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A ruthenium complexes of monastrol and its pyrimidine analogues: Synthesis and biological properties

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ABSTRACT

A new series of mononuclear ruthenium(II) complexes of the type $[Ru(PPh_3)_2(N,S\text{-}L^{1-3})_2]2H_3O^+.(Cl^-)_2.XH_2O$, $[RuCl(dmso)_3(N,S\text{-}L^{1-3})]$, and $[Ru_2(Cl^-)_2(N,S\text{-}L^{1-3})_2].XH_2O$, where **L¹** is ethyl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-pyrimidine-5-carboxylate (monastrol), and **L²** and **L³** are the 4-hydroxyphenyl and 4-bromophenyl analogs of monastrol have been prepared and characterized by elemental analysis, ^1H, and ^{13}C NMR spectroscopy. All the complexes were assayed for their anti-HIV-1 and HIV-2 activity in MT-4 cells, and cytotoxicity was also investigated in mock-infected in MT-4 cells by using MTT assay. All the complexes exhibited no anti-HIV activity, however complexes $[RuCl(dmso)_3(N,S\text{-}L^1)]$ (**7**) and $[RuCl(dmso)_3(N,S\text{-}L^2)]$ (**8**) showed cytotoxicity values of $>$ 0.21 and $>$ 2.14 μM, respectively against mock-infected MT-4 cells. In addition, complexes $[Ru(PPh_3)_2(N,S\text{-}L^3)_2].2H_3O^+.2Cl^-.H_2O$ (**4**), **7**, and $[RuCl_2(N,S\text{-}L^1)]$ (**10**) have been selected for evaluation of their dual inhibition activity against dual-specificity tyrosine phosphorylation-regulated kinase (Dyrk1A).

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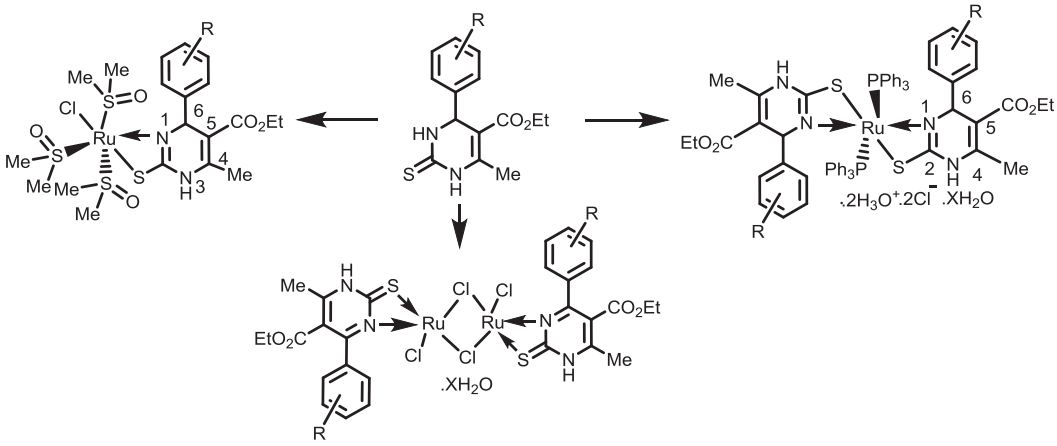
Anti-HIV activity; anti-Dyrk1A activity; cytotoxicity; ruthenium(II) complexes; monastrol and analogs

GRAPHICAL ABSTRACT



Introduction

In recent years, ruthenium complexes have received much attention due to their interesting chemical and biological properties.[1,2] Ruthenium complexes are particularly attractive in cancer therapy.[3–5] Mestroni et al.[6] developed hexacoordinated Ru(II) complexes with dimethylsulfoxide and chloride ligands with anticancer activity. In 1992, Tocher et al.[7] observed the cytotoxicity and anticancer activity of $[(\eta^6\text{-}C_6H_6)RuCl_2(metronidazole)]$. Following this initial study, Dyson and Sadler[8,9] have focused on the antitumor and antimetastatic activity of arene ruthenium complexes such as $(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)Ru(P\text{-}pta)](Cl_2)$ (pta =1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane), termed RAPTA-C,[10] and $[(\eta^6\text{-}C_6H_5Ph)Ru(\kappa^2N,N\text{-}en)Cl]^+ PF_6^-$ (en= 1,2-ethylenediamine) and related analogs.[11] Currently, two ruthenium compounds, NAMI-A (Figure 1, **1**)[12,13] **1** and KP1019 (Figure 1, **2**),[10,14,15] which were developed by Sava and Keppler, have displayed favorable solubilities and anticancer activities in clinical trials.[16] Meggers et al.[17] developed ruthenium anti-cancer drugs designed to act as analogs of staurosporine, an effective organic drug protein kinase inhibitor rather than cancer chelation therapy. Recently we have synthesized new ruthenium(II)-arene complexes of the general formula $[\{(\eta^6\text{-}p\text{-cymene})Ru(L)\}_2](Cl)_2$ (Figure 1, **3**)

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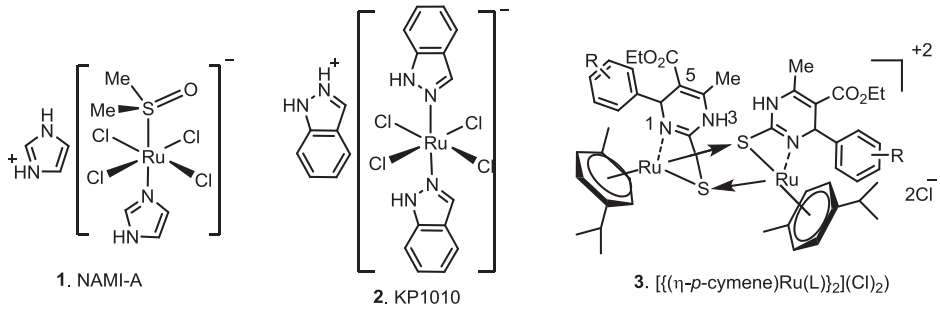
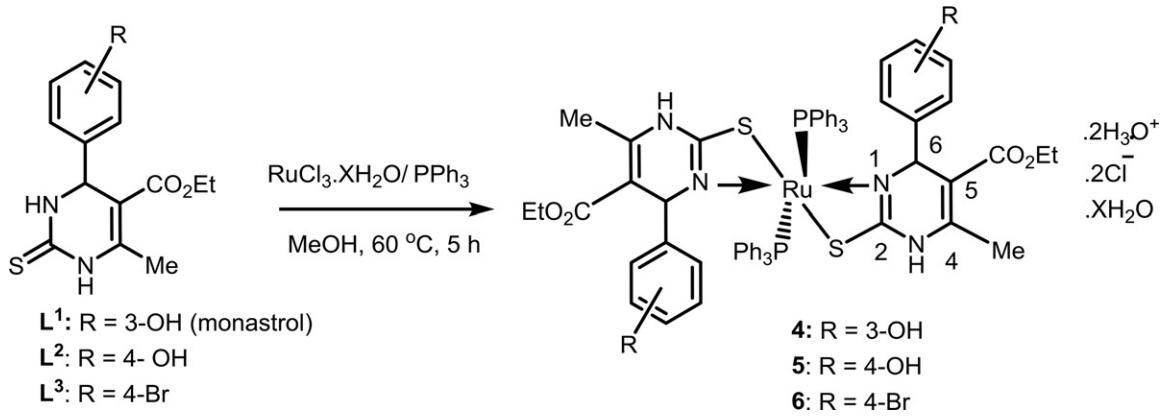


Figure 1. Some promising anticancer (organo)ruthenium complexes.



Scheme 1. Synthesis of ruthenium complexes of ethyl 4-aryl-6-methyl-2-thioxo-pyrimidine-5-carboxylate (**4–6**).

with evaluation of their *in vitro* HIV and kinesin Eg5 inhibition activities.[18]

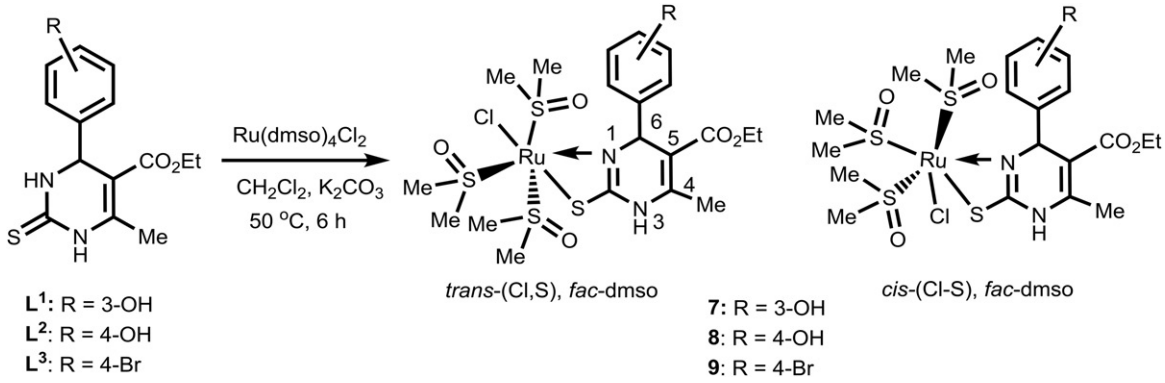
Taking into account the pharmacological properties of arene ruthenium complexes as potential antitumor and antimetastatic agents and the bioactivity of the pyrimidine derivative monastrol as a specific kinesin spindle protein (KSP),[19] we describe here the preparation, characterization and *in vitro* anti-HIV and the dual specificity tyrosine phosphorylation-regulated kinase (Dyrk 1A) inhibitory activity of ruthenium(II) complexes of some pyrimidines related to monastrol.

Results and discussion

Chemistry

Recently, Cini et al.[20] have reported the synthesis and cytotoxic properties of selected Ru(II) complexes with thiopyrimidine and/or thiopurine (antileukemic drugs) ligands. These complexes effected incorporation of thiobases into DNA followed by *N,S*-chelation to Ru(II). In our present work, ethyl-4-aryl-6-methyl-2-thioxo-pyrimidine-5-carboxylates, ligands $\mathbf{L^1}$–$\mathbf{L^3}$, have been selected for the coordination to a ruthenium(II) salt, aiming for evaluation of their *in vitro* anti-HIV activity and the selective dual specificity tyrosine phosphorylation-regulated kinase (Dyrk1A) inhibition activity. Thus, treatment of ligands $\mathbf{L^1}$–$\mathbf{L^3}$ with $RuCl_3{\cdot}3H_2O$/ PPh_3 in MeOH (M:L 1:2 molar ratio) at 60 °C afforded, after purification, ruthenium complexes **4–6** in 51, 60 and 50% yield. respectively (Scheme 1). In these reactions, MeOH serves as the solvent and the reducing agent towards Ru(III), being oxidized to formaldehyde with the concomitant formation of HCl.[21] In complexes **4–9**, the formed HCl is trapped by the iminothiolate ligands, converting them to their corresponding hydrochlorides. The oxidation state Ru(II) follows unambiguously from the diamagnetic NMR spectra (note that Ru(III) is paramagnetic).

The characterization of **4–6** was carried out by elemental analysis, IR, and NMR spectroscopy. The IR spectra of complexes **4–6** showed a similar peak pattern. In the 3214–2810 cm^{-1} region, the IR spectra showed the ν(N-H) and ν(C-H) bands. The absorption bands at 1715–1710 cm^{-1} are attributed to the ν(C = O) of the ethyl ester group. The IR spectra of the ligands ($\mathbf{L^1}$–$\mathbf{L^3}$) exhibited a sharp band at 1600–1620 cm^{-1} assigned to ν(C = N). This band shifts by ~ 15–35 cm^{-1} to lower frequencies upon chelating coordination of these ligands with the ruthenium ion.[22] The ligands $\mathbf{L^1}$–$\mathbf{L^3}$ exhibit thione-thiol tautomerism in solution but adopt the thione form in the solid state. They are readily deprotonated to the corresponding thionate anion in solution.[23] Furthermore, complexes **4** and **5** showed frequencies in the 3415–3410 cm^{-1} range, which are attributed to ν(OH) of the hydroxyl group. The band observed in the 1434–1436 cm^{-1} range is due to the coordinated PPh_3 ligands of the complexes **4–6**.[24] The new bands in the 529–475 cm^{-1} range are due to the ν(Ru–N)) vibrations.[25] Coordination of of ligands $\mathbf{L^1}$ to $\mathbf{L^3}$ to ruthenium is accompanied by a substantial modification of their IR spectra with pertubation of the two thioamide bands, which is indicative of *S,N*-coordination of the ligands in their monoanionic forms.[26] These bands were shifted from ca. 1326 cm^{-1} in the free ligands to ca. 1306 cm^{-1} in the corresponding



L^1: R = 3-OH
L^2: R = 4-OH
L^3: R = 4-Br

7: R = 3-OH
8: R = 4-OH
9: R = 4-Br

Scheme 2. Synthesis of ruthenium complexes of ethyl 4-aryl-6-methyl-2-thioxo-pyrimidine-5-carboxylate derivatives (**7–9**).

complexes, indicating the involvement of the N-1 atom and C-S in coordination to the ruthenium metal ion. Elemental analyses confirmed the Ru(II)L_2 composition of the complexes **4–6**, using a M:L (1:2) molar ratio.

Next, the biological importance of dichlo rotetrakis(dimethylsulfoxide)ruthenium(II) [$RuCl_2(dmso)_4$] as a potential anticancer agent,[27–29] as well as the significant importance of ruthenium-dmso complexes as radiosensitizing agents,[30] and antitumor activity against several murine tumor models[31–33] prompted us to explore the synthesis of new ruthenium(II) complexes of monastrol and its pyrimidine analogs. Thus, the new ruthenium(II) pyrimidine complexes **7–9** were prepared in 56 to 57% yield, respectively, by treatment of ligands $\mathbf{L^1}$–$\mathbf{L^3}$ with [$RuCl_2(dmso)_4$] in a 1:1 molar ratio (Scheme 2).

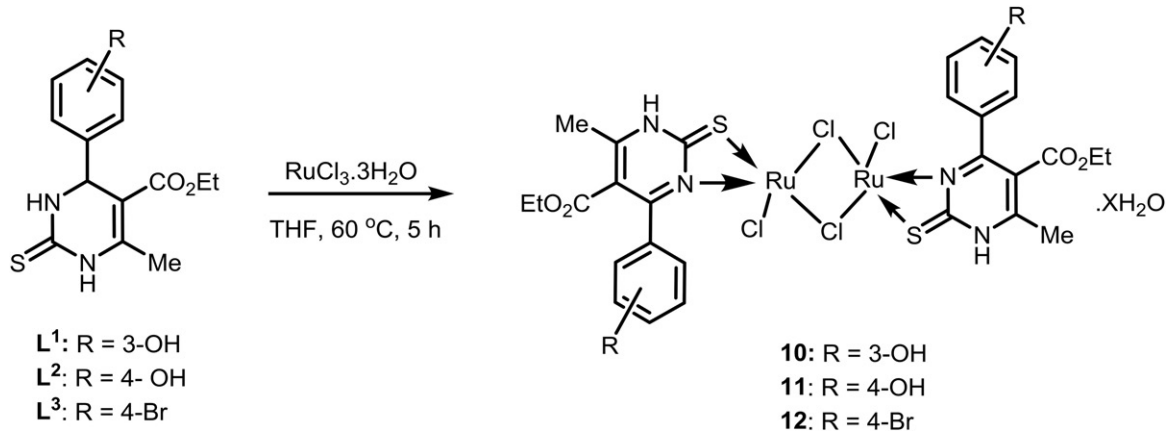
The IR spectra of the complexes **7–9** are characterized by the appearance of peaks at 420–422 cm^{-1} and 1092–1095 cm^{-1} attributable to the ν(Ru-S) and ν(S-O) stretch, respectively, and these strechings indicate coordination of the DMSO through their sulfur atom (κS). Due to the π-acceptor properties of the dmso ligand, the Ru-S bond is favored because of π -retrodonation from the Ru ion to the S acceptor orbitals.[34] Elemental analyses of the complexes **7–9** confirmed the presence of three coordinated dmso molecules and a Ru:L (1:1) molar ratio.

Alessio et al.[35] reported the synthesis of various Ru(II) complexes coordinated with dmso and nitrogen ligands like *cis*,*fac*-$RuCl_2(dmso)_3(L)$ (L = NH_3, imidazole) and similar N-donar ligands. They found that all complexes have exclusively S-bonded dmso ligands and are present as the *fac*-coordination isomers, as confirmed by X-ray crystallography. Based on these observations, we suggest that complexes **7–9** exist as either in the *trans*(Cl-S),*fac*-dmso or the *cis*(Cl-S),*fac*-dmso geometry (Scheme 2).

The structures of complexes **4–9** were assigned on the basis of ^{1}H and ^{13}C NMR spectroscopy. The spectra showed a similar pattern of resonances of the bound ligands $\mathbf{L^1}$ to $\mathbf{L^3}$ with corresponding coordination-induced shifts when compared to the free ligands. Thus, the ^{1}H NMR spectra of **4–9** showed singlets in the regions δ 9.20–9.48 ppm assigned to the N3H proton. The multiplets assignable to triphenylphosphine protons appeared in the regions δ 7.66–7.29 ppm. These resonances are absent in the other complexes. The resonances at δ 4.42–5.67 ppm of the complexes **4–9** were assigned to H-6 of pyrimidine ligands. Its appearance as a singlet is indicative for coordination of N-1 to ruthenium with concomitant tautomerization to the N3H form. In comparison, this signal appears as a doublet resonance in the free ligands. Due to deprotonation of the ligand, the resonance signal of N1-H of the free ligand is missing in the complexes. The methyl protons at C-4 of the ligands appeared as singlets in the regions δ 2.28–2.42 ppm. The other aromatic and aliphatic protons were fully analyzed (*c.f.* Experimental Section). In particular, the ^{13}C NMR spectra of **4–9** showed the resonances at δ 167.8–165.3 ppm for the carbonyl carbon atom of the ester group (CO_2Et), whereas C-4 of the pyrimidine backbone appeared at δ 160.2–157.3 ppm. The resonances for C-5 (δ 122.6–120.8 ppm), C-6 (δ 54.0–53.3 ppm), C4-*Me* (19.7–17.9 ppm) and other aliphatic and aromatic carbon atoms are practically identical for all complexes since they are positioned far away from the binding site of the ligand. However, the resonance for the C2-S carbon atom is shifted downfield by ~10 ppm in the complexes (156.8–154.7 ppm) compared to the the ligands $\mathbf{L^1}$–$\mathbf{L^3}$ (δ 174.6,[36] 173.7 and 174.7 ppm,[18] respectively), are indicative of their chelating coordination as monoanionic bidentate ligands through their S and N-1 donor atoms.

Our work was extended to prepare new models of ruthenium (II) complexes aiming to evaluate their anti-HIV activity and cytotoxicity. Thus, treatment of the ligands $\mathbf{L^1}$–$\mathbf{L^3}$ with $RuCl_3.XH_2O$ (1:1 molar ratio) in THF-MeOH at 60 °C furnished the complexes **10–12** in 64, 71 and 68% yield, respectively (Scheme 3).

The structures of the complexes were confirmed from the IR, ^{1}H, ^{13}C NMR spectra and elemental analyses. 2-Thiopyrimidine ligands $\mathbf{L^1}$–$\mathbf{L^3}$ usually coordinate with a metal ion either in the neutral thione form or, after deprotonation, in the N,S ligand chelating mode. The IR spectra of complexes **10–12** showed a slight shift of the band at ca 1475 cm^{-1} (predominantly the v(C = S) mode) by ~ 15–35 cm^{-1} to lower frequencies upon chelating coordination of theses ligands with the ruthenium ion. Such shift suggests the involvement of exocyclic sulfur in metal coordination. In addition, a one sharp ν(Ru-Cl) band was observed (~ 329 cm^{-1}), suggesting a *trans*-arrangement of the chlorine atoms.[31,37]



Scheme 3. Synthesis of ruthenium complexes of ethyl 4-aryl-6-methyl-2-thioxo-pyrimidine-5-carboxylate (**10–12**).

Table 1. *In vitro* anti-HIV-1[a] and HIV-2[b] activity of some ruthenium-pyrimidine complexes.

Compd.	Virus strain	av. IC_{50} (μM)[c]	av. CC_{50} (μM)[d]	SI[e]
4	III_B	>21.32	21.32	<1
	ROD	>21.32	13.32	<1
5	III_B	>34.20	34.20	<1
	ROD	>34.20	34.20	<1
6	III_B	>50.60	50.63	<1
	ROD	>50.63	50.63	<1
7	III_B	>0.21	>0.21	<1
	ROD	>0.21	>0.21	<1
8	III_B	>2.14	2.14	<1
	ROD	>2.14	2.14	<1
9	III_B	>10.92	10.92	<1
	ROD	>10.92	10.92	<1
10	III_B	>115.00	115.00	<1
	ROD	>115.00	115.00	<1
11	III_B	>100.38	100.38	<1
	ROD	>100.38	100.38	<1
12	III_B	>85.88	85.88	<1
	ROD	>85.88	85.88	<1
Nevirapin	III	0.05	>4	>80
	ROD	>4	>4	<1
AZT	III_B	0.0019	>25	>13144
	ROD	0.0018	>25	>14245
3TC	III_B	0.51	>20	>39
	ROD	2.02	>20	>10

[a]Anti-HIV-1 activity measured against strain III_B;
[b]Anti-HIV-2 activity measured against strain ROD;
[c]Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 and 2-induced cytopathogenic effect;
[d]Average CC_{50}: compound concentration that reduces the viability of mock-infected MT-4 cells by 50%;
[e]SI: selectivity index (CC_{50}/IC_{50}).
All data represents the mean values of at least two separate experiments.

The ^1H and ^{13}C spectra on the complexes **10–12** contained similar resonance signals to those of **7–9**, except for $C^2 = S$ which resonated at even lower field (δ 146.7, 148.5 and 148.6 ppm, respectively), in comparison for those of **4–9** (δ 156.8–154.7 ppm). The Ru oxidation state of + II and the presence of two chlorido ligands per Ru atom indicates that the heterocyclic N,S ligands are here present in their non-deprotonated forms.

In summary, the coordination of Ru(II) via the N-1 and S donor atoms based on the spectroscopic analyses, in addition to our previous results of the synthesis of the complexes $[\{(\eta^6\text{-}p\text{-cymene})Ru(L)\}_2](Cl)_2$,[18] as confirmed by crystallographic study.

In vitro anti-HIV activity

Complexes **4–12** were evaluated for their inhibitory activity against HIV-1 (strain III_B) and HIV-2 (strain ROD), as monitored by the inhibition of the virus-induced cytopathic effect in the human T-lymphocyte (MT-4) cells, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method.[38] The results are summarized in Table 1, in which the data for nevirapin,[39] and azidothymidine (AZT)[40] are included for comparison purposes. The cytotoxicity of the compounds was determined in parallel. The compounds are largely devoid of antiretroviral activity although they showed cytotoxicity against MT-4 cells at micromolar concentrations. All the compounds showed no activity against HIV-1 and HIV-2, except **7** and **8** which showed $IC_{50} > 0.21$ and > 2.14 µM, respectively, with therapeutic index (SI) <1, meaning no selectivity could be observed. Interestingly, compound **7** exhibited significant cytotoxicity of > 0.21 µM against the human $CD4^+$ lymphocytes (MT-4), and can thus be considered to be a promising antiproliferative agent against human leukemia cell line or as antineoplastic agent, since several laboratories have reported that ruthenium complexes coordinated dmso molecules exhibited significant antitumor or antiproliferative activity.[6,31–33]

In vitro dual inhibition activity of Dyrk1A

Dyrk1A is a member of the dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) family. It is one of the kinase enzymes which was reported to phosphorylate numerous serine/arginine rich (SR) proteins *in vitro* and *in vivo*. These proteins play major roles in splice site recognition and are largely regulated by phosphorylation. Inhibition of Dyrk1A would modulate gene transcripts in cells, then regulate alternative splicing in the nucleus and the connection of both SR proteins and SR protein kinases to human diseases, particularly in cancer cells.[41] Engel et al.[42] reported that dual inhibition of Dyrk1A and another dual kinase inhibitor (C1Ks) increased the efficacy of pre-mRNA splicing modulation, however, they found that hydroxybenzothiophene ketones are efficient pre-mRNA splicing modulators due to dual inhibitions of these enzymes. We have

selected the complexes **4**, **7**, and **10** for evaluation of their dual inhibition activity against Dyrk1A. All tested complexes showed no inhibition activity against Dyrk1A at 10 μM (inhibition%. = −4.56, 1.11 and −19.65) in comparison with the reference compound, 3-[4-(1,3-thiazol-5-yl)thiophenepyridine (IC_{50} = 130 nM).[42]

Experimental

General procedure

Melting points were measured on a Büchi melting point apparatus B-545 (Büchi Labortechnik AG, Switzerland) and are uncorrected. Microanalytical data were obtained using a Vario Elemental Analyzer (Shimadzu, Japan). IR spectra were measured on a 1330 Perkin–Elmer spectrophotometer, using Nujol mull.NMR spectra were recorded on a Bruker Avance III DRX 400 or a Bruker Avance DRX 600 (^{1}H) and 150.91 MHz (^{13}C) spectrometers, with TMS as internal standard and on the δ scale in ppm. Signal assignments for protons were performed by selective proton decoupling or by COSY spectra. Heteronuclear assignments were verified by HSQC and HMBC experiments. TLC plates 60 F254 were purchased from Merck. The chromatograms were visualized under UV 254–366 nm and iodine (solvent: hexane-ethyl acetate 3:2). $RuCl_2(DMSO)_4$ was purchased from Sigma-Aldrich (Germany). The Supplemental Materials contains sample ^{1}H and ^{13}C NMR spectra of products 4–12 (Figures S1–S10).

The atom numbering for the NMR characterization follows that provided in Schemes 1–3.

General procedure for synthesis of complexes (4–6)

A solution of hydrated $RuCl_3$ (65 mg, 0.25 mmol) in MeOH (7 mL) was refluxed under nitrogen for 5 min, then allowed to cool to room temperature. To this solution, triphenylphosphine (40 mg, 0.15 mmol) was added and the brown solution was refluxed under nitrogen for 2 h. After cooling, the ligands (**L^1**–**L^3**) (0.50 mmol) and K_2CO_3 (69 mg, 0.50 mmol) were added. The mixture was heated at 60 °C for 5 h under nitrogen atmosphere. After cooling, the crude precipitate was filtered and dried. Recrystallization from EtOH afforded the desired complexes.

[Ru(PPh$_3$)$_2$(N,S-L^3)$_2$].2H$_3$O$^+$.2Cl$^-$.H$_2$O (4)

From **L^1** (monastrol) (146 mg). Yield: 334 mg (50%) as brown solid; mp 143–146 °C. ^{1}H NMR (DMSO-d_6): δ 9.78 (s, 2H, $C_{arom.}$-OH), 9.32 (s, 2H, N3H), 7.65–7.61 (m, 12H, PPh_3), 7.58–7.52 (m, 6H, PPh_3),7.42–7.38 (m, 12H, PPh_3), 7.33 (m, 4H, $H_{arom.}$), 6.30 (m, 4H, $H_{arom.}$), 5.01 (s, 2H, $H_{pyrimid.}$-6) 4.01 (q, 4H, J = 7.0 Hz, CH_2CH_3), 2.37 (s, 6H, $CH_{3pyrimid.}$), 1.10 (t, 6H, J = 7.0 Hz, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 165.3 (C = O), 157.3 ($C4_{pyrimid.}$+$C3_{arom.}$), 155.3 (C-S), 142.6 ($C1_{arom.}$), 134.0 (C1-PPh_3), 133.3 (C2-PPh_3+C6-PPh_3), 130.8 (C4-PPh_3), 129.4 ($C5_{arom.}$), 128.8 (C3-PPh_3+C5-PPh_3), 124.4 ($C4_{arom.}$), 121.4 ($C5_{pyrimid.}$), 120.5 ($C6_{arom.}$), 117.3 ($C2_{arom.}$+$C4_{arom.}$), 113.5 ($C3_{arom.}$), 59.6 (CH_2CH_3), 54.0 ($C6_{pyrimid.}$), 17.9 ($CH_{3pyrimid.}$), 14.1 (CH_2CH_3). Anal. calcd for $C_{64}H_{70}Cl_2N_4O_9P_2RuS_2$ (1337.32): C, 57.48; H, 5.28; N, 4.20. Found: C, 57.42; H, 5.00; N, 3.90.

[Ru(PPh$_3$)$_2$(N,S-L^2)$_2$].2H$_3$O$^+$.2Cl$^-$.H$_2$O (5)

From **L^2** (146 mg). Yield: 401 mg (60%) as deep-brown solid; mp 137–139 °C. ^{1}H NMR (DMSO-d_6): δ 10.00 (s, 2H, OH), 9.20 (s, 2H, N3H), 7.64–7.60 (m, 12H, PPh_3), 7.57–7.51 (m, 6H, PPh_3), 7.42–7.40 (m, 12H, PPh_3), 7.30 (m, 4H, $H_{arom.}$), 7.19 (m, 4H, $H_{arom.}$), 5.12 (s, 2H, $H_{pyrimid.}$-6) 3.99 (q, 4H, J = 7.1 Hz, CH_2CH_3), 2.35 (s, 6H, $CH_{3pyrimid.}$), 1.09 (t, 6H, J = 7.1 Hz, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 166.8 (C = O), 159.9 ($C4_{pyrimid.}$), 157.3 (C-S), 154.1 (C-OH), 137.9 ($C1_{arom.}$), 133.1 (C2-PPh_3+C6-PPh_3) 131.4 ($C2_{arom.}$+$C6_{arom.}$), 128.6 (C3-PPh_3+C4-PPh_3+C5-PPh_3), 122.0 ($C5_{pyrimid.}$), 117.5 ($C3_{arom.}$+$C5_{arom.}$), 59.1 (CH_2CH_3), 55.4 ($C6_{pyrimid.}$), 18.6 ($CH_{3pyrimid.}$), 14.5 (CH_2CH_3). Anal. calcd for $C_{64}H_{68}Cl_2N_4O_9P_2RuS_2$ (1337.30): C, 57.48; H, 5.28; N, 4.20. Found: C, 57.33; H, 4.98; N, 4.00.

[Ru(PPh$_3$)$_2$(N,S-L^3)$_2$].2H$_3$O$^+$.2Cl$^-$ (6)

From **L^3** (178 mg). Yield: 368 mg (51%) as deep-brown solid; mp 128–131 °C. ^{1}H NMR (DMSO-d_6): δ 9.45 (s, 2H, N3H), 7.66–7.61 (m, 12H, PPh_3), 7.56–7.51 (m, 6H, PPh_3), 7.45–7.29 (m, 12H, PPh_3), 6.84 (m, 4H, $H_{arom.}$), 6.31 (m, 4H, $H_{arom.}$), 4.42 (s, 2H, $H_{pyrimid.}$-6), 3.78 (q, 4H, J = 6.9 Hz, CH_2CH_3), 2.42 (s, 6H, $CH_{3pyrimid.}$), 1.09 (t, 6H, J = 6.9 Hz, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 166.3 (C = O), 160.4 ($C4_{pyrimid.}$), 156.4 (C-S), 139.7 ($C1_{arom.}$), 134.1 ($C1_{PPh3}$), 133.1 (C2-PPh_3+C6-PPh_3), 130.7 (C4-PPh_3+$C3_{arom.}$+$C5_{arom.}$), 129.7 ($C2_{arom.}$+$C6_{arom.}$), 128.7 (C3-PPh_3+C4-PPh_3+C5-PPh_3), 123.5 ($C5_{pyrimid.}$), 122.6 (C-Br), 60.1 (CH_2CH_3), 53.1 ($C6_{pyrimid.}$), 18.3 ($CH_{3pyrimid.}$), 14.2 (CH_2CH_3). for Anal. calcd for $C_{64}H_{64}Br_2Cl_2N_4O_6P_2RuS_2$ (1443.08): C, 53.27; H, 4.47; N, 3.88. Found: C, 53.42; H, 4.35; N, 3.59.

General procedure for synthesis of complexes (7–9)

A solution of the ligands **L^1**–**L^3** (0.23 mmol) and K_2CO_3 (32 mg, 0.23 mmol) in CH_2Cl_2 (15 mL) was added to a suspension of dichlorotetrakis(dimethylsulfoxide) ruthenium [$RuCl_2$(dmso)$_4$] (111 mg, 0.23 mmol), in CH_2Cl_2 (10 mL) and the mixture was heated at 50 °C under nitrogen atmosphere for 6 h. After cooling, the solvent was partially removed under reduced pressure, and a brown solid appeared. It was filtered and washed with diethyl ether followed by drying to give the desired complexes.

[RuCl(dmso)$_3$(N,S-L^1)] (7)

From **L^1** (mg). Yield: mg (56%) as deep-brown solid; mp 177–180 °C. ^{1}H NMR ($CDCl_3$): δ 9.26 (s, 1H, N3H), 7.10 (m, 1H, $H_{arom.}$-5), 6.80 (m, 1H, $H_{arom.}$-4), 6.64 (m, 2H, $H_{arom.}$-2 + $H_{arom.}$-6), 5.61 (s, 1H, OH), 4.95 (s, 1H, $H_{pyrimid.}$-6), 3.97 (q, 4H, J = 7.0 Hz, CH_2CH_3), 2.54 (br s, 18H, 3xMe_2SO), 2.35 (s, 3H, $CH_{3pyrimid.}$), 1.13 (t, 3H, J =

7.0 Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 166.8 (C = O), 158.9 ($C4_{pyrimid.}$), 154.7 (C-S), 153.4 ($C_{arom.}$-OH), 138.8 ($C1_{arom.}$), 130.0 ($C5_{arom.}$), 121.2 ($C5_{pyrimid.}$), 121.2 ($C5_{arom.}$), 119.9. ($C4_{arom.}$), 115.0 ($C4_{arom.}$), 113.1 ($C2_{arom.}$), 59.7 (CH_2CH_3), 53.5 ($C6_{pyrimid.}$), 44.1 (3x$SOMe_2$), 18.6 ($CH_{3pyrimid.}$), 14.1 (CH_2CH_3). Anal. calcd for $C_{20}H_{33}ClN_2O_6RuS_4$ (662.25): C, 36.27; H, 5.02; N, 4.23. Found: C, 36.10; H, 5.03; N, 4.52.

[RuCl(dmso)$_3$(N,S-L^2)] (8)

From **L^2** (mg). Yield: mg (56%) as deep-brown solid; mp 117–119 °C. ^{1}H NMR ($CDCl_3$): δ 9.28 (s, 1H, N3H), 7.03 (d, 2H, J = 8.4 Hz, $H_{arom.}$-2 + H_{arom}-6), 6.72 (d, 2H, J = 8.4 Hz, $H_{arom.}$-3 + H_{arom}-5), 5.18 (s, 1H, $H_{pyrimid.}$-6), 4.04 (q, 4H, J = 7.0 Hz, CH_2CH_3), 2.54 (br s, 18H, 3xMe_2SO), 2.28 (s, 3H, $CH_{3pyrimid.}$), 1.13 (t, 3H, J = 7.0 Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 167.8 (C = O), 157.5 ($C4_{pyrimid.}$), 154.7 (C-S), 153.4 ($C_{arom.}$-OH), 133.8 ($C1_{arom.}$), 127.3 ($C_{arom.}$-2 + $C_{arom.}$-6), 122.2 ($C5_{pyrimid.}$), 117.4 ($C_{arom.}$-3 + $C_{arom.}$-5), 59.8 (CH_2CH_3), 53.5 ($C6_{pyrimid.}$), 44.4 (3x$SOMe_2$), 19.7 ($CH_{3pyrimid.}$), 13.9 (CH_2CH_3). Anal. calcd for $C_{20}H_{33}ClN_2O_6RuS_4$ (662.25): C, 36.27; H, 5.02; N, 4.23. Found: C, 36.34; H, 4.87; N, 4.31.

[RuCl(dmso)$_3$(N,S-L^3)] (9)

From **L^3** (mg). Yield: mg (53%) as brown solid; mp 202–204 °C. ^{1}H NMR ($CDCl_3$): δ 9.37 (s, 1H, N3H), 7.56 (d, 2H, J = 7.9 Hz, $H_{arom.}$-3 + H_{arom}-5), 7.17 (d, 2H, J = 7.9 Hz, $H_{arom.}$-2 + H_{arom}-6), $H_{arom.}$), 5.67 (s, 1H, $H_{pyrimid.}$-6), 3.38 (q, 4H, J = 7.0 Hz, CH_2CH_3), 2.54 (br s, 18H, 3xMe_2SO), 2.29 (s, 3H, $CH_{3pyrimid.}$), 1.12 (t, 3H, J = 7.0 Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 165.7 (C = O), 159.2 ($C4_{pyrimid.}$), 156.8 (C-S), 140.0 ($C1_{arom.}$), 130.4 ($C3_{arom.}$+$C5_{arom.}$), 128.0 ($C2_{arom.}$+$C6_{arom.}$), 121.5 ($C_{arom.}$-Br), 120.8 ($C5_{pyrimid.}$), 59.1 (CH_2CH_3), 53.6 ($C6_{pyrimid.}$), 44.1 (3x$SOMe_2$), 18.7 ($CH_{3pyrimid.}$), 13.9 (CH_2CH_3). Anal. calcd for $C_{20}H_{32}BrClN_2O_5RuS_4$ (725.15): C, 33.13; H, 4.45; N, 3.86. Found: C, 33.29; H, 4.25; N, 3.65.

General procedure for synthesis of complexes (10–12)

A mixture of **L^1–L^3** (0.14 mmol), hydrated $RuCl_3$ (36 mg, 0.14 mmol), and K_2CO_3 (19 mg, 0.14 mmol) in THF-MeOH (1:1) (10 mL) was heated under reflux under nitrogen for 5 h. After cooling, the solvent was evaporated to dryness and the residue was washed with ether (3x10 mL) and dried to give the desired complexes as deep brown solid.

[Ru$_2$(Cl$^-$)$_2$(N,S-L1)$_2$] (10)

From **L^1** (monastrol) (146 mg). Yield: 333 mg (50%) as brown solid; mp 143–146 °C. ^{1}H NMR (DMSO-d_6): δ 10.20 (s, 1H, N1H), 7.28 (m, 2H, $H_{arom.}$-5 + $H_{arom.}$-6), 6.94 (m, 2H, $H_{arom.}$-2 + $H_{arom.}$-4), 5.42 (s, 1H, $C_{arom.}$-OH), 4.24 (q, 2H, J = 7.0 Hz, CH_2CH_3), 2.34 (s, 3H, $CH_{3pyrimid.}$), 1.34 (t, 3H, J = 7.0 Hz, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 161.1 (C = O), 158.2 ($C4_{pyrimid.}$), 154.9 ($C_{arom.}$-OH), 148.7 (C = S), 138.6 ($C1_{arom.}$), 131.1 ($C5_{arom.}$), 120.7 ($C5_{pyrimid.}$), 119.7 ($C6_{arom.}$), 115.1 ($C2_{arom.}$), 113.8 ($C4_{arom.}$), 59.4 (CH_2CH_3), 18.3 ($CH_{3pyrimid.}$), 14.1 (CH_2CH_3). Anal. calcd for $C_{28}H_{28}Cl_4N_4O_6Ru_2S_2$ (924.61): C, 36.37; H, 3.05; N, 6.06. Found: C, 36.55; H, 3.29; N, 5.70.

[Ru$_2$(Cl$^-$)$_2$(N,S-L^2)$_2$].H$_2$O (11)

From **L^2** (41 mg). Yield: 47 mg (71%); mp 192–195 °C. ^{1}H NMR (DMSO-d_6): δ 9.50 (s, 1H, N1H), 7.28 (m, 2H, $H_{arom.}$-5 + $H_{arom.}$-6), 6.94 (m, 2H, $H_{arom.}$-2 + $H_{arom.}$-4), 4.99 (s, 1H, $C_{arom.}$-OH), 4.24 (q, 2H, J = 7.0 Hz, CH_2CH_3), 2.34 (s, 3H, $CH_{3pyrimid.}$), 1.34 (t, 3H, J = 7.0 Hz, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 161.9 (C = O), 158.1 ($C4_{pyrimid.}$), 155.1 ($C_{arom.}$-OH), 148.5 (C = S), 133.7 ($C1_{arom.}$), 127.3 ($C_{arom.}$-2 + $C_{arom.}$-6), 120.3 ($C5_{pyrimid.}$), 119.3 ($C_{arom.}$-3 + $C_{arom.}$-5), 58.7 (CH_2CH_3), 18.3 ($CH_{3pyrimid.}$), 13.2 (CH_2CH_3). Anal. calcd for $C_{28}H_{30}Cl_4N_2O_7Ru_2S_2$ (942.63): C, 35.68; H, 3.21; N, 5.94. Found: C, 35.59; H, 3.42; N, 5.78.

[Ru$_2$(Cl$^-$)$_2$(N,S-L^3)$_2$].H$_2$O (12)

From **L^3** (50 mg). Yield: 51 mg (68%); mp 256–259 °C. ^{1}H NMR (DMSO-d_6): δ 7.52 (d, 2H, J = 8.4 Hz, $H_{arom.}$-3 + $H_{arom.}$-5), 7.19 (d, 2H, J = 8.4 Hz, $H_{arom.}$-2 + $H_{arom.}$-6), 3.99 (q, 2H, J = 7.0 Hz, CH_2CH_3), 2.25 (s, 3H, $CH_{3pyrimid.}$), 1.09 (t, 3H, J = 7.0 Hz, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 164.1 (C = O), 157.9 ($C4_{pyrimid.}$), 148.6 (C = S), 138.7 ($C1_{arom.}$), 130.5 ($C_{arom.}$-2 + $C_{arom.}$-6), 127.3 ($C_{arom.}$-3 + $C_{arom.}$-5), 123.7 (C-Br), 118.8 ($C5_{pyrimid.}$), 59.5 (CH_2CH_3), 17.6 ($CH_{3pyrimid.}$), 13.5 (CH_2CH_3). Anal. calcd for $C_{28}H_{30}Br_2Cl_4N_4O_5Ru_2S_2$ (1068.42): C, 31.48; H, 2.64; N, 5.24. Found: C, 31.42; H, 2.83; N, 5.12.

Conclusions

A series of ruthenium complexes of monastrol and its pyrimidine analogs of the type [Ru(PPh$_3$)$_2$(*N,S*-L$^{1-3}$).2H$_2$O$^+$.2Cl$^-$.XH$_2$O], [RuCl(dmso)$_3$(*N,S*-L$^{1-3}$)], and [Ru$_2$(Cl$^-$)$_2$(*N,S*-L$^{1-3}$)$_2$]XH$_2$O were synthesized and characterized. Spectroscopic data agree with the binding of the ligands as unideprotonated N-S chelates. The *in vitro* anti-HIV-1 and HIV-2 activities of the complexes were investigated, and they were found to be inactive. Complexes **7** and **8** showed a significant cytotoxicity against mock-infected MT-4 cells (C type adult T leukemia cells) led to be promising agents for further structural modification. Additionally, complexes **4**, **7** and **10** have been selected for evaluation of their dual inhibition activity against dual-specificity tyrosine phosphorylation-regulated kinase (Dyrk1A). None of these complexes matched the criteria of a selective inhibitor of Dyrk1A in this assay in comparison to IC$_{50}$ = 130 nM of 3-[4-(1,3-thiazol-5-yl)thiophenepyridine as a reference agent .

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