been established now that the reactivity and antiproliferative properties of the Ru center are strongly dictated by the nature of the donor set of the ligands in the inner coordination sphere. Strategies to coordinate or tether bioactive ligands such as flavonoids, quinones, ethacrynic acid and non-steroidal anti-inflammatory drugs, to the Ru(η^6-arene) fragment resulted in promising bioactive agents [25,28-30].

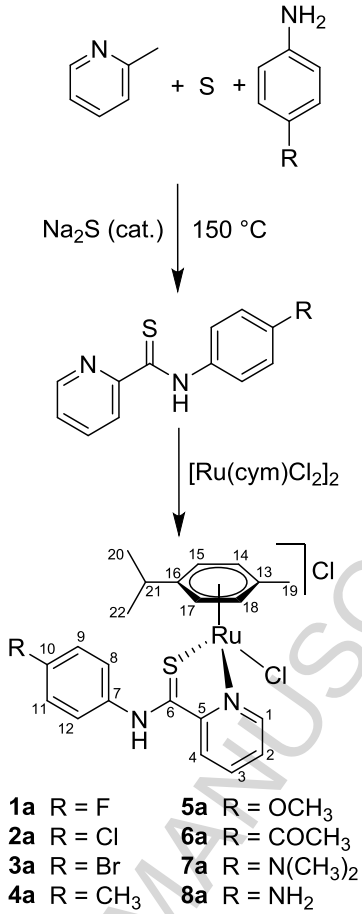
Pyridine-2-carbothioamides (PCAs) are another class of bioactive compounds. We previously reported Ru^{II} and Os^{II} complexes of PCAs that exhibited excellent

antiproliferative activities against different cancer cell lines with IC_{50} values in the low micromolar concentration range [24]. In contrast modification of the PCA ligand with a maleimide moiety rendered them inactive [31]. These compounds demonstrated outstanding stability in acidic conditions and together with significant lipophilicity, this makes them suitable candidates to evaluate the potential for oral administration. Activity *in vivo* after oral administration was recently demonstrated and linked to selective binding to plectin, and therefore they were termed plecstatins [32].

With the aim to establish structure activity relationship and to investigate the influence of the lipophilicity of the coordinated ligand with regard to biological activity, we expanded the series of pyridine-2-carbothioamide complexes substituted at the phenyl ring by varying the substituents in terms of electron-withdrawing and -donating properties as well as considering the protonation potential of the substituents. We established their biological activity against a panel of cell lines while attempting to rationalize their cytotoxicity with regards to the physicochemical properties.

Results and discussion

The PCA ligands **6** and **7** were synthesized by adopting a literature procedure used before for the preparation of **1**–**5** and **8** [24,33,34]. Briefly, the *N*-substituted aniline was refluxed for 48–72 h with an excess of sulfur and 2-picoline in the presence of catalytic amounts of sodium sulfide (Scheme 1). After work up, the ligands were purified by recrystallization from methanol/acetonitrile, to yield the PCAs from 77 to 83% yield, which is in a similar range as reported previously for related compounds [24,33].



Scheme 1. Synthesis of the PCA ligands **1**–**8** and the respective Ru(cym)Cl complexes **1a**–**8a**.

The PCA ligands were characterized by NMR spectroscopy, ESI-MS, elemental and single crystal X-ray diffraction analysis, if crystals were obtained. The ^{1}H NMR spectra of PCAs in deuterated solvents ($CDCl_3$/DMSO-d_6) featured the thioamide proton resonance at *ca*. 12 ppm. Comparison of the chemical shifts found for equivalent 2-picolinamides shows that the amide protons of **6** and **7** were more deshielded which caused a downfield shift of *ca* 2.5 ppm [35]. The chemical shifts of the individual pyridine proton and carbon atoms were observed in the range 7.65–8.70 ppm and 124.2–157.4 ppm, respectively, and both were practically unaffected by the nature of *N*-phenyl substituents which however impacted the proton and carbon atom shifts observed for the phenyl ring. For example, the H-9/H-12 protons as well as H-8/H-12 protons of ligands **3** and **4**, bearing electron-withdrawing chloro and electron-donating methyl substituents, respectively, were shifted by ~1 ppm. A similar trend was observed for the C9/C11 and C8/C12 carbon atoms with chemical shifts of ~3 ppm in the ^{13}C{^{1}H} NMR spectra.

Single crystals of the ligands *N*-(4-bromophenyl)pyridine-2-carbothioamide **3** and *N*-(4-acetylphenyl)pyridine-2-carbothioamide **6**, suitable for X-ray diffraction analysis, were obtained by slow evaporation from methanol and they crystallized in the triclinic and monoclinic space groups *P*-1 and $P2_1/c$, respectively. Selected bond lengths and angles are listed in Table 1 and the crystallographic data are shown in Table S1. In the molecular structures of both **3** and **6** (Figure 1), the pyridine and phenyl ring are co-planar. In general, the structures of both compounds are very similar. The C–S bond lengths are approximately the same, as were the torsion angles for S–C6–C5–N1 at -179.7(1) and -172.1(1)°. Both **3** and **6** showed an offset π-stacking interaction between the phenyl substituents of adjacent molecules.

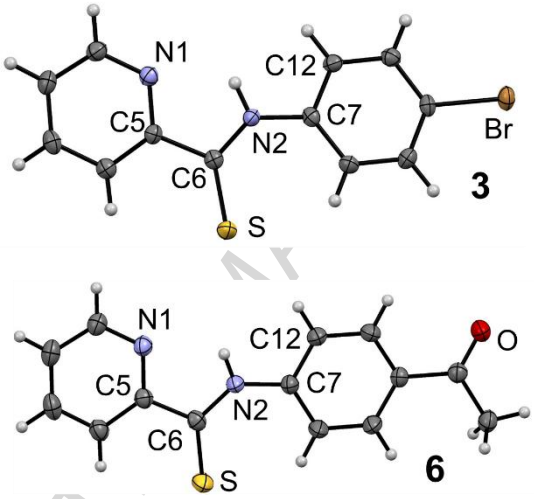


Figure 1. The molecular structures of **3** (top) and **6** (bottom) drawn at 50% probability level.

Table 1. Selected bond lengths (Å) and angles (°) for ligands **3** and **6** and complexes **4a** and **5a**.

	3	**6**	**4a**	**5a**
Ru–S	-	-	2.3469(7)	2.3483(16)
Ru–Cl1	-	-	2.4001(7)	2.4059(17)
Ru–N1	-	-	2.102(2)	2.106(5)
C6–S	1.662(18)	1.656(19)	1.695(3)	1.699(6)
C6–N2	1.341(2)	1.347(2)	1.319(4)	1.318(7)
C6–C5	1.515(2)	1.504(3)	1.484(4)	1.477(8)
C5–N1	1.345(2)	1.341(2)	1.353(3)	1.375(8)
C1–N1	1.331(2)	1.338(2)	1.350(3)	1.342(7)
C7–N2	1.405(2)	1.403(2)	1.433(3)	1.433(7)
N1–Ru–S			81.36(6)	81.53(14)
N1–Ru–Cl1			83.68(6)	83.17(14)
S–Ru–Cl1			89.44(3)	90.40(6)

The *N*-phenyl-substituted pyridine-2-carbothioamides (PCAs) **1**–**8** were used to prepare a series of new Ru(cym) complexes **2a**–**8a** and for comparison plecstatin-1 **1a** [24,32] (Scheme 1) by adding the dimeric precursor $[Ru(cym)Cl]_2$ in absolute dichloromethane to a solution of the respective PCA ligand in absolute tetrahydrofuran. After stirring the reaction mixture for 4 h at 40 °C and workup, the mononuclear complexes were obtained in 62–87% yield.

Surprisingly, conducting this complexation reaction under the same conditions in methanolic solution resulted in the appearance of two species in the ^{1}H NMR spectra. In this protic solvent, the thioamide group was deprotonated which resulted in *N*,*N′*-coordination (10–20%) of the mono-anionic PCA rather than *N*,*S*-coordination as in case of neutral PCA [35,36]. This switch in coordination mode in protic solvents was found to be dependent on time, temperature and the pH value. In an attempt to avoid formation of a mixture of coordination isomers, we aimed to shift the equilibrium to maintain the thioamide in its protonated state. For this purpose, the PCAs were dissolved in 3.3% acetic acid/methanol and Ru(cym) was added. This procedure yielded only one species with PCA acting as a neutral *N,S*-chelating ligand. However, this method resulted in low yield (40–54%) which could be improved to 80–90% when absolute THF and DCM were used. Furthermore, **3a** was also obtained by using absolute DCM as the solvent and stirring the reaction mixture for 4 h at room temperature, following a literature procedure [31]. Unfortunately, the

latter method cannot be applied for all ligands because of their low solubility in DCM, which therefore requires the use of the solvent combinations as mentioned before.

The ^{1}H NMR spectra of the organometallic compounds were recorded in *d*$_4$-MeOD/$CDCl_3$. The H4 and H1 proton of the pyridine ring were most deshielded, which confirms *N,S*-bidentate coordination of the pyridine nitrogen and thioamide moiety to the Ru. The most drastic shift compared to the ligand was observed for H1 at *ca.* 1 ppm (compare Figure 2 for **3** and **3a**). The methyl protons H19 of *p*-cymene appeared as singlets while the isopropyl protons H20 and H22 coupled to H21 and therefore were detected as two doublets in the range of 2.10–2.43 ppm and 1.02–1.21 ppm, respectively. The *p*-cymene aromatic protons H14, H15, H17 and H18 were observed in the range of 5.54–6.94 ppm as four doublets (Figure 2). Signal for the thioamide proton were not observed in all complexes, possibly due to fast exchange of the NH proton in deuterated solvents. In the $^{13}C\{^{1}H\}$ NMR spectra of the Ru complexes, the quaternary carbon atom of the thioamide functionality appeared in the range of ~192–197 ppm for complexes **4a** and **7a**, however, this carbon atom was not detectable for the other complexes. Similarly, C5 and C7 were not visible in **3a**. The pyridine carbon atoms C5 and C1 next to the pyridine nitrogen coordinated to the Ru center were detected most downfield and appeared in the range of 155–160 ppm and 157–160 ppm, respectively. The remaining carbon atoms C2, C3 and C4 of the pyridine ring appeared in the range of 123.4–140.2 ppm.

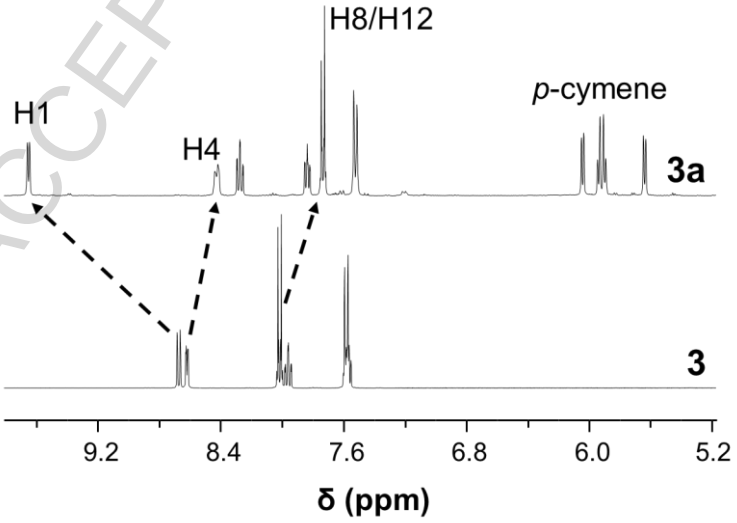


Figure 2. Comparison of the ^{1}H NMR spectra in *d*$_4$-MeOD recorded for ligand **3** and after complexation with $[Ru(cym)Cl_2]_2$. The protons of the PCA ligand were shifted after

coordination to Ru and the most significant change was observed for H1 after complexation as indicated by a shift from 8.67 ppm in **3** to 9.66 ppm in **3a**.

The complexes were also characterized by electrospray ionization mass spectrometry (ESI-MS). The ESI-mass spectra of all complexes featured the $[M - 2Cl - H]^+$ ions in dichloromethane solutions.

The molecular structures of **4a** and **5a** were determined by single crystal X-ray crystallography. Crystallographic parameters including bond lengths and bond angles are given in Tables 1 and S2. Single crystals of **4a** were grown by slow diffusion of diethyl ether into a methanol solution and crystallized in the space group *C*2/*c*. A single crystal of **5a** with a space group of $P2_1/n$ was obtained by slow evaporation of a saturated solution of the complex in methanol and ethyl acetate. The complexes crystallized in monoclinic crystal systems with the Ru center adopting a pseudooctahedral coordination geometry.

In contrast to organometallic *N*-phenyl-picolinamido complexes where an *N*,*N*' coordination mode was found [35], the molecular structures of **4a** and **5a** showed an *N,S*-coordination mode of the PCA ligands towards ruthenium (Figure 3). The charge of these cationic complexes was balanced by chloride as the counterion. The bite angles between adjacent atoms in the coordination sphere of ruthenium were around 85°. The Ru–S bond lengths at *ca*. 2.347 Å were very similar in the complexes and the C6–S bond was elongated as compared to the ligands, indicating more single bond character (Table 2). In line, the C6–N2 distance was shorter than in **3** and **6**, indicating increased double bond character upon coordination of the Ru center to the S atom. The Ru–Cl1 bond lengths observed were 2.4001(7) and 2.4059(17) Å, respectively for **4a** and **5a** (Table 1). The torsion angle S–C6–C5–N1 for a structurally-related osmium complex was 4.1(4)° [24], while it was 17.63 and 19.14° for **4a** and **5a**, and analogous Ru–$PCA_{maleimide}$ derivative [31]. In contrast the analogous torsion angles C6–N2–C7–C12 for the Ru complexes **4a** and **5a** were smaller than in the Os derivative but similar to the Ru–$PCA_{maleimide}$ derivative [31].

In the structures of **4a** and **5a**, two enantiomers were present. In case of **5a** they were linked through π stacking of the pyridine moieties of the PCA ligand (3.958 Å; Figure S1). In addition, the chloride counterions Cl2 were found in both structures to be involved in H bonds with the amide NH and the N2–H···Cl2 distances were 3.078 and 3.071 Å for **4a** and **5a**.

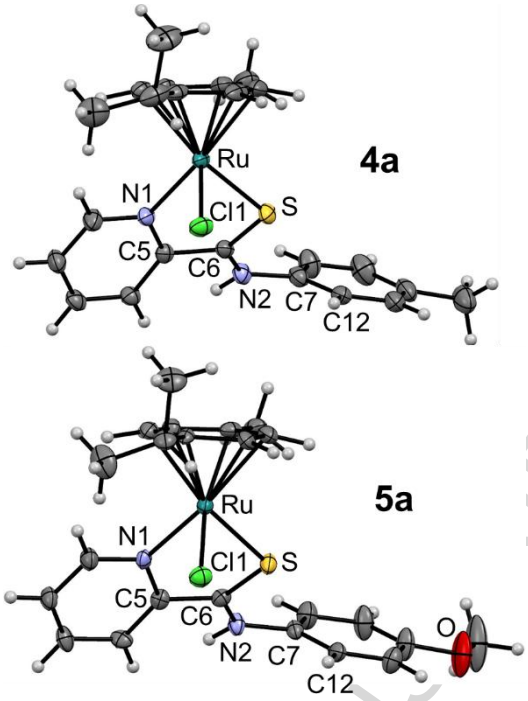


Figure 3. The molecular structures and atom numbering for metal complexes **4a** and **5a** at 50% probability level. Solvent molecules and counterions were omitted for clarity.

Stability in aqueous solution

The parent compounds to this series of PCA–Ru(cym) derivatives were shown to be very stable under acidic conditions [24], while they undergo a chlorido/aqua ligand exchange reaction in water. To determine the aqueous stability of complexes **1a** and **2a**, they were dissolved in D_2O and ^1H NMR spectra were recorded over a time course of 0.5, 3, 24, 48 and 72 h (Figure 4). The compounds hydrolyzed very quickly to form an aqua complex and even after 30 mins of incubation in D_2O, more than 60% of the complex was already hydrolyzed. While after 2 h two sets of peaks for the chlorido and aqua complexes can be detected, the ^1H NMR spectrum recorded after 24 h shows the conversion to the aqua complex to be complete, as indicated by a well-resolved spectrum. The formed aqua species were stable for more than a week as demonstrated by ^1H NMR spectroscopy.

The NMR experiments were complemented by ESI-MS studies with a special focus on the stability in presence of 60 mM HCl, and compared to that in aqueous solutions. The former environment was chosen to resemble stomach conditions, and estimate stability in acidic media as one of the beneficial conditions for potential oral administration. The incubation mixtures were analyzed after 0.5, 24, 72 h and 7

days. The spectrum of **1a** dissolved in water featured a peak at *m/z* 467.0556 as the base peak which was assigned to [**1a** – H – 2Cl]$^+$ (*m/z*$_{calc}$ 467.0531; Figure S2). The spectrum hardly changed over the time course of a week and the latter peak was still the most abundant. Incubation of **1a** in 60 mM HCl on the other hand gave a mass spectrum in which the peak assigned to the [**1a** – H – 2Cl]$^+$ was still the most abundant, but in addition a peak at *m/z* 503.0302 was detected and assigned to [**1a** – Cl]$^+$ (*m/z*$_{calc}$ 503.0295). In HCl solution an exchange of the thiocarbamide *S* with an *O* atom was observed with peaks at m/z 451.0778 and 487.0541 for [**1a**^{O} – H – 2Cl]$^+$ and [**1a**^{O} – Cl]$^+$ respectively (Figure S2).

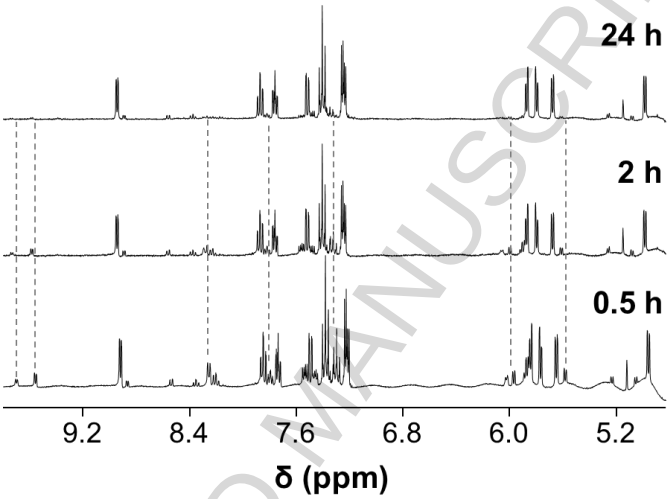


Figure 4. ^{1}H NMR spectra of **1a** in D_2O recorded after 0.5, 2 and 24 h, showing the chlorido/aqua ligand exchange reaction to occur very rapidly. The dashed grey lines indicate the positions of the protons of the chlorido complex **1a**.

In vitro antiproliferative activity and lipophilicity

Carbothioamides are potent gastric mucosal protectants [37]. The fluoro-substituted PCA **1** and structurally-related *N*-(2,6-difluorophenyl)-pyridine-2-carbothioamide exhibited very low acute toxicities in mouse models, indicating high tolerability *in vivo* [37]. We reported earlier that the coordination of Ru or Os centers to PCAs results in potent antiproliferative agents in human ovarian teratocarcinoma (CH1), colon carcinoma (SW480) and non-small cell lung cancer (A549) cells after 96 h exposure with the *p*-fluoro derivative **1a** being the most potent Ru compound in the MTT assay [24]. This derivative was included in this study as a benchmark and compared to its ligand **1** and the analogous **2**–**8** as well as their respective complexes **2a**–**8a** in

terms of their antiproliferative activity in sulforhodamine B (SRB) assays with human colorectal carcinoma (HCT116), non-small cell lung carcinoma (H460), cervical carcinoma (SiHa) and colon carcinoma (SW480) cells. The ability of ligands and complexes to inhibit the growth of cancer cells is summarized in Table 2.

The Ru(cym) complexes **1a**–**5a** and **7a** exhibited potent cytotoxic activity in HCT116, NCI-H460 and SiHa cells with IC_{50} values in the low micromolar range, which is clearly associated with the cytotoxic activity of their respective PCA ligands and gave similar IC_{50} values as the complexes in these cell lines. However, in case of **6a** and **8a**, complexation reduced the cytotoxic potency of the ligands, with **8a** being the least active derivative. The SW480 human colon carcinoma cells were the most chemo-resistant cells included in this assay. However, with the exception of **8a**, complexation significantly enhanced the cytotoxicity of ligands **1**–**7** and the complexes **1a**–**7a** gave IC_{50} values in the range 7.8–15 μM in this cell line. Surprisingly, the ruthenium complex **6a** bearing the most active ligand **6** was less cytotoxic than its uncoordinated ligand. It should be noted that the chloride ions present in the cell culture medium should prevent chlorido/aqua ligand exchange reactions to occur.

Table 2. *In vitro* anticancer activity (mean IC_{50} values ± standard deviations) of PCA ligands **1**–**8** and their respective Ru(cym) complexes **1a–8a** in human colorectal (HCT116), non-small cell lung (NCI-H460) and cervical (SiHa) carcinoma cell lines (exposure time 72 h).

Compounds	IC_{50} value (μM)			
	HCT116	**NCI-H460**	**SiHa**	**SW480**
1	5.7± 0.7	7.8 ± 1.8	16 ± 6	33 ± 2
2	4.3 ±1.3	3.8 ± 0.3	10 ± 1	23 ± 2
3	5.2 ± 1.3	5.0 ± 0.2	11 ± 1	23 ± 6
4	9.2 ± 2.3	9.5 ± 0.5	28 ± 3	149 ± 69
5	9.8 ± 3.4	11 ± 1	35 ± 6	77 ± 20
6	1.1 ± 0.2	1.1 ± 0.1	5.9 ± 2.1	25 ± 12
7	13 ± 3	12 ± 1	38 ± 5	96 ± 15
8	59 ± 7	52 ± 1	97 ± 0.2	>300
1a	6.5 ± 0.3	10 ± 2	8.3 ± 0.7	9.9 ± 0.7
2a	5.5 ± 0.4	6.2 ± 0.5	13 ± 1	7.8 ± 0.7
3a	7.1 ± 1.2	8.2± 0.8	15 ± 1	9.9 ± 1.3
4a	8.7 ± 2.5	9.4 ± 1.0	19 ± 1	8.8 ± 1.5
5a	12 ± 1	15± 2	35 ± 4	11 ± 1
6a	17 ± 2	23± 4	50 ± 3	15 ± 1
7a	10 ± 0.4	15 ± 1	33 ± 2	12 ± 1

8a	146 ± 19	> 300	> 300	> 300

As the cytotoxicity of anticancer agents is often linked to their ability to accumulate in cells, the lipophilicity of **1**–**8** was calculated. Higher lipophilicity allows compounds to pass through membranes more efficiently and is often given as octanol/water partition coefficient (log*P*). The octanol/water partition coefficient was calculated (clog*P*) using Chemdraw 12.0, molinspiration (www.molinspiration.com) and ALOGPS 2.1 (Table S3). As the Ru(cym)Cl moiety is present in all the organoruthenium complexes **1a**–**8a**, the clog*P* values should depend on ligands **1**–**8** only. In general, the most lipophilic ligands **1**–**4** were the most potent cytotoxins when coordinated to a Ru moiety. The least lipophilic ligand **8** resulted in the least active anticancer agent **8a**, suggesting that the lipophilicity indeed plays a major role in the bioactivity of these compounds.

Quantitative estimate of drug-likeness of ligands

As the compounds were developed with the aim to achieve oral application, the quantitative estimate of druglikeness was calculated to predict their potential as orally active compounds. The weighted quantitative estimate of drug-likeness of the ligands based on maximum information content (QED_w^{mo}) was determined for ligands **1**–**8** (Table S4). The PCAs **1**–**8** showed excellent druglikeness with QED_w^{mo} values around 0.8–0.9. The overall highest QED_w^{mo} value was found for **6** and **7** with a value of 0.91. It was also ligand **6** which showed the highest antiproliferative activity, while surprisingly their complexes were only moderately active in the cytotoxicity assay. Interestingly, **1–4** were found to have fairly similar QED_w^{mo} and IC_{50} values in all cell lines. Furthermore, their respective complexes also shared the same trend in cytotoxic studies.

Conclusions

In this structure-activity relationship study, we have expanded on our series of *N*-phenyl substituted pyridine-2-carbothioamides and their organometallic Ru^{II}(cym) complexes, which we reported to be potent anticancer agents in previous studies [24]. The new derivatives were modified at the phenyl ring by introducing electron-withdrawing and -donating substituents and offering the option of protonation. The

optimization of the synthesis of the complexes resulted in the development of three procedures to rule out the formation of coordination isomers and purely obtain complexes in the desired *N*,*S*-coordination mode, as was demonstrated by X-ray diffraction analysis for two derivatives as well as spectroscopic studies. Compound **1a** was found to be stable in aqueous solution over a period of 1 week after undergoing a chlorido/aqua ligand exchange reaction after dissolution. Incubation of **1a** in 60 mM HCl, to resemble stomach conditions, resulted in sulfur/oxygen exchange of the PCA. Most of the PCAs and their organoruthenium compounds were shown to be potent anticancer agents in human cancer cell lines. The biological activity was correlated with the clog*P* values calculated for the PCAs and the most lipophilic compounds were shown to be most potent in the *in vitro* anticancer activity assays as well. QED_w^{mo} of the PCAs supported their potential for development as orally active metallodrugs.

Acknowledgments

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EXPERIMENTAL SECTION

Materials and Methods

All air- and moisture-sensitive reactions were carried out under nitrogen atmosphere using standard Schlenk techniques. Chemicals obtained from commercial suppliers were used as received and were of analytical grade. Tetrahydrofuran (THF) and dichloromethane (DCM) were first dried through a solvent purification system (LC Technology Solutions Inc., SP-1 solvent purifier), degassed under a N_2 flow, and the stored in a Schlenk flask. Methanol (MeOH) was dried using standard procedures and stored over activated molecular sieves (3Å).

4-Fluoroaniline, α-terpinene, 2-picoline, and $Na_2S{\cdot}9H_2O$ were purchased from Merck, 4-chloroaniline, 4-bromoaniline, *p*-toluidine, *p*-anisidine, 4-aminoacetophenone, *N*,*N*-dimethyl-*p*-phenylenediamine and sulfur from Sigma-Aldrich, and $RuCl_3{\cdot}3H_2O$ (99%) from Precious Metals Online.

Bis[dichlorido(η^6-*p*-cymene)ruthenium(II)] [38] and the ligands *N*-(4-fluorophenyl)pyridine-2-carbothioamide **1** [24], *N*-(4-chlorophenyl)pyridine-2-carbothioamide **2** [34], *N*-(4-bromophenyl)pyridine-2-carbothioamide **3**, *N*-(*p*-tolyl)pyridine-2-carbothioamide **4** [34], *N*-(4-methoxyphenyl)pyridine-2-carbothioamide **5** [39], *N*-(4-aminophenyl)pyridine-2-carbothioamide **8** [33], and [chlorido(η^6-p-cymene)(*N*-(4-fluorophenyl)pyridine-2-carbothioamide)ruthenium(II)] chloride **1a** [24] were synthesized by adopting standard procedures.

^1H and $^{13}C\{^1H\}$ and 2D (COSY, HSQC, HMBC) NMR spectra were recorded on Bruker Avance AVIII 400 MHz NMR spectrometer at ambient temperature at 400.13 MHz (^1H) or 100.61 MHz ($^{13}C\{^1H\}$). Chemical shifts are reported versus $SiMe_4$ and were determined by reference to the residual solvent peaks.

High resolution mass spectra were recorded on a Bruker micrOTOF-QII mass spectrometer in positive electrospray ionization (ESI) mode. Elemental analyses were carried out on an Exeter Analytical Inc-CE-440 Elemental Analyser. X-ray diffraction measurements of single crystals were carried out on a Bruker SMART APEX2 diffractometer with a CCD area detector using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). Structure solution were performed with the SHELXL-2013 program package [40], structure refinements with the Olex2 program package [41,42]. The molecular structures were visualized using Mercury 3.5.1.

General Procedure for the Synthesis of Ligands

For the synthesis of carbothioamide ligands **6** and **7**, a mixture of *N*-substituted aniline (25 mmol), sulfur (75 mmol), $Na_2S·9H_2O$ (0.5 mol %) and 2-picoline (15 mL) was refluxed at 150 °C for 72 h [24]. After cooling, the solvent was evaporated under vacuum. The dark solid residue was dissolved in dichloromethane and twice filtered through a bed of silica gel. The solvent was evaporated using a rotary evaporator. Pure product was obtained after recrystallization from methanol.

***N*-(4-Acetylphenyl)pyridine-2-carbothioamide (6)**

Compound **6** was prepared following general procedure using 4-acetylaniline (3.37 g, 25 mmol), sulfur (2.40 g, 75 mmol), $Na_2S·9H_2O$ (0.12 g, 0.5 mol%) and 2-picoline (15 mL). Yield: 77% (4.93 g, yellow-orange solid). Elemental analysis found: C, 64.77; H, 4.67; N, 10.81, calculated for $C_{14}H_{12}N_2OS·0.2H_2O$: C, 64.69; H, 4.81; N, 10.78. ^{1}H NMR (400.13 MHz, DMSO-d_6, 25 °C): δ = 12.47 (s, 1H, N*H*), 8.70 (d, ^3J = 6 Hz, 1H, H-4), 8.52 (d, ^3J = 8 Hz, 1H, H-1), 8.19 (d, ^3J = 8 Hz, 2H, H-9/H-11), 8.05 (m, 3H, H-3/H-8/H-12), 7.68 (ddd, ^3J = 7 Hz, ^3J = 5 Hz, ^4J = 1 Hz, 1H, H-2), 2.59 (s, 3H, COC$\underline{H}_3$) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, DMSO-d_6, 25 °C): δ = 196.8 ($\underline{C}OCH_3$), 190.7 (C-6), 152.6 (C-5), 147.4 (C-1), 143.1 (C-7), 137.8 (C-3), 134.3 (C-10), 128.7 (C-9/C-11), 126.6 (C-8/C-12), 124.7 (C-2), 123.4 (C-4), 26.7(C_{ar}-$COCH_3$) ppm. MS (ESI$^+$): *m/z* 279.0568 $[M + Na]^+$ (m_{ex} = 279.0563).

***N*-(4-(Dimethylamino)phenyl)pyridine-2-carbothioamide (7)**

Compound **7** was prepared following general procedure using *N*,*N*-dimethyl-*p*-phenylenediamine (3.40 g, 25 mmol), sulfur (2.40 g, 75 mmol), $Na_2S·9H_2O$ (0.12 g, 0.5 mol%) and 2-picoline (15 mL). Yield: 5.34 g (83%, red needles). Elemental analysis found: C, 64.33; H, 5.71; N, 15.64, calculated for $C_{14}H_{15}N_3S·0.3H_2O$: C, 63.99; H, 5.98; N, 15.99. ^{1}H NMR (400.13 MHz, DMSO-d_6, 25 °C) δ = 12.09 (s, 1H, N*H*), 8.65 (d, ^3J = 7 Hz, 1H, H-4), 8.53 (d, ^3J = 8 Hz, 1H, H-1), 8.02 (td, ^3J = 7 Hz, ^4J = 1 Hz, 1H, H-3), 7.90 (m, 2H, H-8/H-12), 7.62 (ddd, ^3J = 7 Hz, ^4J = 1 Hz, 1H, H-2), 6.76 (d, ^3J = 9 Hz, 2H, H-9/H-11), 2.93 (s, 6H, N($C\underline{H}_3$)$_2$) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, DMSO-d_6, 25 °C): δ = 186.5 (C-6), 152.8 (C-5), 148.7 (C-10), 147.2 (C-1), 137.7 (C-3), 128.4 (C-7), 126.0 (C-8/C-12), 124.4 (C-2), 124.2 (C-4), 111.5 (C-9/C-11), 40.1 (C_{ar}-$N(CH_3)_2$) ppm. MS (ESI$^+$): *m/z* 280.0884 $[M + Na]^+$ (m_{ex} = 280.0879).

General procedures for the syntheses of metal complexes 2a–8a

Method A. A solution of $[Ru(cym)Cl_2]_2$ in dry DCM was added to a stirred solution of carbothioamide ligand in dry THF. The reaction mixture was stirred for 4 h at 40 °C under nitrogen atmosphere. A change in color from brown to deep red was observed immediately after the addition of dimer. The solvent was evaporated and the residue was dissolved in a minimal volume of DCM, followed by addition of *n*-hexane that resulted in immediate precipitation. After placing it in the fridge overnight, the precipitate was filtered, and dried under reduced pressure.

Method B. The respective carbothioamide was dissolved in absolute DCM (20 mL) and a solution of $[Ru(cym)Cl_2]_2$ in absolute DCM (20 mL) was added. The reaction mixture was stirred for 4 h at room temperature under nitrogen atmosphere. The solvent was concentrated *in vacuo* to *ca.* 5 mL and *n*-hexane was added for precipitation in the fridge. The solvent was decanted and subsequent drying *in vacuo* yielded analytically pure solid product.

Method C. The carbothioamide ligand was dissolved in dry MeOH (30 mL) followed by addition of 1 mL acetic acid. $[Ru(cym)Cl_2]_2$ was added to the stirred solution of the ligand and stirred for another 4 h under nitrogen atmosphere. The solvent was evaporated using a rotary evaporator. The solid residue was washed with ethyl acetate (2 × 10 mL) followed by with diethyl ether (2 × 10 mL) and dried under vacuum to isolate the desired product.

[Chlorido(η^6-*p*-cymene)(*N*-(4-chlorophenyl)pyridine-2-carbothioamide)ruthenium(II)] chloride (2a)

Compound **2a** was synthesized following the general synthetic procedure **A** using *N*-(4-clorophenyl)pyridine-2-carbothioamide (100 mg, 0.40 mmol) and $[Ru(cym)Cl_2]_2$ (122 mg, 0.20 mmol). Yield: 77% (171 mg, red solid). Elemental analysis found: C, 48.63; H, 4.24, N, 4.97, calculated for $C_{22}H_{23}Cl_3N_2RuS{\cdot}0.15C_6H_{14}$: C, 48.44; H, 4.46; N, 4.93. ^{1}H NMR (400.13 MHz, d_4-MeOD, 25 °C): δ = 9.63 (d, ^3J = 6 Hz, 1H, H-4), 8.40 (d, ^3J = 8 Hz, 1H, H-1), 8.25 (t, ^3J = 8 Hz, 1H, H-3), 7.81 (t, ^3J = 7 Hz, 1H, H-2), 7.56 (m, 4H, H-9/H-11/H8/12), 6.02 (d, ^3J = 6 Hz, 1H, H-15), 5.92 (d, ^3J = 6 Hz, 1H, H-17), 5.87 (d, ^3J = 6 Hz, 1H, H-18), 5.61 (d, ^3J = 6 Hz, 1H, H-14), 2.73 (sept, ^3J = 6

Hz, 1H, H-21), 2.20 (s, 3H, H-19), 1.20 (d, ^{3}J = 6 Hz, 3H, H-20), 1.13 (d, ^{3}J = 7 Hz, 3H, H-22) ppm. $^{13}C\{^{1}H\}$ NMR (100.61 MHz, d_4-MeOD, 25 °C): δ = 159.9 (C-1), 155.5 (C-5), 140.9 (C-3), 139.8 (C-7), 134.9 (C-10), 130.8 (C-9/C-11), 130.5 (C-2), 127.6 (C-8/C-12), 125.1 (C-4), 107.1 (C-16), 105.2 (C-13), 89.2 (C-15), 89.1 (C-17), 86.5 (C-18), 84.8 (C-14), 32.4 (C-21), 22.9 (C-20), 21.9 (C-22), 18.8 (C-19) ppm. MS (ESI^+): *m/z* 483.0236 $[M - 2Cl - H]^+$ (m_{ex} = 483.0231).

[Chlorido(η^6-*p*-cymene)(*N*-(4-bromophenyl)pyridine-2-carbothioamide)ruthenium(II)] chloride (3a)

Compound **3a** was synthesized following the general synthetic procedure **B** using *N*-(4-bromophenyl)pyridine-2-carbothioamide (100 mg, 0.34 mmol) and $[Ru(cym)Cl_2]_2$ (104 mg, 0.17 mmol). Yield: 70% (143 mg, dark red solid). Elemental analysis found: C, 44.39; H, 3.90; N, 4.63, calculated for $C_{22}H_{23}BrCl_2N_2RuS$: C, 44.09; H, 3.87; N, 4.67. ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ = 9.34 (d, ^{3}J = 6 Hz, 2H, H-4/H-1), 8.06 (t, ^{3}J = 8 Hz, 1H, H-3), 7.64 (d, ^{3}J = 8 Hz, 2H, H-8/H-12), 7.57 (m, 3H, H-2/H-9/H-11), 5.69 (d, ^{3}J = 6 Hz, 1H, H-15), 5.59 (d, ^{3}J = 6 Hz, 1H, H-17), 5.52 (d, ^{3}J = 6 Hz, 1H, H-18), 5.37 (d, ^{3}J = 6 Hz, 1H, H-14), 2.76 (sept, ^{3}J = 6 Hz, 1H, H-21), 2.20 (s, 3H, H-19), 1.21 (d, ^{3}J = 7 Hz, 3H, H-20), 1.14 (d, ^{3}J = 7 Hz, 3H, H-22) ppm. $^{13}C\{^{1}H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ = 157.1 (C-1), 139.7 (C-3), 136.1 (C-10), 132.4 (C-8/C-12), 128.6 (C-2), 127.0 (C-9/111),126.4 (C-4), 106.1 (C-16), 102.8 (C-13), 87.6 (C-15), 87.2 (C-17), 84.6 (C-18), 83.8 (C-14), 31.1 (C-21), 22.8 (C-20), 22.0 (C-22), 18.9 (C-19) ppm. MS (ESI^+): *m/z* 528.9731 $[M - 2Cl - H]^+$ (m_{ex} = 528.9723).

[Chlorido(η^6-*p*-cymene)(*N*-(*p*-tolyl)pyridine-2-carbothioamide)ruthenium(II)]chloride (4a)

Compound **4a** was synthesized following the general synthetic procedure **C** using *N*-(*p*-tolyl)pyridine-2-carbothioamide (100 mg, 0.44 mmol) and $[Ru(cym)Cl_2]_2$ (134 mg, 0.22 mmol).Yield: 47% (111 mg, dark red solid). Elemental analysis found: C, 52.04; H, 5.08; N, 5.00, calculated for $C_{23}H_{26}Cl_2N_2RuS{\cdot}0.1C_6H_{14}$: C, 52.19; H, 5.08; N, 5.16. ^{1}H NMR (400.13 MHz, d_4-MeOD, 25 °C): δ = 9.67 (d, ^{3}J = 6 Hz, 1H, H-4), 8.44 (d, ^{3}J = 8 Hz, 1H, H-1), 8.30 (td, ^{3}J = 8 Hz, ^{4}J = 1.5 Hz, 1H, H-3), 7.85 (td, ^{3}J = 7 Hz, ^{4}J = 1 Hz, 1H, H-2), 7.51 (d, ^{3}J = 8 Hz, 2H, H-8/H-12), 7.41 (d, ^{3}J = 8 Hz, 2H, H-8/H-12), 6.05 (d, ^{3}J = 6 Hz, 1H, H-15), 5.94 (d, ^{3}J = 6 Hz, 1H, H-17), 5.91 (d, ^{3}J = 6 Hz, 1H, H-18), 5.65 (d, ^{3}J = 6 Hz, 1H, H-14), 2.74 (sept, ^{3}J = 6 Hz, 1H, H-21), 2.24 (s, 3H, -

CH_3), 2.21 (s, 3H, H-19), 1.21 (d, ^3J = 7 Hz, 3H, H-20), 1.13 (d, ^3J = 7 Hz, 3H, H-22) ppm. $^{13}C\{^1H\}$ NMR (100.61 MHz, d_4-MeOD, 25 °C): δ = 193.7 (C-6), 160.2 (C-1), 154.7 (C-5), 141.1 (C-3), 140.8 (C-10), 136.4 (C-7), 131.4 (C-9/C-11), 130.8 (C-2), 126.1 (C-4), 125.0 (C-8/C-12), 107.3 (C-16), 105.5 (C-13), 89.3 (C-15), 89.2 (C-17), 86.7 (C-18), 85.0 (C-14), 32.4 (C-21), 22.9 (C-20), 21.9 (C-22), 21.3 (C-19), 18.8 (C_{ar}-CH_3) ppm. MS (ESI^+): *m/z* 463.0782 $[M - 2Cl - H]^+$ (m_{ex} = 463.0777).

[Chlorido(η^6-*p*-cymene)(*N*-(4-methoxyphenyl)pyridine-2-carbothioamide)ruthenium(II)] chloride (5a)

Compound **5a** was synthesized following the general synthetic procedure **A** using *N*-(4-methoxyphenyl)pyridine-2-carbothioamide (90 mg, 0.37 mmol) and $[Ru(cym)Cl_2]_2$ (113 mg, 0.18 mmol). Yield: 87% (183 mg, dark red solid). Elemental analysis found: C, 49.86; H, 4.53; N, 5.24; calculated for $C_{23}H_{26}Cl_2N_2ORuS$: C, 50.18; H, 4.76; N, 5.09. ^1H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ = 9.59 (d, ^3J = 9 Hz, 1H, H-4), 9.53 (d, ^3J = 5 Hz, 1H, H-1), 8.04 (t, ^3J = 9 Hz, 1H, H-3), 7.83 (d, ^3J = 8 Hz, 2H, H-8/H-12), 7.57 (t, ^3J = 6 Hz, 1H, H-2), 6.98 (d, ^3J = 9 Hz, 2H, H-9/H-11), 5.72 (d, ^3J = 6 Hz, 1H, H-15), 5.65 (d, ^3J = 6 Hz, 1H, H-17), 5.59 (d, ^3J = 6 Hz, 1H, H-18), 5.42 (d, ^3J = 6 Hz, 1H, H-14), 3.84 (s, 3H, -OCH_3), 2.76 (sept, ^3J = 6 Hz, 1H, H-21), 2.20 (s, 3H, H-19), 1.20 (d, ^3J = 7 Hz, 3H, H-20), 1.14 (d, ^3J = 7 Hz, 3H, H-22) ppm. $^{13}C\{^1H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ = 159.6 (C-5), 157.7 (C-1), 154.0 (C-10), 140.0 (C-3), 130.9 (C-7), 129.0 (C-8/C-12), 127.3 (C-2), 126.8 (C-4), 114.5 (C-9/C-11), 106.4 (C-16), 103.0 (C-13), 87.7 (C-15), 87.3 (C-17), 84.8 (C-18), 84.0 (C-14), 55.7 (-OCH_3), 31.1 (C-21), 22.8 (C-20), 22.0 (C-22), 18.9 (C-19) ppm. MS (ESI^+): *m/z* 479.0731 $[M - 2Cl - H]^+$ (m_{ex} = 479.0732).

[Chlorido(η^6-*p*-cymene)(*N*-(4-acetylphenyl)pyridine-2-carbothioamide)ruthenium(II)] chloride (6a)

Compound **6a** was synthesized following the general synthetic procedure **A** using *N*-(4-acetylphenyl)pyridine-2-carbothioamide (100 mg, 0.39 mmol) and $[Ru(cym)Cl_2]_2$ (116 mg, 0.19 mmol). Yield: 84% (197 mg, red solid). Elemental analysis found: C, 51.21; H, 4.68; N, 4.91, calculated for $C_{24}H_{26}Cl_2N_2ORuS$: C, 51.24; H, 4.66; N, 4.98. ^1H NMR (400.13 MHz, d_4-MeOD, 25 °C): δ = 9.66 (d, ^3J = 6 Hz, 1H, H-4), 8.44 (d, ^3J = 8 Hz, 1H, H-1), 8.29 (td, ^3J = 8 Hz, ^4J = 2 Hz, 1H, H-3), 8.19 (d, ^3J = 9 Hz, 2H, H-9/H-11), 7.84 (td, ^3J = 6 Hz, ^4J = 1 Hz, 1H, H-2), 7.74 (d, ^3J = 9 Hz, 2H, H-8/H-12),

6.05 (d, ^3J = 6 Hz, 1H, H-15), 5.94 (d, ^3J = 6 Hz, 1H, H-17), 5.90 (d, ^3J = 6 Hz, 1H, H-18), 5.65 (d, ^3J = 6 Hz, 1H, H-14), 3.77 (s, 3H, OCH_3), 2.75 (sept, ^3J = 6 Hz, 1H, H-21), 2.66 (s, 3H, $COCH_3$), 2.21 (s, 3H, H-19), 1.21 (d, ^3J = 7 Hz, 3H, H-20), 1.13 (d, ^3J = 7 Hz, 3H, H-22) ppm. $^{13}C\{^1H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ = 197.2 (CO), 159.2 (C-5), 157.3 (C-1), 139.9 (C-3), 136.0 (C-7),134.4 (C-10), 129.5 (C-9/C-11), 128.8 (C-8/C-12), 127.4 (C-2), 124.8 (C-4), 106.3 (C-16), 103.0 (C-13), 87.7 (C-15), 87.3 (C-17), 84.7 (C-18), 83.9 (C-14), 31.1 (C-21), 26.8($CO\underline{C}H_3$), 22.8 (C-20), 22.0 (C-22), 18.9 (C-19) ppm. MS (ESI^+): *m/z* 491.0731 $[M - 2Cl - H]^+$ (m_{ex} = 491.0721).

[Chlorido(η^6-*p*-cymene)(*N*-(4-(dimethylamino)phenyl)pyridine-2-carbothioamide)ruthenium(II)] chloride (7a)

Compound **7a** was synthesized following the general synthetic procedure **A** using *N*-(4-(dimethylamino)phenyl)pyridine-2-carbothioamide (100 mg, 0.39 mmol) and $[Ru(cym)Cl_2]_2$ (116 mg, 0.19 mmol). Yield: 74% (182 mg, red solid). Elemental analysis found: C, 49.47; H, 5.28; N, 6.36, calculated for $C_{24}H_{29}Cl_2N_3RuS{\cdot}0.33C_6H_{14}{\cdot}0.66CH_2Cl_2$: C, 49.36; H, 5.44; N, 6.48. ^1H NMR (400.13 MHz, d_4-MeOD, 25 °C): δ = 9.63 (d, ^3J = 5 Hz, 1H, H-4), 8.39 (d, ^3J = 8 Hz, 1H, H-1), 8.25 (t, ^3J = 7 Hz, 1H, H-3), 7.80 (t, ^3J = 6 Hz, 1H, H-2), 7.56 (d, ^3J = 9 Hz, 2H, H-8/H-12), 6.94 (d, ^3J = 8 Hz, 2H, H-9/H-11), 6.01 (d, ^3J = 6 Hz, 1H, H-15), 5.92 (d, ^3J = 6 Hz, 1H, H-17), 5.87 (d, ^3J = 6 Hz, 1H, H-18), 5.61 (d, ^3J = 6 Hz, 1H, H-14), 3.07 (s, 6H, $N(CH_3)_2$), 2.74 (sept, ^3J = 6 Hz, 1H, H-21), 2.21 (s, 3H, H-19), 1.20 (d, ^3J = 7 Hz, 3H, H-20), 1.12 (d, ^3J = 7 Hz, 3H, H-22) ppm. $^{13}C\{^1H\}$ NMR (100.61 MHz, $CDCl_3$, 25 °C): δ = 197.2 (C-6), 159.6 (C-5), 157.2 (C-1), 152.3 (C-10), 140.0 (C-3), 135.8 (C-7), 129.5 (C-8/C-12), 128.8 (C-2), 127.3 (C-4), 124.8 (C-9/C-11), 106.2 (C-16), 102.9 (C-13), 87.7 (C-15), 87.3 (C-17), 84.7 (C-18), 83.9 (C-14), 31.1 ($N(CH_3)_2$), 26.8 (C-21), 22.8 (C-20), 22.0 (C-22), 18.9 (C-19) ppm. MS (ESI^+): *m/z* 492.1047 $[M - 2Cl - H]^+$ (m_{ex} = 492.1041).

[Chlorido(η^6-p-cymene)(*N*-(4-aminophenyl)pyridine-2-carbothioamide)ruthenium(II)] chloride (8a)

Compound **8a** was synthesized following the general synthetic procedure **A** using *N*-(4-aminophenyl)pyridine-2-carbothioamide (50 mg, 0.22 mmol) and $[Ru(cym)Cl_2]_2$ (67 mg, 0.11 mmol). Yield: 57% (73 mg, black/dark red solid). Elemental analysis found:

C, 45.94; H, 5.15; N, 6.75, calculated for $C_{22}H_{25}Cl_2N_3RuS \cdot 0.33CH_2Cl_2 \cdot 1.33H_2O$: C, 45.63; H, 4.86; N, 7.15. ^{1}H NMR (400.13 MHz, d_4-MeOD, 25 °C): δ = 9.60 (d, ^3J = 6 Hz, 1H, H-4), 8.32 (d, ^3J = 8Hz, 1H, H-1), 8.20 (t, ^3J = 8 Hz, 1H, H-3), 7.76 (t, ^3J = 6 Hz, 1H, H-2), 7.37 (d, ^3J = 9 Hz, 2H, H-8/H-12), 6.93 (d, ^3J = 8 Hz, 2H, H-9/H-11), 5.97 (d, ^3J = 6 Hz, 1H, H-15), 5.88 (d, ^3J = 6 Hz, 1H, H-17), 5.82 (d, ^3J = 6 Hz, 1H, H-18), 5.56 (d, ^3J = 6 Hz, 1H, H-14), 2.73 (sept, ^3J = 6 Hz, 1H, H-21), 2.20 (s, 3H, H-19), 1.20 (d, ^3J = 7 Hz, 3H, H-20), 1.13 (d, ^3J = 7 Hz, 3H, H-22) ppm. ^{13}C{^{1}H} NMR (100.61 MHz, $CDCl_3$ (0.3mL) / d_4-MeOD (0.1mL), 25 °C): δ = 158.8 (C-1), 148.7 (C-10), 140.2 (C-3), 136.6 (C-7), 129.5 (C-8/C-12), 126.0 (C-2), 124.4 (C-4), 117.1 (C-9/C-11) 106.1 (C-16), 104.1 (C-13), 88.3 (C-15), 88.2 (C-17), 85.4 (C-18), 83.9 (C-14), 31.7 (C-21), 22.9 (C-20), 21.9 (C-22), 18.9 (C-19) ppm. MS (ESI$^+$): *m/z* 464.0734 $[M - 2Cl - H]^+$ (m_{ex} = 464.0768).

Stability in aqueous solution

Hydrolytic stability of **1a** and **2a** was carried out by dissolving the compounds (1–2 mg/mL) in D_2O and ^{1}H NMR spectra were recorded after 0.5, 2, 24, 48, 72 h and 7 d and ESI-mass spectra after 0.5, 24, 72 h and 7 days. To determine the stability in acidic medium, **1a** was dissolved in 60 mM HCl and the incubation mixture was analyzed by ESI-MS after 0.5, 24, 72 h and 7 days.

Sulforhodamine B Cytotoxicity Assay

The antiproliferative activity of the compounds in HCT116, NCI-H460, SW480 and SiHa cells was determined using the sulforhodamine B assay as described previously [43,44].

Calculated logarithmic octanol/water partition coefficient (clog*P*)

ChemBioDrawUltra 15.0 was used to estimate the lipophilicity based on calculated logarithmic octanol-water partition coefficients (clogP) of **1**–**8**.

Quantitative estimate of druglikeness

The QED for **1**–**8** was determined as described previously [24].

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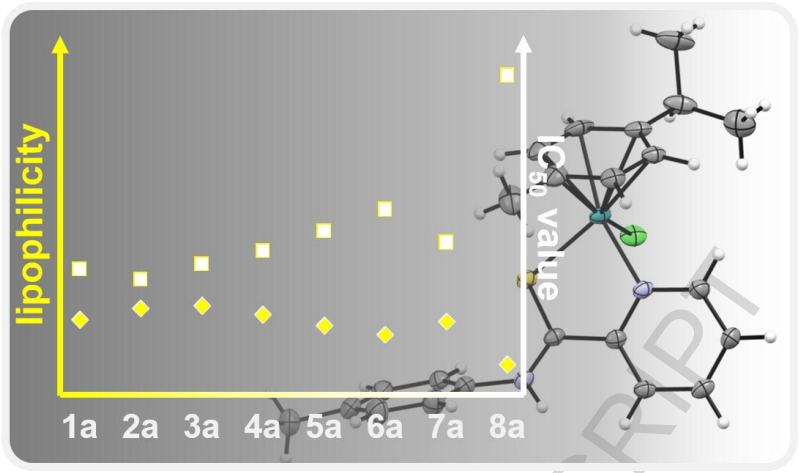
Graphical Abstract



This paper aimed to develop structure-activity relationships for pyridine-2-carbothiamide-based organometallics that are orally active anticancer agents. The lipophilic nature of the ligands correlated well with the cytotoxicity of the complexes prepared.

Highlights

- Preparation of pyridine-2-carbothioamides and their organoruthenium complexes
- Structural characterization of the ligands and complexes
- increased in vitro anticancer activity of the complexes as compared to the ligands
- lipophilicity correlates with anticancer activity
- high stability under acidic conditions