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Structural characterization and cytotoxicity studies of ruthenium(II)–dmso–chloro complexes of chalcone and flavone derivatives

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ABSTRACT

A synthetic precursor *cis*-[$Ru^{II}Cl_2(dmso)_4$] is complexed separately with 3-(4-benzyloxyphenyl)-1-(2-hydroxylphenyl)-prop-2-en-1-one (L^1H) and 2-(4-benzyloxyphenyl)-3hydroxy-chromen-4-one (L^2H). The resulting complexes are assigned the composition *fac*-[$RuCl(S\text{-}dmso)_3(L^1)$] **1** and *fac*-[$RuCl(S\text{-}dmso)_3(L^2)$] **2** using elemental analyses, FAB mass data and spectroscopic (IR, ^1H NMR, UV–Vis, emission) spectral properties. The X-ray diffraction analysis shows that complexes self-associate through non-covalent interactions and provide 1D and 2D supramolecular structures. These complexes are assayed for their cytotoxicity studies on Dalton Lymphoma cell lines.

1. Introduction

Ruthenium complexes introduced almost two decades ago for antitumor therapy have great potential as alternative drugs to cisplatin in view of their low toxicity and good selectivity for solid tumor metastasis [1–4]. Among several synthetic ruthenium-based anticancer agents, ruthenium–dmso complexes are believed to have great potential owing to their selectivity for solid tumor metastases and low toxicity against host [5]. Among the coordination complexes of ruthenium–chloro–dmso containing heterocyclic ligands *viz.* NAMI, Na[*trans*-$RuCl_4$(S-dmso)(im)] (dmso = dimethyl sulfoxide; im = imidazole) [6] and NAMI-A, [Him][*trans*-$RuCl_4$(S-dmso)(im)] [7] have proved their potential candidature as drug in the treatment of cancer cells. Moreover, NAMI-A successfully finished a Phase I clinical trial. It is worth to mention that in contrast to cisplatin and other platinum based compounds, biological testing of ruthenium–dmso compounds have indicated that DNA is not the only responsible target for their antimetastatic activity [8,9].

Additionally, chalcones and flavones are reported to exhibit a wide spectrum of biological activities, which include potential applications as new drugs, and agrochemicals [10–12]. Compounds such as 4′-ethoxy-2′-hydroxy-4,6′-dimethoxy chalcone and 4′,6-dichloroflavone interact directly with viral capsid proteins causing their uncoating and subsequently liberating viral RNA. Flavone derivatives especially [5,7-dihydroxy-2-(3-hydroxy-4-methoxy phenyl)-4-1-benzopyran-4-one]-1 possess potential therapeutic activities hence are considered leading synthetic targets in the research area of medicinal chemistry [13].

Several metal complexes from our laboratory have already been reported as cytotoxic [14] *in vitro* as well as *in vivo* inducing apoptosis in Dalton's Lymphoma (DL) cells [15]. Thus in persuasion of our studies on cytotoxic ruthenium complexes, attempts are made to complex a representative chalcone (L^1H) and flavone (L^2H) with well known precursor *cis*-[$Ru(dmso)_4Cl_2$] in anticipation to substitute its either two dmso or a dmso and a Cl substituent. The resulting complexes *fac*-[$RuCl(S\text{-}dmso)_3(L^1)$] **1** and *fac*-[$RuCl(S\text{-}dmso)_3(L^2)$] **2**, are structurally characterized. The preliminary level *in vitro* anticancer screening of these complexes using MTT assay on DL cell lines is also described. The DL cell lines have been chosen as tumor model in view of their successful applications for other anticancer drugs like cisplatin [16–18].

2. Experimental

2.1. Materials and methods

Starting materials were purchased from Sigma–Aldrich and used without further purification. Compounds were analyzed for C, H, N measurements from Central Drug Research Institute, Lucknow, India whereas Infrared, UV–Vis and luminescence spectra were recorded on VARIAN 3100 FTIR, Shimadzu UV-1601 and Perkin–Elmer LS-45 spectrophotometer, respectively. However, ^1H NMR spectra were recorded on JEOL AL 300 MHz spectrometer

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using TMS as internal reference. The starting material *cis*-[$Ru^{II}Cl_2(dmso)_4$] was prepared from $RuCl_3{\cdot}3H_2O$ using reported procedure [19].

2.2. X-ray crystallographic studies

Suitable X-ray quality crystals of the complexes **1** and **2** were grown from dichloromethane/petroleum ether (40–60 °C) solvent mixture at room temperature, and X-ray crystallographic data are recorded by mounting a single-crystal of complex **1** ($0.33 \times 0.26 \times 0.21$) mm^3 and complex **2** ($0.33 \times 0.29 \times 0.27$) mm^3 on glass fibers. Oxford diffraction XCALIBUR-S CCD area detector diffractometer equipped with an LN-2 low-temperature attachment was used for the cell determination and intensity data collection. Appropriate empirical absorption corrections were applied using multi-scan programs. Monochromated Mo Kα radiation (λ = 0.71073 Å) was used for the measurements. The crystal structures were solved by direct methods and refined by full matrix least squares SHELXL-97 [20]. Drawings were carried out using MERCURY [21], DIAMOND [22] and special computations were carried out with PLATON [23] Pertinent crystallographic data are presented in Table 1 whereas important bond lengths and bond angles for complexes **1** and **2** are listed in Table 2.

2.3. In vitro cytotoxicity assay experiment

The cell toxicity *in vitro* The DL (Dalton's Lymphoma: a transplantable T cell lymphoma) cells were collected from the mouse ascite as described earlier [15]. The viable DL cells, determined by trypan blue exclusion test, were seeded onto 96 well plates in 100 μl of the RPMI-1640 culture medium supplemented with 10% fetal bovine serum and allowed to grow in a CO_2 incubator with 5% CO_2 at 37 °C. Stock solutions of test compounds were prepared in DMSO. After 24 h incubation, different concentrations (10^{-7}–10^{-3} M) of the compounds, made by serial dilutions in the culture medium, were added in 48 and 72 h experimental sets for all the compounds separately. The final concentration of dmso was 0.01% in each well. A separate well containing 0.01% dmso only was run also as dmso control.

Cell growth was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which is based on ability of the viable cells to reduce a soluble yellow tetrazolium salt to a blue formazan crystals [24,25]. Briefly, after 48 and 72 h of the treatment, the MTT dye (10 μl/100 μl of medium), prepared in phosphate buffered saline (PBS) was added to all the wells. The plates were then incubated for 4 h at 37 °C, medium was discarded and 100 μl of dmso was added to each well. Optical density was measured at 570 nm. Percent of viable cells are determined by taking the cell counts in the untreated sets as 100%. By the help of semi logarithmic dose-response plots, constructed using GRAPHPAD PRISM5 software [26], the IC_{50} values were determined as concentration of the compound that inhibited DL cell growth by 50%.

2.4. Preparation of 3-(4-benzyloxyphenyl)-1-(2-hydroxylphenyl)-prop-2-en-1-one (L^1H) and 2-(4-benzyloxyphenyl)-3hydroxy-chromen-4-one (L^2H)

The synthetic details for the preparation of chalcone (L^1H) and flavones derivative (L^2H) is described by us elsewhere [27].

2.5. Synthesis of fac-[RuCl(S-dmso)$_3$(L^1)] **1**

A methanolic solution (30 cm^3) of *cis*-[$Ru^{II}Cl_2(dmso)_4$] (484 mg, 1 mmol) was added dropwise to a solution of L^1H (330 mg, 1 mmol) in methanol (25 mL) containing equimolar NEt_3, while stirring was continued for 12 h at room temperature. The red crystalline solid thus obtained was filtered and washed with methanol followed by diethyl ether and then dried *in vacuo*. Yield: 0.419 g (60%). M.p.: >200 °C. *Anal.* Calc. for $C_{28}H_{35}O_6S_3ClRu$: C, 48.0; H, 5.0; S, 13.7. Found: C, 48.4; H, 5.1; S, 13.6%. FAB-MS: *m/z*: 700

Table 1
Summary of crystallographic data for complexes **1** and **2**.

Parameters	1	2
CCDC deposition number	656402	650283
Empirical formula	$C_{28}H_{35}ClO_6RuS_3$	$C_{28}H_{33}ClO_7RuS_3$
Formula weight	700.26	714.24
Temperature (K)	150(2)	150(2)
Crystal system	triclinic	monoclinic
Space group	$P\bar{1}$	$P2_1/n$
a (Å)	8.323(16)	15.549(14)
b (Å)	12.323(15)	12.132(2)
c (Å)	15.269(3)	17.028(6)
α (°)	81.06(13)	90.00
β (°)	89.87(16)	107.30(2)
γ (°)	73.09(14)	90.00
Volume (Å^3)	1478.80(4)	2259.77(14)
Z, calculated density (mg m^{-3})	2, 1.573	4, 1.547
Absorption coefficient (mm^{-1})	0.873	0.846
F(0 0 0)	720	1464
Reflections collected/unique	13880/5182	19101/5394
Data/restraints/parameters	5182/0/358	5394/0/367
R_{int}	0.0390	0.0588
Index ranges	$-9 \le h \le 9$ $-14 \le k \le 14$ $-18 \le l \le 18$	$-18 \le h \le 18$ $-14 \le k \le 14$ $-19 \le l \le 20$
θ Range for data collection (°)	3.45–25.00	3.02–25.00
Completeness to θ = 25.00 (%)	99.7	99.8
Maximum and minimum transmission	0.8380 and 0.7616	0.8038 and 0.7677
Refinement method	Full-matrix, least squares on F^2	
Final *R* indices [$I > 2\sigma(I)$] R_1, wR_2	0.0371, 0.1038	0.0340, 0.0639
R indices (all data) R_1, wR_2	0.0449, 0.1111	0.0752, 0.0722
Goodness-of-fit (GOF)	1.061	0.848
Largest differences in peak and hole (e Å^{-3})	0.832 and −0.932	0.455 and −0.316

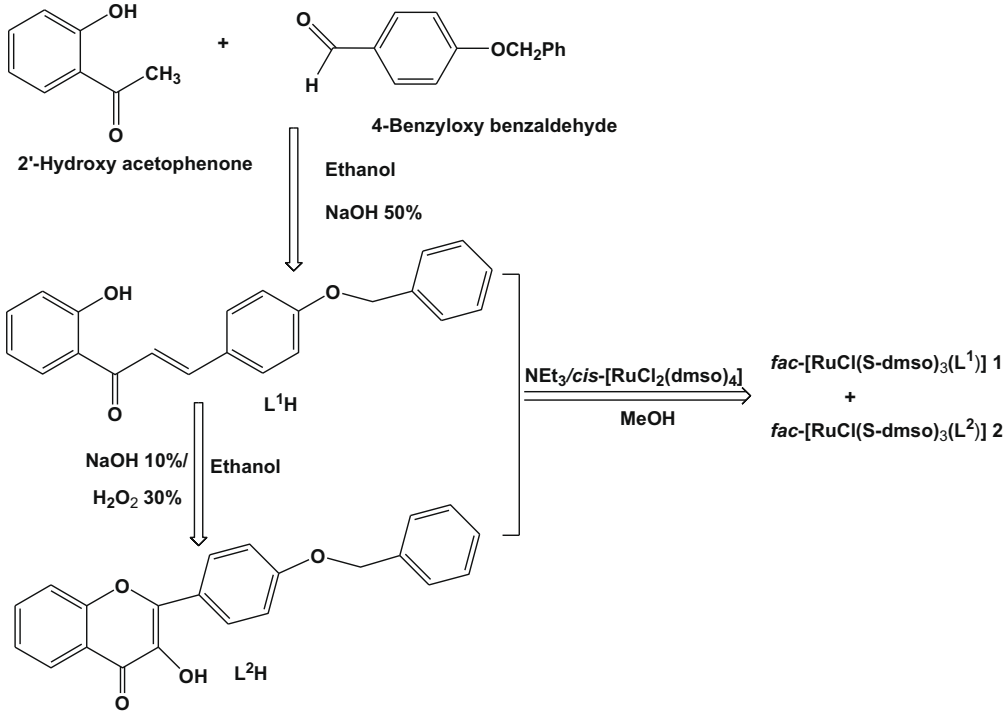
Table 2
Selected bond lengths (Å) and bond angles (°) for complexes **1** and **2**.

Compound ***1***					
Ru(1)–O(1)	2.026(2)	O(1)–Ru(1)–O(2)	88.93(9)	O(1)–Ru(1)–S(1)	178.51(7)
Ru(1)–O(2)	2.061(2)	O(2)–Ru(1)–S(1)	89.73(7)	O(1)–Ru(1)–S(3)	85.70(7)
Ru(1)–S(1)	2.246(10)	O(2)–Ru(1)–S(3)	173.67(7)	S(1)–Ru(1)–S(3)	95.59(4)
Ru(1)–S(3)	2.255(10)	O(1)–Ru(1)–S(2)	88.30(7)	O(2)–Ru(1)–S(2)	88.14(7)
Ru(1)–S(2)	2.266(9)	S(1)–Ru(1)–S(2)	92.31(3)	S(3)–Ru(1)–S(2)	95.05(3)
Ru(1)–Cl(1)	2.404(9)	O(1)–Ru(1)–Cl(1)	86.17(7)	O(2)–Ru(1)–Cl(1)	87.16(7)
O(1)–C(1)	1.296(4)	S(1)–Ru(1)–Cl(1)	93.11(3)	S(3)–Ru(1)–Cl(1)	89.13(3)
O(2)–C(7)	1.261(4)	S(2)–Ru(1)–Cl(1)	172.81(3)	O(6)–C(16)–C(17)	111.60(3)
C(8)–C(9)	1.329(5)	C(13)–O(6)–C(16)	119.3(3)	O(6)–C(16)–C(17)	111.6(3)
S(1)–O(3)	1.477(3)	C(1)–C(6)–C(7)	122.8(3)	C(9)–C(8)–C(7)	122.6(3)
Compound ***2***					
Ru(1)–O(4)	2.086(2)	O(4)–Ru(1)–O(5)	80.69(9)	O(4)–Ru(1)–S(1)	89.94(7)
Ru(1)–O(5)	2.098(2)	O(5)–Ru(1)–S(1)	170.63(7)	O(4)–Ru(1)–S(3)	171.82(7)
Ru(1)–S(1)	2.246(10)	O(5)–Ru(1)–S(3)	92.14(7)	S(1)–Ru(1)–S(3)	97.22(4)
Ru(1)–S(3)	2.245(11)	O(4)–Ru(1)–S(2)	89.08(7)	O(5)–Ru(1)–S(2)	87.30(7)
Ru(1)–S(2)	2.275(13)	S(1)–Ru(1)–S(2)	92.38(4)	S(3)–Ru(1)–S(2)	94.55(4)
Ru(1)–Cl(1)	2.418(13)	O(4)–Ru(1)–Cl(1)	84.93(7)	O(5)–Ru(1)–Cl(1)	86.07(7)
O(4)–C(7)	1.271(4)	S(1)–Ru(1)–Cl(1)	93.35(4)	S(3)–Ru(1)–Cl(1)	90.68(4)
O(5)–C(15)	1.354(4)	S(2)–Ru(1)–Cl(1)	171.70(4)	O(7)–C(22)–C(23)	110.1(4)
C(14)–C(15)	1.384(5)	C(7)–O(4)–Ru(1)	111.5(2)	C(15)–O(5)–Ru(1)	109.3(2)
O(7)–C(22)	1.443(4)	C(13)–O(6)–C(14)	122.9(3)	C(19)–O(7)–C(22)	116.0(3)

$[M]^+$, 664 $[M-Cl]^+$, 587 $[M-Cl\text{-}dmso]^+$, 508 $[M-Cl\text{-}2dmso]^+$. IR (KBr pellet, cm^{-1}): 3016(m) υ(C–H), 1606(s) υ(C=O), 1096(s) υ(S=O), 426(m) υ(Ru–S). ^1H NMR ($CDCl_3$, δ ppm): 5.12 (s, 2H; OCH_2), 3.09–3.55 (m, 18H; dmso), 7.76 (s, 2H; HC=CH), 6.52–7.74 (m, 13H; phenyl). UV–Vis (dmso, 10^{-4} M): λ_{max} (nm) (ε_{max} $M^{-1}\,cm^{-1}$) 234 (39800), 364 (37800), 498^{sh} (8400). Emission at λ_{ex} 498 nm (dmso, 10^{-4} M): non-emissive.

2.6. Synthesis of *fac*-[RuCl(S-dmso)$_3$(L^2)] **2**

This complex was also prepared using procedure similar to that used for complex **1** by the addition of a methanolic solution of *cis*-$[Ru^{II}Cl_2(dmso)_4]$ (484 mg, 1 mmol) (25 mL) to a solution of L^2H (344 mg, 1 mmol) in methanol containing equimolar amount of NEt_3 at pH ~9.0. The colour of reaction mixture changes from light brown to dark reddish brown. The reaction mixture was then stirred on a steam bath for 0.5 h. The reddish solution was then reduced to one fourth of its initial volume and left at room temperature for 24 h. The crystalline solid thus obtained was filtered and then washed with methanol followed by diethyl ether and finally dried in *vacuo*. Yield: 0.464 g (65%). M.p.: >200 °C. *Anal.* Calc. for $C_{28}H_{33}O_7S_3ClRu$: C, 47.1; H, 4.6; S, 13.4. Found: C, 47.1; H, 4.5; S, 13.3%. FAB-MS: *m*/*z*: 715 [M+1], 679 $[M-Cl]^+$, 602 $[M-Cl\text{-}dmso]^+$, 524 $[M-Cl\text{-}2dmso]^+$. IR (KBr pellet, cm^{-1}): 3034(m) υ(C–H), 1605(s) υ(C=O), 1107(s) υ(C–O–C), 1080(s) υ(S=O), 428(m) υ(Ru–S). ^1H NMR (dmso-d_6, δ ppm): 5.16 (s, 2H; OCH_2), 7.07–8.65 (m, 13H; phenyl), 3.16–3.69 (m, 18H; dmso). UV–Vis (dmso, 10^{-4} M): λ_{max} (nm) (ε_{max} $M^{-1}\,cm^{-1}$) 228 (31600), 342 (17430),



Scheme 1. Synthesis of ligands (L^1H and L^2H) and their complexes (**1** and **2**).

469^{sh} (16200). Emission at λ_{ex} 469 nm (dmso, 10^{-4} M): λ_{max} (nm) (intensity in a.u.) 531 (24.1).

3. Result and discussion

3.1. Structural characterization

Synthetic routes for the preparation of ligands and their Ru(II) complexes are depicted in Scheme 1. The complexes are found air stable and soluble in organic solvents such as acetonitrile, dimethylformamide (dmf), dimethyl sulfoxide (dmso) and dichloromethane (dcm). Electrical conductivity of the complexes measured in dmso (10^{-3} M) solution shows that complexes are non-electrolyte in nature. The IR spectrum of L^1H displays characteristic peaks at 3450 and 1637 cm^{-1} assigned to υ(OH) and υ(C=O) vibrations, respectively. However, L^2H shows sharp and strong peaks at 3441 and 1607 cm^{-1} assigned as υ(OH) and υ(C=O) vibrations, respectively. Another peak observed at 1110 cm^{-1} is assigned to υ(C–O–C) vibration [28].

The υ(C=O) vibration observed at 1606 and 1605 cm^{-1} in the IR spectra of complexes **1** and **2**, respectively show that C=O group of the corresponding ligand had coordinated to Ru(II) ion. However, no peak due to υ(O–H) is observed in corresponding IR spectra of complexes. Thus, it may be considered that OH group is deprotonated during coordination with the metal ion. Additional peaks observed at 1100–1050 and 425–430 cm^{-1} are assigned to υ(S=O) and υ(Ru–S) vibrations [29], respectively. The structures of complexes are further supported by their ^{1}H NMR spectral data. The ^{1}H NMR spectrum of complex **1** shows peaks at δ (ppm) 5.12 (s, 2H; OCH_2), 3.09–3.55 (m, 18H; dmso), 7.76 (s, 2H; HC=CH), 6.52–7.74 (m, 13H; phenyl). No peak corresponding to phenolic OH group is observed in the spectra of both the complexes **1** and **2**. Hence it again supports that deprotonated OH group had coordinated with Ru(II) ion. Additional peaks observed in ^{1}H NMR

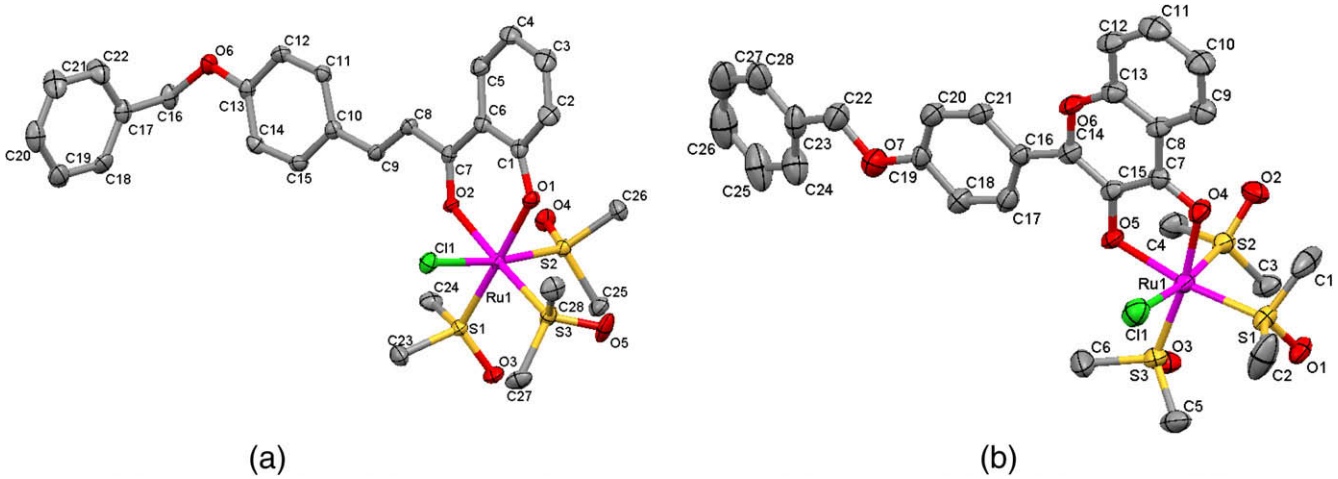


Fig. 1. ORTEP view of complexes **1** (a) and **2** (b), with atom numbering scheme and thermal ellipsoids drawn at the 50% probability level. H-atoms have been removed for clarity.

Table 3
Selected parameters for weak interactions in complexes **1** and **2**.

D–H···A (Å)	D–H (Å)	H···A (Å)	D···A (Å)	DHA (°)	Symmetry code
*Compound **1***					
C(9)–H(9)···O(2)	0.93	2.38	2.724(4)	102	–
C(12)–H(12)···O(4)	0.93	2.46	3.386(4)	171	$-x, 1-y, -z$
C(14)–H(14)···O(3)	0.93	2.54	3.404(4)	155	$1-x, -y, -z$
C(18)–H(18)···O(3)	0.93	2.49	3.266(4)	141	$1-x, -y, -z$
C(20)–H(20)···O(4)	0.93	2.52	3.440(5)	170	$x, y, -1+z$
C(23)–H(23B)···Cl(1)	0.96	2.76	3.625(4)	151	$-x, -y, -z$
C(25)–H(25A)···O(3)	0.96	2.53	3.142(5)	121	–
C(25)–H(25B)···O(5)	0.96	2.25	3.057(5)	141	–
C(26)–H(26A)···O(5)	0.96	2.43	3.172(4)	134	$-x, -y, 1-z$
C(28)–H(28B)···Cl(1)	0.96	2.80	3.376(5)	120	–
C(28)–H(28C)···O(1)	0.96	2.41	2.922(5)	113	–
*Compound **2***					
C(1)–H(1A)···O(1)	0.96	2.55	3.487(5)	165	$-x, 1-y, 1-z$
C(1)–H(1B)···O(4)	0.96	2.36	2.873(5)	113	–
C(3)–H(3B)···O(3)	0.96	2.31	3.093(5)	138	–
C(3)–H(3C)···O(1)	0.96	2.47	3.153(5)	128	–
C(4)–H(4A)···O(5)	0.96	2.49	2.999(4)	113	–
C(6)–H(6B)···Cl(1)	0.96	2.72	3.382(5)	127	–
C(11)–H(11)···O(1)	0.93	2.33	3.217(5)	158	$x, -1+y, z$
C(12)–H(12)···O(3)	0.93	2.57	3.439(5)	156	$x, -1+y, z$
C(17)–H(17)···O(5)	0.93	2.28	2.924(4)	126	–
C(20)–H(20)···O(2)	0.93	2.53	3.427(5)	161	$1-x, -y, 1-z$
C(21)–H(21)···O(6)	0.93	2.26	2.630(5)	103	–
C(24)–H(24)···O(7)	0.93	2.38	2.736(6)	102	–

spectrum of complex **2** are assigned at δ 5.16 (s, 2H) OCH_2, 7.07–8.65 (m, 13H) phenyl and at δ 3.16–3.69 ppm (m, 18H) are methyl protons of dmso.

In the UV–Vis spectra, the peaks are observed at λ_{max} 234, 364, 498 nm and 228, 342, 469 nm (Fig. S1, Supplementary Information). These peaks are assigned to intra-ligand and MLCT transitions, respectively. The MLCT transition of complex **1** occurs at lower energy as compared to its position in complex **2**. This could be considered owing to highly conjugated skeleton of the ligand present in the complex **1**.

Thus, on the basis of spectral (IR, ^{1}H NMR) data along with elemental analysis and FAB mass data, the composition of the complexes are assigned as *fac*-[RuCl(S-dmso)$_3$(L^1)] **1** and *fac*-[RuCl(S-dmso)$_3$(L^2)] **2**. However, their molecular structures are further investigated using their X-ray diffraction data.

The molecular structures along with crystallographic numbering schemes of the complexes (**1** and **2**) are illustrated in Fig. 1. A computational program PLATON [23] is used to study the involvement of weaker interactions and bond distances data are shown in Table 3.

Complex **1** crystallizes in a triclinic crystal system with space group $P\bar{1}$. The unit cell contains two discrete molecules; arranged in a head to tail fashion, extending along the axis. No solvent accessible voids could be seen from its packing behaviour. The Kitaigorodskii packing index [30] is found to be 69.8% with the presence of 6 grid points. The non-classical hydrogen bond (11) distances are listed in Table 3.

The coordination core (O_2S_3Cl) consisting of a deprotonated bidentate ligand (L^1; O, O), three dmso (S, S, S) and one chlorine atom provides a distorted octahedral geometry around Ru(II) ion. Three S-dmso molecules display facial geometry. Thus, hetero-coordination core specially of weaker ligands surrounding Ru(II) ion makes this complex quite interesting in view of the possibility of making selective substitutions by another ligand especially of bio-molecules. The bond distances varies as (Ru–Cl) > (Ru–S) > (Ru–O). The C1–O1–Ru1–O2–C7 atoms lie in same plane. However, C1–C6 and C17–C22 rings are bent towards C10–C15 ring with a dihedral angle of 10.5(5)° and 76.4(2)°, respectively. Benzyl ring is almost perpendicular to C10–C15 ring. The two oxygen atoms of ligand are bonded to Ru(II) centre at distances Ru(1)–O(1) 2.026(2) Å and Ru(1)–O(2) 2.061(2) Å which are found in consistence with the reported values [31,32]. Additionally, Ru–S distance varies from 2.246(10) to 2.266(9) Å whereas, Ru–Cl bond length is found to be 2.404(9) Å which are again consistent with the values reported for *cis*-[$RuCl_2$(dmso)$_4$] complex [33].

The crystal packing behaviour of the complex **1** consists of 2D layered structure along the crystallographic [0 1 0] direction (Fig. 2). The adjacent two Ru(II) centers are separated by a distance of 8.32(2) Å in the chain. However, the shortest interlayer distance (calculated between the two ruthenium atom centers between the adjacent chains) is found as 12.32(2) Å.

PLATON analysis of the structre confirms the presence of eleven non-conventional hydrogen bonds, out of which; in two cases, Cl(1) acts as acceptor and is involved in weak intearcation with H(23B) and H(28B), at H···A distance of 2.76(2) Å H(23B)–Cl(1) (intermolecular) and 2.80(2) Å H(28B)–Cl(1) (intramolecular) as depicted in Fig. 3.

Molecular structure of the complex **2** is depicted in Fig. 1. The monoclinic crystal contains four discrete complex molecules and are arranged in a chain and showing no solvent accessible voids in the packing of this complex. However, Kitaigorodskii packing index [30] is found 67.2% with the presence of 4 grid points. The weaker force study using PLATON programme indicates that 12 non-conventional H-bonds are formed in its packing structure. These non-conventional bonds involve C–H as H-donor and O as H-acceptor in most of the cases whereas in one case Cl acts as H-acceptor.

In this complex too, each Ru(II) ion is surrounded by hetero-coordination core consisting of one chlorine atom, three dmso molecules (S, S, S) and one deprotonated bidentate (O, O) flavone ligand in a distorted octahedral arrangement. Coordinated three S-dmso molecules are found to be in facial geometry. The distances Ru(1)–O(4) 2.086(2) to Ru(1)–O(5) 2.098(2) Å, Ru–S 2.246(10)–2.275(13) Å and Ru–Cl at 2.418(13) Å are found in the range of reported values. The C7–O4–Ru1–O5–C15 are co-planar with the three phenyl rings while benzyl ring deviate from planarity with a dihedral angle of 18.4(3)°.

The crystal packing diagram of complex **2** as depicted in Fig. S2 (Supplementary Information) shows that a 1D chain like structure

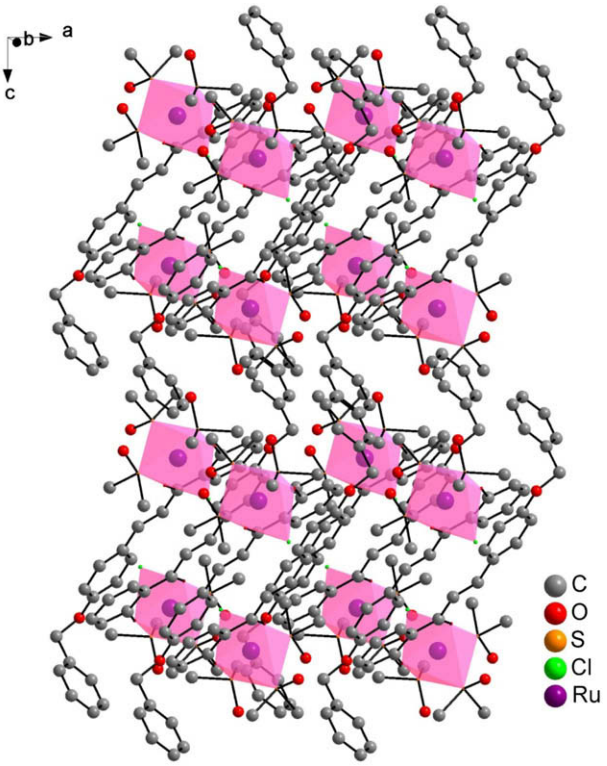


Fig. 2. Perspective view of **1** along the [0 1 0] direction, showing the 2D layered structure (H-atoms are omitted for clarity).

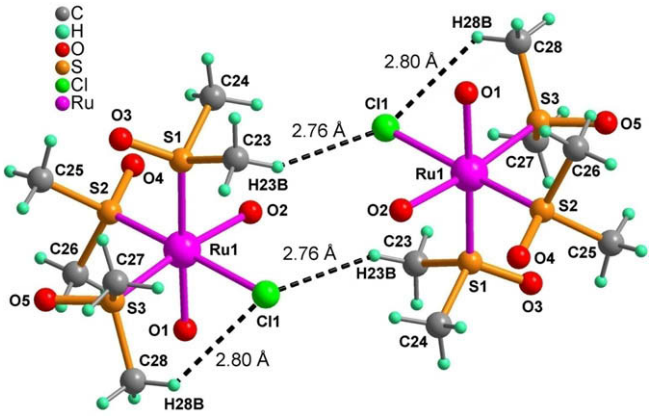


Fig. 3. Intermolecular C–H···Cl interaction in **1** (ligand framework are omitted for clarity).

with Ru(II) centers separated at 19.72(2) Å is formed. The inter chain Ru···Ru distance is found to be 8.54(9) Å.

Thus, both complexes crystallize in different crystal system providing different mode of packing with different metal–ligand bond distances. However, it is quite interesting to observe that both assemble structures are getting stabilized by non-covalent hydrogen bonds.

3.2. In vitro cytotoxicity assay

The cell toxicity was measured using MTT assay technique as this is reported as a reliable method to determine bioactivity of the compounds [34]. Dalton lymphoma (DL) has been successfully used earlier to estimate the anticancer potential of a novel Ru(II) complex both *in vitro* and *in vivo* [15]. Therefore, to have a preliminary level screening of these newly synthesized compounds, MTT assay is performed for the evaluation of their anticancer potential on DL cells *in vitro*. DMSO has been reported to be non-toxic to the DL cells *in vitro* [35]. We also performed MTT assay in the well containing 0.01% dmso and it showed 100% cell viability thus suggested no toxicity of dmso on DL cells. However, as shown in Table 4, all the compounds show different responses on DL cells (as anticipated by their structural difference) in culture when incubation was restricted to 48 h. The complex **1** is found to be the most cytotoxic with IC_{50} value of 0.319 μM followed by the activity of compounds **2** and L^1H with the IC_{50} values of 0.816 μM and 1.337 μM, respectively. Nonetheless, L^2H was found to be the least cytotoxic with the IC_{50} of >5 μM. After 72 h treatment the pattern of IC_{50} values of all the compounds show a similar trend, however, within a very narrow range of 0.032 μM for **1** to 0.054 μM for L^2H. Similar type of dose and incubation time dependent cytotoxic pattern is also observed when data is presented as percentage of viable DL cells in all experimental sets (Fig. 4).

Table 4
IC_{50} values (μM) of L^1H, **1**, L^2H and **2** on Dalton lymphoma cells after 48 h and 72 h of incubation.

Compound	48 h	72 h
Dmso control[a]	none	none
L^1H	1.337	0.034
1	0.319	0.032
L^2H	>5	0.054
2	0.816	0.042

[a] DL cells incubated with 0.01% dmso, as solvent control, did not produce any cytotoxicity.

Some novel dinuclear ruthenium–arene compounds have also been tested on human ovarian carcinoma (A2780) and colon adenocarcinoma (SW480), however, showing 4–5 times higher IC_{50} values than the cytotoxicity of cisplatin (IC_{50} = 0.33 μM) and oxaliplatin (IC_{50} = 0.40 μM) [36]. *In vitro* cytotoxicity of some ruthenium(III) dimethyl sulfoxide pyridinehydroxamic acid complexes have also been evaluated on KB oral carcinoma cells which after 48 and 72 h of treatment showed IC_{50} of 246–301 μM and 284–211 μM, respectively [37]. The IC_{50} of another well known anticancer compound 5-fluorouracil, tested on the DL cells, has been reported to be 37.4 μM [38]. Recently, we have reported a concentration dependent cytotoxicity of a Ru(II) complex against the DL cells [15], however, in comparison, IC_{50} values of L^1H, **1**, L^2H and **2** are in the range of micro- to nano-molar concentrations (Table 4) which is not only much lower than these reported compounds but are also several times lower than the cisplatin and oxaliplatin and thus suggesting potent cytotoxicity of the compounds reported here. Furthermore, the compounds **1** and **2** show lower IC_{50} values than those of the corresponding ligands when tested alone. This suggests that interaction of both the ligands with Ru(II)-metal centre enhances their anticancer activity *in vitro*. Some Ru(II) complexes have also been reported to show their IC_{50} in micromolar range [39]. However, in comparison, Ru(II) complexes in the present study show significantly lower IC_{50} values

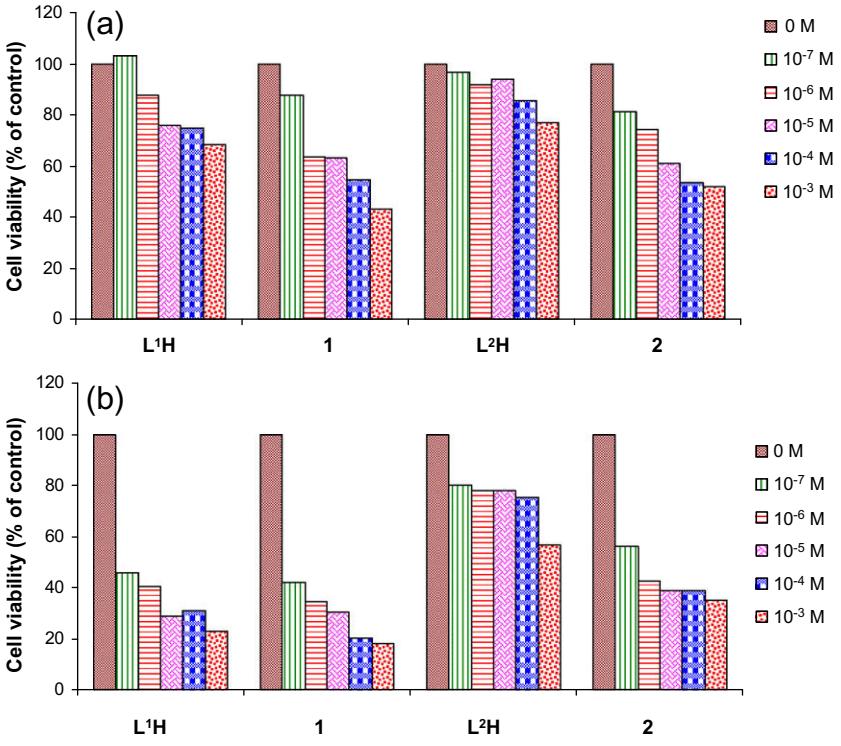


Fig. 4. Effect of L^1H, **1**, L^2H and **2** on DL cell viability. DL cells were treated with L^1H, **1**, L^2H and **2** for 48 h (a) and 72 h (b) at the indicated concentrations, and viability was measured by MTT assay.

against the DL cells, even lower than cisplatin on other cell lines, and hence show better anticancer activity.

4. Conclusion

Two new complexes bearing hetero-coordination core (S, S, S; O, O; Cl) around Ru(II) ion are prepared and characterized using spectroscopic as well as X-ray crystallographic techniques. These complexes show significant cytotoxicity against Dalton Lymphoma cells.

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Appendix A. Supplementary data

CCDC 656402 and 650283 contain the supplementary crystallographic data for **1** and **2**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.poly.2009.11.012.

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