Inorg. Chem. **2003**, *42*, 7579–7586

Ruthenium(II) Sulfoxide–Maltolato and –Nitroimidazole Complexes: Synthesis and MTT Assay

Adam Wu, David C. Kennedy, Brian O. Patrick, and Brian R. James*

Department of Chemistry, The University of British Columbia, 2036 Main Mall, Vancouver, British Columbia, Canada V6T 1Z1

Received April 7, 2003

Ru^{II} sulfoxide–maltolato complexes, $Ru(ma)_2(L)_2$ (L = DMSO (**1a**) and TMSO (**1b**) or L_2 = BESE (**1c**)), were synthesized, as well as the analogous ethylmaltolato derivatives, $Ru(etma)_2(L)_2$ (**2a**–**c**) (ma = 3-hydroxy-2-methylpyran-4-onate, etma = 2-ethyl-3-hydroxypyran-4-onate, TMSO = tetramethylene sulfoxide, BESE = 1,2-bis(ethylsulfinyl)ethane). A Ru^{II} bidentate sulfoxide–metronidazole complex, $RuCl_2(BESE)(metro)_2$ (**3**), was also synthesized (metro = metronidazole = 2-methyl-5-nitroimidazole-1-ethanol). The complexes were characterized generally by ^1H NMR, UV–vis, and IR spectroscopies, as well as MS, elemental analysis, solution conductivity, and cyclic voltammetry. The molecular structures of $Ru(ma)_2$(*S*,*R*-BESE) (**1c**) and *trans*-$RuCl_2$(*R*,*R*-BESE)$(metro)_2$ (**3**) were determined by X-ray crystallography. All sulfoxide ligands are S-bonded. The complexes were tested against human breast cancer cells (MDA-MB-435S) using an in vitro MTT assay, a colorimetric determination of cell viability: **2a**,**b** exhibit the lowest IC_{50} values of 190 ± 10 and 220 ± 10 μM, respectively. Cisplatin exhibits an IC_{50} value of 30 ± 5 μM.

Introduction

cis- and *trans*-$RuCl_2(DMSO)_4$ exhibit anticancer activity,[1,2] most likely due to DNA coordinating to the Ru via intrastrand cross-linking between two adjacent purines, and both the cis and trans isomers have been shown to react in water with nucleoside and nucleotide components.[3] Other potent Ru complexes include (ImH)[*trans*-$Ru(Im)_2Cl_4$], where the anticancer mechanism is thought to be transferrin-mediated (Im = imidazole),[4] and (Na or ImH)[*trans*-$Ru(Im)(DMSO)Cl_4$] (called NAMI and NAMI-A, respectively) that prevent tumor metastases via a mechanism thought not to involve Ru−DNA binding as the complexes lack cytotoxicity.[5,6] NAMI-A is currently undergoing phase I clinical trials.[7] A general review on the biological activities of Ru complexes appeared in 1999.[1]

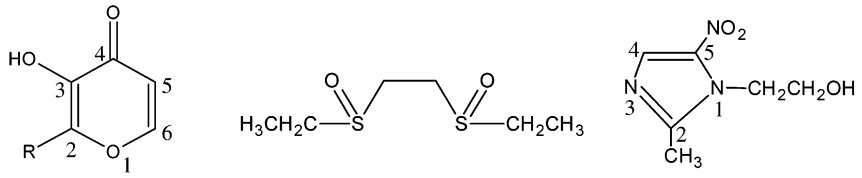
Our group was the first to synthesize *cis*-$RuCl_2(DMSO)_4$[8] but for use as an olefin hydrogenation catalyst, this then leading to the first chiral sulfoxide systems for asymmetric hydrogenation.[9] We later synthesized Ru^{II} sulfoxide−nitroimidazole complexes as potential radiosensitizers, with $RuCl_2(DMSO)_2(4\text{-}NO_2Im)_2$ being the most effective, although the configuration of such complexes was never

* To whom correspondence should be addressed. E-mail: brj@chem.ubc.ca.

(1) Clarke, M. J.; Zhu, F.; Frasca, D. R. *Chem. Rev.* **1999**, *99*, 2511.
(2) (a) Alessio, E.; Mestroni, G.; Nardin, G.; Attia, W. M.; Calligaris, M.; Sava, G.; Zorzet, S. *Inorg. Chem.* **1988**, *27*, 4099 and references therein. (b) Farrell, N. P. *Transition Metal Complexes as Drugs and Chemotherapuetic Agents*; Ugo, R., James, B. R., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1989; p 147. (c) Coluccia, M.; Sava, G.; Loseto, F.; Nassi, A.; Boccarelli, A.; Giordano, D.; Alessio, E.; Mestroni, G. *Eur. J. Cancer* **1993**, *29A*, 1873 and references therein.
(3) (a) Cauci, S.; Viglino, P.; Esposito, G.; Quadrifoglio, F. *J. Inorg. Biochem.* **1991**, *43*, 739. (b) Esposito, G.; Cauci, S.; Fogolsri, F.; Alessio, E.; Scocchi, M.; Quadrifoglio, F.; Viglino, P. *Biochemistry* **1992**, *31*, 7094. (c) Tian, Y.; Yang, P.; Li, Q.; Guo, M.; Zhao, M. *Polyhedron* **1997**, *16*, 1993. (d) Davey, J. M.; Moerman, K. L.; Ralph, S. F.; Kanitz, R.; Sheil, M. M. *Inorg. Chim. Acta* **1998**, *281*, 10.
(4) (a) Keppler, B. K.; Rupp, W.; Juhl, U. M.; Endres, H.; Niebl, R.; Balzer, W. *Inorg. Chem.* **1987**, *26*, 4366. (b) Kratz, F.; Hartmann, M.; Keppler, B.; Messori, L. *J. Biol. Chem.* **1994**, *269*, 2581.
(5) (a) Sava, G.; Pacor, S.; Coluccia, M.; Mariggio, M.; Cocchietto, M.; Alessio, E.; Mestroni, G. *Drug Invest.* **1994**, *8*, 150. (b) Sava, G.; Pacor, S.; Bergamo, A.; Cocchietto, M.; Mestroni, G.; Alessio, E. *Chem.-Biol. Interact.* **1995**, *95*, 109. (c) Sava, G.; Capozzi, I.; Clerici, K.; Gagliargi, G.; Alessio, E.; Mestroni, G. *Clin. Exp. Metastasis* **1998**, *16*, 371. (d) Sava, G.; Gagliardi, R.; Bergamo, A.; Alessio, E.; Mestroni, G. *Anticancer Res.* **1999**, *19*, 969.
(6) (a) Bergamo, A.; Gagliardi, R.; Scarcia, V.; Furlani, A.; Alessio, E.; Mestroni, G.; Sava, G. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 559. (b) Bergamo, A.; Zorzet, S.; Gava, B.; Sorc, A.; Alessio, E.; Iengo, E.; Sava, G. *Anti-Cancer Drugs* **2000**, *11*, 665.
(7) Sava, G.; Bergamo, A.; Zorzet, S.; Gava, B.; Casarsa, C.; Cocchietto, M.; Furlani, A.; Scarcia, V.; Serli, B.; Iengo, E.; Alessio, E.; Mestroni, G. *Eur. J. Cancer* **2002**, *38*, 427.
(8) James, B. R.; Ochiai, E.; Rempel, G. L. *Inorg. Nucl. Chem. Lett.* **1971**, *7*, 781.

10.1021/ic030119j CCC: $25.00
Published on Web 10/10/2003

Chart 1



R = Me, maltol (Hma);
R = Et, ethylmaltol (Hetma)

BESE

metronidazole (metro)

established by a crystal structure.[10] Bidentate-sulfoxide complexes of the type *cis*- or *trans*-$RuCl_2(S-S)_2$, where S−S = sulfur-bonded $RS(O)(CH_2)_nS(O)R$ (R = alkyl or aryl), were then made, and in vitro assays indicated that they accumulated in Chinese hamster ovary cells without hypoxic selectivity or toxicity, while the trans species accumulated in DNA to a greater degree than the cis complexes.[11,12]

We more recently synthesized several Ru β-diketonato−imidazole complexes, including *cis*-$[Ru(acac)_2(L)_2](CF_3SO_3)$ (L = Im or N-MeIm), that exhibit hypoxia-selective toxicity toward some mouse carcinoma cells.[13] We have now extended our studies by using maltolato ligands (Chart 1) in place of diketonato; both monoanionic ligand types utilize similar η^2(O−O) bonding modes, giving five- and six-membered chelate rings, respectively, but maltol is nontoxic and is used as a food additive.[14] The promise of Ru sulfoxide complexes for anticancer activity encourages us to present this paper that focuses on the characterization of Ru^{II} maltolato and ethylmaltolato complexes (**1** and **2**) with ancillary monodentate (DMSO and TMSO) and bidentate (BESE) sulfoxide ligands (see Chart 1), as well as a BESE/metro complex (**3**); the MTT assay data for **1**−**3** against a human breast cancer cell line are also presented. Metro itself (Chart 1) has been used clinically as a radiosensitizer,[15] and for treating anaerobic bacterial infections.[16] We have recently published related papers on the synthesis and MTT assay results of Ru^{II} *p*-cymene complexes containing BESE[17a] and Ru^{II} acac complexes containing sulfoxides.[17b]

Experimental Section

Materials for Synthesis. Reagent grade solvents (Fisher Scientific) were dried using standard procedures[18] under N_2 before use, and deuterated solvents (Cambridge Isotope Laboratories) were used as received. $RuCl_3 \cdot 3H_2O$ (Colonial Metals), maltol (Cultor Food Science), ethylmaltol (Pfizer Food Science), KO^{t}Bu (Acros Organics), TMSO, metronidazole, $[^nBu_4N](PF_6)$, $FeCp^*_2$, cisplatin, and silica gel preparative TLC plates with fluorescent indicator (20 × 20 cm^2, Uniplate from Analtech) were purchased from Aldrich, unless stated otherwise. *meso*-BESE,[11,17] K(ma),[19,20] *cis*-$RuCl_2(DMSO)_4$,[21] *cis*-$RuCl_2(TMSO)_4$,[22] and $[RuCl(H_2O)(BESE)]_2(\mu\text{-}Cl)_2$[12] were prepared by literature methods. K(etma) was synthesized by following the maltol/KO^{t}Bu procedure used for K(ma),[19,20] except ethylmaltol was used. Standard Schlenk techniques were used for synthesis of the complexes.

Physical Techniques and Instrumentation. ^{1}H NMR spectra were recorded at room temperature (rt, ∼20 °C) on Bruker AV300 or AV400 instruments (s = singlet, d = doublet, br = broad, and m = multiplet; *J* values are given in Hz), with chemical shifts being calibrated using residual proton resonances from deuterated solvents. Elemental analyses were performed by P. Borda of this department or by M. K. Yang of the Simon Fraser University Chemistry Department on Carlo Erba EA 1108 CHN-O analyzers. Mass spectral data (reported as *m*/*z* values) were acquired on a Kratos Concept IIHQ LSIMS instrument using a thioglycerol matrix or on a Bruker Esquire ES spectrometer in this department (c/o G. Eigendorf). UV−vis spectra were recorded at rt on a Hewlett-Packard 8452A diode-array spectrometer, and data are presented as λ_{max}, nm ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}). IR spectra (KBr) were recorded on ATI Mattson Genesis or Bomem-Michelson MB-100 FT-IR spectrometers; selected ν values (cm^{-1}) are given with assigned functional groups.[23] Conductivity measurements, carried out on a RCM151B Serfass conductance bridge (A. H. Thomas Co. Ltd.) with a 3403 cell (Yellow Springs Instrument Co.), were calibrated using 0.01000 M aqueous KCl solution (Λ_M = 141.3 Ω^{-1} cm^2 mol^{-1} at 25 °C, cell constant = 1.016 cm^{-1}), and data are given in units of Ω^{-1} cm^2 mol^{-1}.[24] CV was performed in CH_2Cl_2 containing 0.1 M $[^nBu_4N](PF_6)$ as supporting electrolyte, voltammograms being

(9) (a) McMillan, R. S.; Mercer, A.; James, B. R.; Trotter, J. *J. Chem. Soc., Dalton Trans.* **1975**, 1006. (b) James, B. R.; McMillan, R. S.; Reimer, K. J. *J. Mol. Catal.* **1975/76**, *1*, 439. (c) James, B. R.; McMillan, R. S. *Can. J. Chem.* **1977**, *55*, 3927. (d) Davies, A. R.; Einstein, F. W. B.; Farrell, N. P.; James, B. R.; McMillan, R. S. *Inorg. Chem.* **1978**, *17*, 1965. (e) James, B. R.; McMillan, R. S.; Morris, R. H.; Wang, D. K. W. *Adv. Chem. Ser.* **1978**, *No. 167*, 122.

(10) (a) Chan, P. K. L.; Skov, K. A.; James, B. R.; Farrell, N. P. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1059. (b) Chan, P. K. L.; Chan, P. K. H.; Frost, D. C.; James, B. R.; Skov, K. A. *Can. J. Chem.* **1988**, *66*, 117 and references therein. (c) Chan, P. K. L.; James, B. R.; Frost, D. C.; Chan, P. K. H.; Hu, H.-L.; Skov, K. A. *Can. J. Chem.* **1989**, *67*, 508 and references therein.

(11) Yapp, D. T. T.; Rettig, S. J.; James, B. R.; Skov, K. A. *Inorg. Chem.* **1997**, *36*, 5635.

(12) Cheu, E. L. S. Ph.D. Dissertation, The University of British Columbia, Vancouver, BC, Canada, 2000.

(13) (a) Baird, I. R. Ph.D. Dissertation, The University of British Columbia, Vancouver, BC, Canada, 1999. (b) Baird, I. R.; Rettig, S. J.; James, B. R.; Skov, K. A. *Can. J. Chem.* **1999**, *77*, 1821.

(14) LeBlanc, D. T.; Akers, H. A. *Food Technol.* **1989**, *43*, 78.

(15) (a) Fowler, J. F.; Denekamp, J. *Pharmacol. Therapeut.* **1979**, *7*, 413. (b) Denny, W. A.; Roberts, P. B.; Anderson, R. F.; Brown, J. M.; Wilson, W. R. *Int. J. Radiat. Biol. Oncol. Phys.* **1992**, *22*, 553.

(16) Brogden, R. N.; Heel, R. C.; Speight, T. M.; Avery, G. S. *Drugs* **1978**, *16*, 387.

(17) Huxham, L. A.; Cheu, E. L. S.; Patrick, B. O.; James, B. R. *Inorg. Chim. Acta* **2003**, *352*, 238. (b) Wu, A.; Kennedy, D. C.; Patrick, B. O.; James, B. R. *Inorg. Chem. Commun.* **2003**, *6*, 996.

(18) Gordon, A. J.; Ford, R. A. *The Chemist's Companion: A Handbook of Practical Data, Techniques, and References*; John Wiley & Sons: New York, 1972; p 429.

(19) Fryzuk, M. D.; Jonker, M. J.; Rettig, S. J. *Chem. Commun.* **1997**, 377.

(20) Jonker, M. J. M.Sc. Dissertation, The University of British Columbia, Vancouver, BC, Canada, 1993.

(21) Evans, I. P.; Spencer, A.; Wilkinson, G. *J. Chem. Soc., Dalton Trans.* **1973**, 204.

(22) (a) Alessio, E.; Milani, B.; Mestroni, G.; Calligaris, M.; Faleschini, P.; Attia, W. M. *Inorg. Chim. Acta* **1990**, *177*, 255. (b) Yapp, D. T. T.; Jaswal, J.; Rettig, S. J.; James, B. R.; Skov, K. A. *Inorg. Chim. Acta* **1990**, *177*, 199.

(23) Pavia, D. L.; Lampman, G. M.; Kriz, G. S. *Introduction to Spectroscopy*, 2nd ed.; Harcourt Brace & Co: Orlando, FL, 1996.

(24) (a) Geary, W. J. *Coord. Chem. Rev.* **1971**, *7*, 81. (b) Huheey, J. E. *Inorganic Chemistry: Principles of Structure and Reactivity*, 3rd ed.; Harper Collins Publishers: New York, 1983; p 362.

Table 1. Crystallographic Data for **1c**·H_2O and **3**

	1c·H_2O	**3**
formula	$C_{18}H_{26}O_9S_2Ru$	$C_{18}H_{32}N_6O_8S_2Cl_2Ru$
fw	551.59	696.58
cryst color, habit	orange, prism	orange, platelet
cryst size (mm)	0.15 × 0.10 × 0.05	0.25 × 0.10 × 0.04
cryst system	triclinic	orthorhombic
space group	$P\bar{1}$ (No. 2)	*Pbca* (No. 61)
a (Å)	7.5998(3)	13.4946(7)
b (Å)	9.8229(4)	19.628(1)
c (Å)	15.3305(4)	20.746(1)
α (deg)	71.618(6)	90.00
β (deg)	82.902(8)	90.00
γ (deg)	89.238(8)	90.00
V (Å^3)	1077.34(8)	5495.1(5)
Z	2	8
D_{calcd} (g cm^{-3})	1.700	1.684
F_{000}	564	2848
no. of observns ($I > 0.00\sigma(I)$)	4403	6361
no. of variables	271	366
μ(Mo Kα) (cm^{-1})	9.69	9.70
R1[a]	0.029 ($I > 3\sigma(I)$, 3396 observns)	0.042 ($I > 2\sigma(I)$, 3674 observns)
wR2[b]	0.076	0.099
goodness of fit	0.88	0.87

[a] $R1 = \sum||F_o| - |F_c||/\sum|F_o|$ (observed data). [b] $wR2 = (\sum(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2)^{1/2}$ (all data).

recorded on a Pine Bipotentiostat (model AFCBP1) and PineChem v2.00 software; the scan rate was 200 mV s^{-1} using a Pt working electrode, a Pt wire counter electrode, and a Ag wire reference electrode, with $FeCp^*_2$ (−0.13 V vs SCE in CH_2Cl_2) used as an internal calibrant.[25] $E_{1/2}$ values are given vs SCE.

X-ray Crystallography. Measurements were made at 173(1) K on a Rigaku/ADSC CCD area detector with graphite-monochromated Mo Kα radiation (0.710 69 Å). Some crystallographic data for **1c**·H_2O and **3** are shown in Table 1. The final unit-cell parameters were based on 6720 reflections with $2\theta = 4.4-55.7°$ for **1c** and 20 589 reflections with $2\theta = 4.2-55.8°$ for **3**. The data were collected and processed using the d*TREK program,[26] and the structures were solved using direct methods[27] and expanded using Fourier techniques.[28] The non-hydrogen atoms were refined anisotropically, and the H atoms were included but not refined. For **3**, one Et of the BESE ligand, C(1)−C(2), was disordered and was subsequently modeled in two orientations, the populations of the disordered fragments being refined to roughly 0.58 and 0.42.

Ru(ma)$_2$(DMSO)$_2$ (1a). Complex **1a** was synthesized by a literature procedure[19] but using EtOH rather than toluene as solvent. A suspension of *cis*-$RuCl_2(DMSO)_4$ (100 mg, 0.21 mmol) and K(ma) (85 mg, 0.52 mmol) in EtOH (20 mL) was refluxed in air at 80 °C for 16 h to give a dark red solution. The solvent was removed under vacuum, and the residue was then extracted with C_6H_6 (2 × 20 mL); the extract was filtered through Celite, the filtrate was reduced to 10 mL, and then hexanes (60 mL) was added to yield a yellow precipitate that was collected under N_2 and dried in vacuo at rt. The hygroscopic product was stored in a desiccator over anhydrous $CaSO_4$.

Yield: 55 mg (53%). ^{1}H NMR (C_6D_6; δ): 2.07, 2.13, 2.14, 2.18 (s, 6H, CH_3-ma); 2.77, 2.86, 2.87, 2.94, 2.98, 3.07, 3.13, 3.19, 3.21, 3.28, 3.30, 3.34 (s, 12H, CH_3-DMSO); 6.03−6.15 (multiple d, 2H, *H*(5), $^3J_{HH} = 5.1$); 6.47−6.59 (multiple d, 2H, *H*(6), $^3J_{HH} = 5.1$). Anal. Calcd for $C_{16}H_{22}O_8S_2Ru$: C, 37.86; H, 4.37. Found: C, 38.00; H, 4.55. LR-MS (+LSIMS): 508 (M^+), 430 (M^+ − DMSO), 352 (M^+ − 2 DMSO). UV−vis (H_2O): 212 (32.0), 270 (10.7), 356 (6.03). IR (KBr): $\nu_{S=O}$ 1094; $\nu_{C=O} + \nu_{C=C}$ 1547; $\nu_{C=O}$ 1595. Λ_M (H_2O) = 8 (nonconducting). $E_{1/2}(Ru^{III/II}) = 0.52$ V. The NMR and IR ($\nu_{S=O}$) data agree with those reported.[19,20]

Ru(etma)$_2$(DMSO)$_2$ (2a). The complex was synthesized as for **1a**, except K(etma) (92 mg, 0.52 mmol) was used.

Yield: 50 mg (45%). ^{1}H NMR (C_6D_6; δ): 1.02 (m, 6H, CH_3-etma); 2.49−2.85 (br m, 4H, CH_2); 2.79, 2.88, 2.95, 2.99, 3.07, 3.09, 3.13, 3.18, 3.20, 3.28, 3.30, 3.36 (s, 12H, CH_3-DMSO); 6.02−6.16 (multiple d, 2H, *H*(5), $^3J_{HH} = 5.1$); 6.51−6.64 (multiple d, 2H, *H*(6), $^3J_{HH} = 5.1$). Anal. Calcd for $C_{18}H_{26}O_8S_2Ru$: C, 40.36; H, 4.89. Found: C, 40.38; H, 4.88. LR-MS (+LSIMS): 536 (M^+), 458 (M^+ − DMSO), 380 (M^+ − 2 DMSO). UV−vis (H_2O): 212 (29.5), 272 (10.1), 356 (5.82). IR (KBr): $\nu_{S=O}$ 1097; $\nu_{C=O} + \nu_{C=C}$ 1546; $\nu_{C=O}$ 1592. Λ_M (H_2O) = 15 (essentially nonconducting). $E_{1/2}(Ru^{III/II}) = 0.51$ V.

Ru(ma)$_2$(TMSO)$_2$ (1b). The complex **1b** was synthesized as for **1a**, except *cis*-$RuCl_2(TMSO)_4$ (100 mg, 0.17 mmol) and K(ma) (70 mg, 0.43 mmol) were used.

Yield: 50 mg (53%). ^{1}H NMR (C_6D_6; δ): 1.50−2.50 (br m, 8H, CH_2CH_2S); 2.07, 2.18, 2.20, 2.24 (s, 6H, CH_3-ma); 3.00−4.60 (br m, 8H, CH$_2$CH_2S); 6.05−6.25 (multiple d, 2H, *H*(5), $^3J_{HH} = 5.1$); 6.45−6.65 (multiple d, 2H, *H*(6), $^3J_{HH} = 5.1$). Anal. Calcd for $C_{20}H_{26}O_8S_2Ru \cdot H_2O$: C, 41.59; H, 4.89. Found: C, 41.49; H, 4.71. LR-MS (+LSIMS): 560 (M^+), 456 (M^+ − TMSO), 352 (M^+ − 2 TMSO). UV−vis (H_2O): 210 (31.0), 270 (9.60), 354 (5.44). IR (KBr): $\nu_{S=O}$ 1056, 1117; $\nu_{C=O} + \nu_{C=C}$ 1549; $\nu_{C=O}$ 1594. Λ_M (H_2O) = 30. $E_{1/2}(Ru^{III/II}) = 0.52$ V.

Ru(etma)$_2$(TMSO)$_2$ (2b). The complex was synthesized as for **1b**, except that K(etma) (76 mg, 0.43 mmol) was used.

Yield: 50 mg (50%). ^{1}H NMR (C_6D_6; δ): 1.00 (m, 6H, CH_3-etma); 1.40−2.10 (br m, 8H, CH_2CH_2S); 2.40−2.90 (br m, 4H, CH_2-etma); 3.00−4.50 (br m, 8H, CH$_2$CH_2S); 6.00−6.25 (multiple d, 2H, *H*(5), $^3J_{HH} = 5.1$); 6.45−6.70 (multiple d, 2H, *H*(6), $^3J_{HH} = 5.1$). Anal. Calcd for $C_{22}H_{30}O_8S_2Ru$: C, 44.96; H, 5.15. Found: C, 44.78; H, 5.08. LR-MS (+LSIMS): 588 (M^+), 484 (M^+ − TMSO), 380 (M^+ − 2 TMSO). UV−vis (H_2O): 214 (31.0), 272 (10.6), 358 (5.95). IR (KBr): $\nu_{S=O}$ 1055, 1116; $\nu_{C=O} + \nu_{C=C}$ 1546; $\nu_{C=O}$ 1592. Λ_M (H_2O) = 20. $E_{1/2}(Ru^{III/II}) = 0.52$ V.

Ru(ma)$_2$(BESE) (1c). The complex was made exactly as for **1a**, except that $[RuCl(H_2O)(BESE)]_2(\mu\text{-}Cl)_2$ (100 mg, 0.13 mmol) and K(ma) (110 mg, 0.67 mmol) in EtOH (20 mL) were used. Crystals suitable for X-ray analysis were grown by slow evaporation of an acetone solution of the complex layered with hexanes.

Yield: 57 mg (40%). ^{1}H NMR (yellow product, D_2O; δ): 1.15−1.50 (br m, 6H, CH_3–BESE); 2.23, 2.26, 2.34, 2.37 (s, 6H, CH_3-ma); 2.60−3.90 (br m, 8H, CH_2S(O)CH_2); 6.47−6.71 (multiple d, 2H, *H*(5), $^3J_{HH} = 5.0$); 7.82−7.95 (multiple d, 2H, *H*(6), $^3J_{HH} = 5.0$). ^{1}H NMR (crystal, D_2O; δ): 1.20−1.50 (m, 6H, CH_3-BESE); 2.35, 2.39 (s, 6H, CH_3-ma); 2.60−3.90 (m, 8H, CH_2S(O)CH_2); 6.53, 6.55 (d, 2H, *H*(5), $^3J_{HH} = 5.1$); 7.84, 7.88 (d, 2H, *H*(6), $^3J_{HH} = 5.1$). Anal. Calcd for $C_{18}H_{24}O_8S_2Ru$: C, 40.52; H, 4.53. Found: C, 40.39; H, 4.53. LR-MS (+LSIMS): 534 (M^+), 352 (M^+ − BESE). UV−vis (H_2O): 208 (34.7), 266 (13.9), 354 (6.94). IR (KBr): $\nu_{S=O}$ 1079, 1113; $\nu_{C=O} + \nu_{C=C}$ 1549, 1560; $\nu_{C=O}$ 1595. Λ_M (H_2O) = 4 (nonconducting). $E_{1/2}(Ru^{III/II}) = 0.55$ V.

(25) Connelly, N. G.; Geiger, W. E. *Chem. Rev.* **1996**, *96*, 877.
(26) *d*TREK: Area Detector Software*, version 7.1I; Molecular Structure Corp: The Woodlands, TX, 2001.
(27) Altomare, A.; Burla, M. C.; Cammalli, G.; Cascarano, M.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, A. *J. Appl. Crystallogr.* **1999**, *32*, 115.
(28) Beurskens, P. T.; Admiraal, G.; Beurskens, G.; Bosman, W. P.; de Gelder, R.; Israel, R.; Smits, J. M. M. *The DIRDIF-94 Program System*; Technical Report of the Crystallography Laboratory; University of Nijmegen: Nijmegen, The Netherlands, 1994.

Ru(etma)$_2$(BESE) (2c). The complex was synthesized as for **1c**, except K(etma) (120 mg, 0.52 mmol) was used.

Yield: 50 mg (33%). ^{1}H NMR (D_2O; δ): 1.06 (m, 6H, CH_3-etma); 1.15−1.50 (br m, 6H, CH_3–BESE); 2.55−3.95 (br m, 12H, CH_2-etma and CH_2S(O)CH_2); 6.50−6.70 (multiple d, 2H, *H*(5), $^3J_{HH}$ = 5.1); 7.83−7.97 (multiple d, 2H, *H*(6), $^3J_{HH}$ = 5.1). Anal. Calcd for $C_{20}H_{28}O_8S_2Ru$: C, 42.77; H, 5.03. Found: C, 43.03; H, 5.00. LR-MS (+LSIMS): 562 (M^+), 380 (M^+ − BESE). UV−vis (H_2O): 210 (32.1), 268 (13.1), 358 (6.71). IR (KBr): $\nu_{S=O}$ 1079, 1114; $\nu_{C=O}$ + $\nu_{C=C}$ 1545, 1559; $\nu_{C=O}$ 1593. Λ_M (H_2O) = 9 (nonconducting). $E_{1/2}(Ru^{III/II})$ = 0.55 V.

RuCl$_2$(BESE)(metro)$_2$ (3). A suspension of [RuCl(H_2O)(BESE)]$_2$-(μ-Cl)$_2$ (150 mg, 0.20 mmol) and metronidazole (207 mg, 1.2 mmol) in MeOH (60 mL) was refluxed in air at 75 °C for 16 h to give a yellow mixture. The volume was reduced to 5 mL, and the mixture was loaded onto a preparative TLC plate. The solvent was allowed to evaporate, and product separation was achieved using CH_2Cl_2/MeOH (90:10). The major (yellow) band was extracted with MeOH (3 × 20 mL), and the mixture then filtered through Celite. The filtrate was reduced to 5 mL, and Et_2O (60 mL) was added to give a product that was collected and dried in vacuo at rt. Crystals suitable for X-ray analysis were grown from evaporation of MeOH/Et_2O solutions.

Yield: 72 mg (26%). ^{1}H NMR (D_2O; δ): 1.00−1.60 (br m, 6H, CH_3-BESE); 2.34, 2.47, 2.60, 2.79 (s, 6H, CH_3-metro); 3.15−4.00 (br m, 12H, CH_2S(O)CH_2 and CH_2OH); 4.30−4.80 (br m, 4H, N−CH_2CH_2OH); 8.09, 8.14, 8.30, 8.49 (s, 2H, *H*(4)-metro). Anal. Calcd for $C_{18}H_{32}N_6O_8Cl_2S_2Ru\cdot 2H_2O$: C, 29.51; H, 4.95; N, 11.47. Found: C, 29.87; H, 4.70; N, 10.69. LR-MS (+ES ion trap, MeOH): 661 (M^+ − Cl), 491 (M^+ − Cl − metro), 456 (M^+ − 2 Cl − metro). UV−vis (H_2O): 310 (13.9). IR (KBr): $\nu_{S=O}$ 1079, 1114; $\nu_{N=O}$(symm) 1364; $\nu_{N=O}$(asymm) 1480; ν_{OH} 3422. Λ_M (H_2O) = 180 (5 min), 200 (30 min), 210 (3 h), 220 (24 h) (2:1 electrolyte). $E_{1/2}(NO_2/NO_2^-)$ = −1.16 V. $E_{1/2}(Ru^{III/II})$ = 1.18 V.

MTT Assay. All reagents were handled in a sterile fume hood. Leibovitz's L-15 medium with L-glutamine (L-15), fetal bovine serum (FBS), Zn bovine insulin, phosphate-buffered saline 7.4 (PBS), trypsin−EDTA (0.25% trypsin and 1 mM Na_4(EDTA)), and trypan blue stain (0.4%) were purchased from Gibco. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was an Aldrich product. The growth medium (L-15 medium with 10% FBS and 0.01 mg/mL insulin), Zn bovine insulin, and MTT were stored at 4 °C, while trypsin−EDTA and FBS were stored frozen at −10 °C and thawed before use; PBS was stored at rt.

Human breast cancer cells (MDA-MB-435S),[29] purchased from American Type Culture Collection (ATCC), were plated in a T-75 flask (Becton Dickinson and Co.) and incubated at 37 °C in air. The cells were transferred to a new flask biweekly and treated with trypsin−EDTA to detach them from the flask. Cells were counted under a microscope using a hemacytometer (Hausser Scientific, 0.100 mm deep), after the addition of trypan blue that stains and excludes dead cells. Cell solutions were diluted with growth medium to a concentration of 1 × 10^5 cells/mL and transferred to a 96-well plate, by plating the wells in columns C and 1 to 8 (Chart 2) with 100 μL (1 × 10^4 cells). Growth medium (100 μL) was added to column B, as a blank. To each of the perimeter wells was added deionized water (200 μL), and the plate was then incubated at 37 °C for 24 h. PBS solutions (100 μL) of the test Ru complex at 8 different concentrations (4000 to 2 μM) were then added to the well in columns 1 (highest [Ru]) to 8 (lowest [Ru]). PBS (100 μL) was also added to the wells in column B and C, and the plate was incubated at 37 °C for 3 days.

Chart 2. A 96-well Plate Showing Columns B, C, and 1−8 in the Shaded Wells

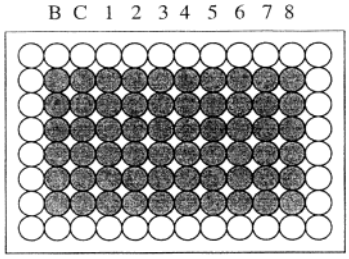
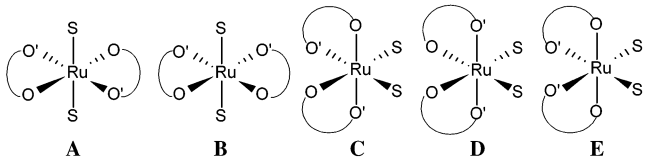


Chart 3. Five Possible Stereoisomers of **1a** or **2a**[a]



[a] S represents S-bonded DMSO, and O−O′ represents the chemically inequivalent hydroxy (O) and carbonyl (O′) oxygen atoms of maltolato or ethylmaltolato ligands.

A modified procedure of Mosmann was used for the MTT assay.[30] A PBS solution of MTT (50 μL, 2.5 mg/mL) was added to each well of the plate that was then incubated for 3 h, by which time a purple precipitate of formazan formed at the bottom of certain wells. The contents of each well were carefully aspirated off to leave the formazan that was then dissolved in DMSO (150 μL); the plate was shaken and analyzed by a plate reader (Spectra Max 190 from Molecular Devices) to determine the absorbance of each well at 570 nm. The percentage cell viability was calculated by dividing the average absorbance of the cells treated with a Ru complex by that of the control; % cell viability vs drug concentration (logarithmic scale) was plotted to determine the IC_{50} (drug concentration at which 50% of the cells are viable relative to the control), with its estimated error derived from the average of 3 trials.

Results and Discussion

Complexes 1a and 2a. The solid-state molecular structure of **1a** was reported[19] to be a cis isomer with S-bonded DMSO ligands (structure **C** in Chart 3; the IR $\nu_{S=O}$ value, 39 cm^{-1} > that of 1055 cm^{-1} for free DMSO, supports solely S-bonded sulfoxide[10,31]), but the solution structure is more complex.[19,20] Because of the inequivalence of the O atoms of the maltolato (ma) ligand, three cis and two trans stereoisomers are possible for the solely S-bonded sulfoxide species **1a** (and **2a)** (Chart 3). The ^{1}H NMR spectrum of **1a** in C_6D_6 exhibits four singlets in the δ 2.1 region, each assignable to a Me of ma, while two sets of four doublets centered at ~ δ 6.1 and 6.5 are assigned to the ma *H*(5) and *H*(6), respectively. These data are consistent with the presence of the three cis isomers, the inequivalent ma ligands in **C** giving rise to two Me singlets and the equivalent Me groups in **D** and **E** each giving rise to one; the doublets are assigned similarly to *H*(5) and *H*(6). The 12 singlets between

(29) (a) Brinkley, B. R.; Beall, P. T.; Wible, L. J.; Mace, M. L.; Turner, D. S.; Cailleau, R. M. *Cancer Res.* **1980**, *40*, 3118. (b) Siciliano, M. J.; Barker, P. E.; Cailleau, R. *Cancer Res.* **1979**, *39*, 919. (c) Cailleau, R.; Olive, M.; Cruciger, Q. V. J. *In Vitro* **1978**, *14*, 911.

(30) Mosmann T. *J. Immunol. Methods* **1983**, *65*, 55.

(31) Davies, J. A. *Adv. Inorg. Chem. Radiochem.* **1981**, *24*, 115.

δ 2.7 and 3.4 correspond to the Me groups of the S-bonded DMSO ligands.[10,31] The formation of the trans isomers (**A** and **B**), which like **D** and **E** also have equivalent ma ligands, is not favored because the π-accepting sulfoxides would prefer when possible not to be mutually trans.[32] Our interpretation of the ^{1}H NMR spectrum agrees with that discussed in an M.Sc. thesis.[20] Relative integrations of the resonances suggest that in solution there is ~55% of **C**, with the other two isomers being present in a ratio of ~2:1.The acac analogue of **1a**, *cis*-Ru(acac)$_2$(DMSO)$_2$, exists as a single isomer in solution and is readily characterized by ^{1}H NMR.[17b] All the cis isomers are chiral at Ru, and *racemic* mixtures must also be present in solution. Complex **2a** also contains S-bonded DMSO ligands ($\nu_{S=O}$ = 1097 cm^{-1}); its ^{1}H NMR solution spectrum is essentially similar to that of **1a**, except for coupling between the etma Me and C*H*$_2$ groups that theoretically gives a triplet and a quartet, respectively, for each isomer, and because of the presence of three cis isomers, multiplets are observed for overlapping signals. The C*H*$_2$ etma multiplets also appear to overlap with the Me singlets of DMSO. Complexes **1a** and **2a** are soluble in H_2O, immediately forming yellow, essentially nonconducting solutions; their UV−vis spectra that do not change over 24 h are considered to refer to a mixture of the cis isomers.

The ma and etma $\nu_{C=O}/\nu_{C=C}$ values for **1a** and **2a** (as well as for complexes **1b**,**c** and **2b**,**c** discussed below) are between 1545 and 1595 cm^{-1}, ~60 cm^{-1} below those of the free maltolato anions,[33] reasonable values for binding via the carbonyl group.

Complexes 1b and 2b. The IR spectra of the TMSO complexes show $\nu_{S=O}$ values greater than that for free TMSO (1023 cm^{-1}) and again imply the presence of S-bonded TMSO ligands.[22] In the ^{1}H NMR spectrum of *cis*-RuCl$_2$(TMSO)$_4$, the α-proton signals shift downfield to δ 3.4 and 4.0, while the β-proton resonances shift slightly upfield to δ 2.3, compared to the signals of free TMSO.[22] Similar trends are seen in the ^{1}H NMR spectra of **1b** and **2b**, but these are more complicated because of the presence of multiple isomers. The ^{1}H signals appear as broad multiplets between δ 3.0 and 4.5 for the α-protons and between δ 1.5 and 2.5 for the β-protons. The four Me singlets of **1b** are centered at δ 2.2 and give a pattern similar to that observed for **1a**. On the basis of the available spectroscopic data, solution structures of **1b** and **2b** in C_6D_6 are tentatively assigned as all cis, similar to those of **1a** and **2a**. Complexes **1b** and **2b** dissolve in H_2O to give weakly conducting solutions (Λ_M = 30 and 20 Ω^{-1} cm^2 mol^{-1}, respectively), presumably because of partial dissociation of the maltolato ligands.

Complexes 1c and 2c. The crystal structure for **1c** and IR data for **1c** and **2c** with $\nu_{S=O}$ values greater than that for free BESE (1015 cm^{-1}) again reveal S-bonded sulfoxide moieties.[11,31] Of the three stereoisomers possible **C′**, **D′** and **E′**, (equivalent to **C**−**E** in Chart 3 but with connected S atoms), the structure of **1c** (Figure 1) reveals isomer **D′** where the carbonyl O atoms of ma are mutually trans, in contrast to structure **C** determined for **1a** (see above);[19] the disulfoxide

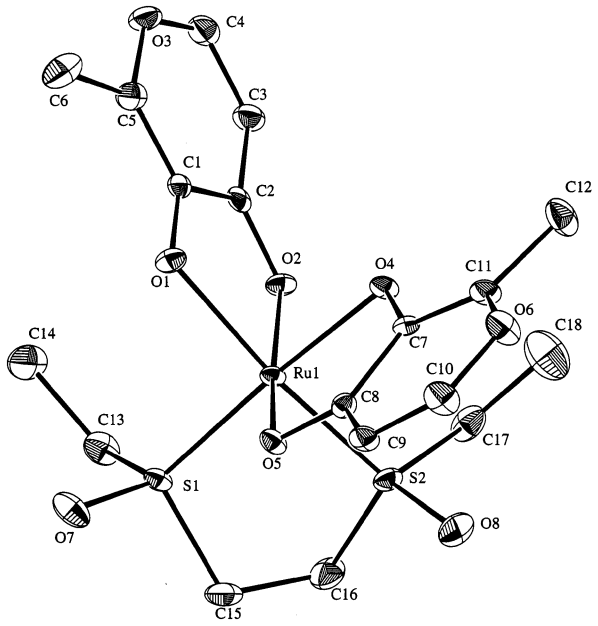


Figure 1. ORTEP diagram of Ru(ma)$_2$(*S*,*R*-BESE) (**1c**) with 50% probability ellipsoids.

is found as the meso form (S1 = *S*, S2 = *R*; Figure 2), which is the predominant form used in the synthesis.[12,17] The structure is the first reported for a complex containing both ma and a bidentate disulfoxide. Selected bond lengths and angles for **1c** are shown in Table 2; **1c** and **1a** have similar Ru−S bond lengths (2.18−2.21 Å) and Ru−O bond lengths (2.08−2.15 Å), and both have distorted octahedral geometries with the ma ligand having a bite of ~80°. The geometry of the coordinated ma is close to that found in RuCl(η^6-mesitylene)(ma).[34] The Ru−S bond lengths in **1c** are shorter than those in *cis*- and *trans*-RuCl$_2$(BESE)$_2$[11,12] and [RuCl(*p*-cymene)(BESE)]PF$_6$,[17] when a S atom is trans to another S or a hydrocarbon fragment (2.288−2.329 Å) as opposed to being trans to oxygen in **1c**. The bite angle of the BESE in **1c** is 88.27°, close to those noted for the complexes mentioned above (83.73−87.69°).

Complexes **1c** and **2c** are very soluble in water, immediately forming yellow, nonconducting solutions, whose UV−vis spectra do not change over 24 h at rt. The time-independent ^{1}H NMR spectrum of the crystal of **1c** in D_2O shows two, equal-intensity ma-Me singlets, in addition to two sets of doublets for the *H*(5) and *H*(6) protons, consistent with the presence of just isomer **D′**, the solid-state structure. The ^{1}H NMR for the yellow powder product (**1c**) in D_2O shows four major singlets centered at δ 2.3 for the ma-Me resonances, and multiple sets of doublets are for the *H*(5) and *H*(6) nuclei centered at δ 6.6 and 7.9, respectively. For the *meso*-BESE ligand system, the three possible isomers (**C′**, **D′** and **E′**) would exhibit inequivalent ma ligands (i.e., give six singlets for the Me protons and six sets of doublets for *H*(5) and *H*(6)); the four ma-Me singlets observed indicate the presence of two major isomers. The etma-Me and -C*H*$_2$ groups of **2c** give rise to overlapping

(32) Calligaris, M.; Carugo, O. *Coord. Chem. Rev.* **1996**, *153*, 83.
(33) Greaves, S. J.; Griffith, W. P. *Polyhedron* **1988**, *7*, 1973.
(34) Capper, G.; Carter, L. C.; Davies, D. L.; Fawcett, J.; Russell, D. R. *J. Chem. Soc., Dalton Trans.* **1996**, 1399.

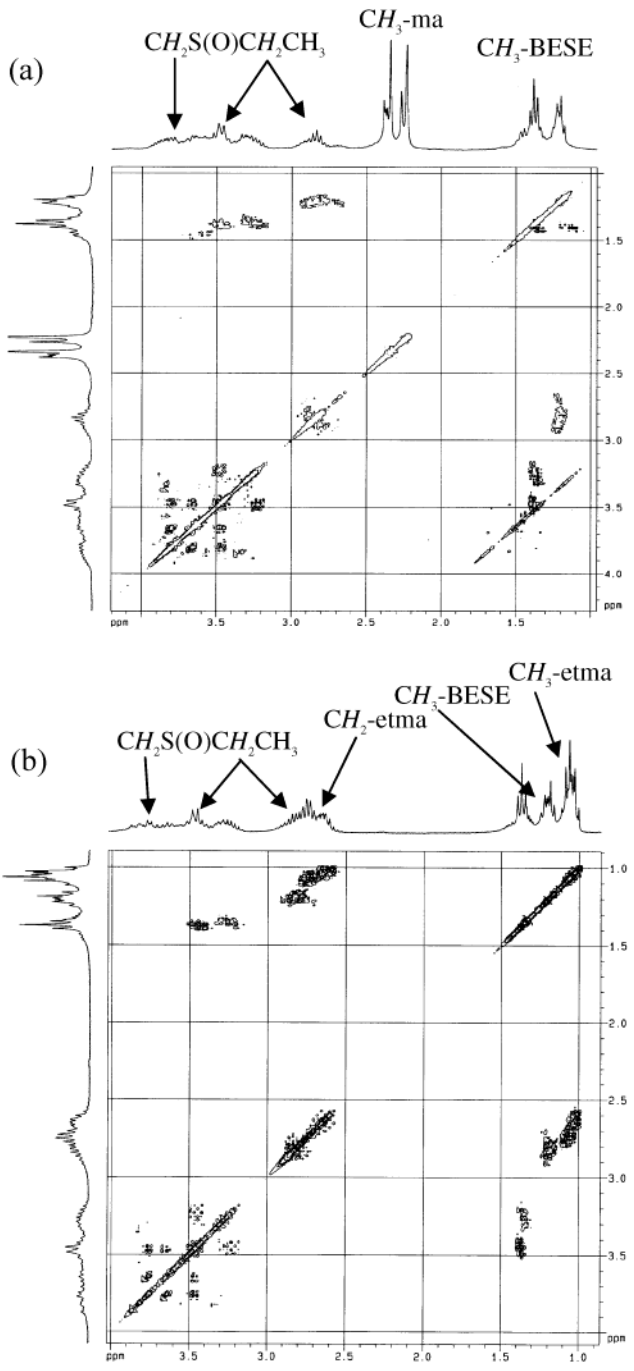


Figure 2. The ^{1}H COSY NMR spectra of **1c** (a) and **2c** (b) in D_2O.

Table 2. Selected Bond Lengths and Angles of **1c**·H_2O

bond	length (Å)	bond	angle (deg)
Ru(1)−O(1)	2.141(2)	S(1)−Ru(1)−O(4)	174.52(5)
Ru(1)−O(2)	2.082(2)	S(2)−Ru(1)−O(1)	172.93(6)
Ru(1)−O(4)	2.098(2)	O(2)−Ru(1)−O(5)	168.24(7)
Ru(1)−O(5)	2.085(2)	O(1)−Ru(1)−O(2)	80.37(7)
Ru(1)−S(1)	2.2054(7)	O(4)−Ru(1)−O(5)	81.17(7)
Ru(1)−S(2)	2.1807(7)	S(1)−Ru(1)−S(2)	88.27(3)
S(1)−O(7)	1.487(2)	Ru(1)−O(1)−C(1)	107.9(2)
S(2)−O(8)	1.476(2)	Ru(1)−O(2)−C(2)	111.1(2)
O(1)−C(1)	1.318(3)	O(7)−S(1)−C(15)	105.9(1)
O(2)−C(2)	1.281(2)	C(13)−S(1)−C(15)	100.9(1)

triplets ($\sim \delta$ 1.1) and quartets ($\sim \delta$ 2.6), respectively. The BESE-Me signals of **1c** and **2c** result in multiplets between δ 1.2−1.5, while the $CH_3CH_2S(O)CH_2$ protons, occurring

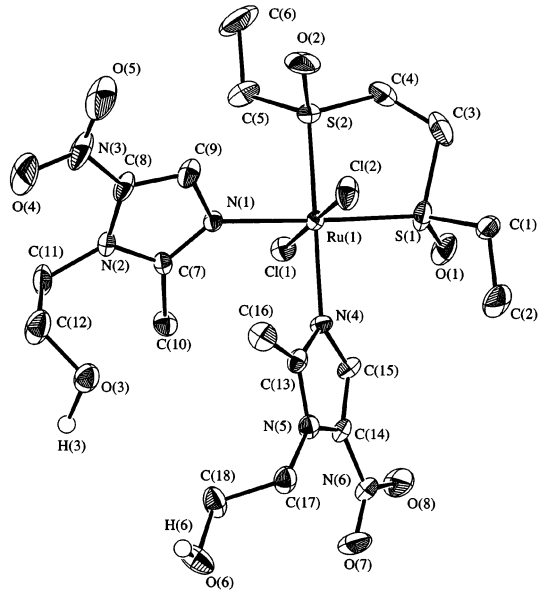


Figure 3. ORTEP diagram of *trans*-$RuCl_2$(*R*,*R*-BESE)$(metro)_2$ (**3**) with 50% probability ellipsoids.

Table 3. Selected Bond Lengths and Angles of **3**

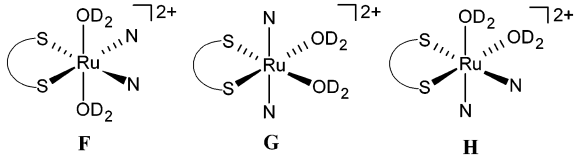
bond	length (Å)	bond	angle (deg)
Ru(1)−N(1)	2.139(3)	N(1)−Ru(1)−S(1)	177.93(9)
Ru(1)−N(4)	2.143(3)	N(4)−Ru(1)−S(2)	179.03(8)
Ru(1)−S(1)	2.2267(11)	Cl(2)−Ru(1)−Cl(1)	179.41(4)
Ru(1)−S(2)	2.2174(11)	S(2)−Ru(1)−S(1)	87.23(4)
Ru(1)−Cl(1)	2.4148(10)	N(1)−Ru(1)−N(4)	89.26(12)
Ru(1)−Cl(2)	2.4006(11)	N(1)−Ru(1)−S(2)	90.86(9)
S(1)−O(1)	1.477(3)	N(1)−Ru(1)−Cl(1)	90.69(8)
S(2)−O(2)	1.495(3)	S(1)−Ru(1)−Cl(1)	90.11(4)
O(4)−N(3)	1.238(5)	O(1)−S(1)−C(1)	108.4(3)
O(5)−N(3)	1.214(5)	C(3)−S(1)−C(1)	92.6(3)

as overlapping multiplets between δ 2.6−4.0, have been assigned from ^{1}H COSY NMR data (Figure 2). Qualitatively, the NMR data for **2c** appear consistent also with the presence of two major isomers. The **C′**, **D′**, and **E′** isomers are all chiral at Ru, and so the *racemic* mixtures are present as well; more complex spectra would be expected if the *R*,*R* or *S*,*S* form of BESE were present in **1c** or **2c**.

The presence of maltolato ligands certainly increases the water solubility of Ru sulfoxide complexes; e.g., **1c** is much more water-soluble than either *cis*- or *trans*-$RuCl_2(BESE)_2$,[11,12] and this represents a potential advantage for medicinal use, with the added benefit that maltol itself is approved for therapeutic use because of its nontoxicity as a food additive.[14] The incorporation of maltolate ligands does not always give water solubility as $Ru(ma)_2(PPh_3)_2$ (cis or trans geometry unknown) and $Ru(ma)_2$(COD) are reported to be water-insoluble.[19]

Complex 3. The structure of **3** is shown in Figure 3, with selected bond lengths and angles given in Table 3. The essentially octahedral structure reveals trans chlorides and S-bonded BESE, as indicated also by the IR $\nu_{S=O}$ values. The conformation at both S atoms is *R*, an unexpected result as the complex $[RuCl(H_2O)(BESE)]_2(\mu\text{-}Cl)_2$, from which **3** was made, contained *meso*-BESE.[17] It is possible that the product (obtained in 26% yield) contains *meso*-BESE, while the crystal investigated happens to contain *R*,*R*-BESE. The

Chart 4. Three Stereoisomers of $[Ru(D_2O)_2(BESE)(metro)_2]^{2+}$ [a]



[a] S−S and N represent S-bonded BESE and metronidazole, respectively.

other possibility, that epimerization at a S-atom occurs during the synthesis of **3**, is unlikely. An equal amount of the *S*,*S* conformer must also be present in the crystallographic unit cell of **3**. The Ru−S bonds, where the S atoms are trans to N atoms, and the bite angle are very similar to those found for **1c** (see above). The Ru−Cl lengths are in the range noted for other Ru^{II}−BESE complexes (2.385−2.449 Å).[11,12,17]

The $\nu_{N=O}$ values (symm and asymm) of the coordinated metro are within 5 cm^{-1} of those for free metro; shifts of ∼20 cm^{-1} are seen when coordination via the NO_2 group occurs.[10b,c] Complex **3** represents the first structurally characterized Ru complex containing both nitroimidazole and sulfoxide ligands, which is significant in view of the biological properties of such species (see Introduction).[10]

Complex **3** rapidly dissociates both chlorides in aqueous solutions, based on conductivity data (180 Ω^{-1} cm^2 mol^{-1} after 5 min) showing an approximate 2:1 electrolyte; in D_2O, three isomers of the putative $[Ru(D_2O)_2(BESE)(metro)_2]^{2+}$ (**F**−**H** in Chart 4) are possible. The ^1H NMR spectrum in D_2O shows four major singlets for Me and *H*(4) protons of metro, centered at δ 2.6 and 8.3, respectively. The nature of the BESE within the sample used for the ^1H NMR is unclear. If the BESE is meso, all three isomers would exhibit inequivalent metro ligands, and six singlets for each of these two sets of protons would be expected; thus, the NMR data would indicate the presence of mainly two isomers. If the BESE is racemic, the two *H*(4) protons in **F** and **G** would be equivalent and those in **H** inequivalent; thus, the observed singlets could correspond to the presence of all three isomers. The CV data in CH_2Cl_2 (below) are not definitive in drawing conclusions about possible isomer mixtures. The BESE-Me ^1H NMR signals are seen at δ 1.0−1.6, while the CH_3CH_2S-(O)CH_2 signals overlap with those of the metro-CH_2OH protons, giving multiplets between δ 3.2−4.0; the metro-CH_2CH_2OH resonance (δ 4.3−4.8) partially overlaps with the residual D_2O solvent signal. The ^1H NMR data show no dissociation of either BESE or metro ligands over 24 h.

Cyclic Voltammetry. The cyclic voltammograms for **1** and **2** in CH_2Cl_2 do not reveal the presence of isomers: the observed waves were about twice as broad as that for the $[FeCp^*_2]^+/[FeCp^*_2]$ couple and are thought to result from mixtures of the cis formulations discussed. The $Ru^{III/II}$ reduction potentials of the ma- and etma-sulfoxide complexes are very similar, with the BESE-containing complexes exhibiting a 40−50 mV more positive value than the DMSO and TMSO complexes (0.51−0.52 V vs SCE). The potentials strongly reinforce the conclusion that the DMSO and TMSO exist as cis isomers (as for the BESE complexes), because within Ru systems cis isomers have reduction potentials ∼0.2 V higher than those of the corresponding trans isomers.[35]

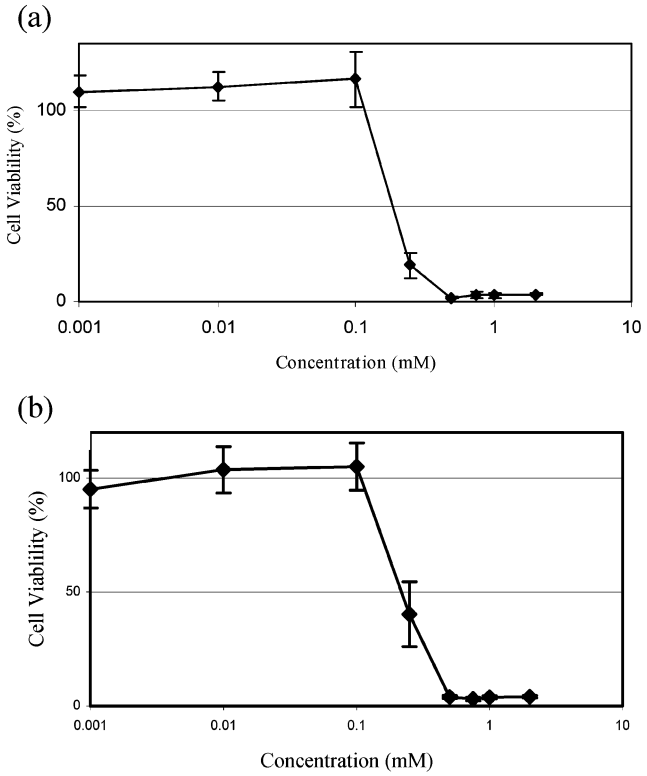


Figure 4. MTT plots for **2a** (a) and **2b** (b), with IC_{50} values equal to 190 ± 10 and 220 ± 10 μM, respectively. The error bars indicate one standard deviation of the averaged cell percent viability.

Table 4. IC_{50} Values

complex	IC_{50} (μM)	complex	IC_{50} (μM)
1a	370 ± 20	**1c**	1300 ± 100
2a	190 ± 10	**2c**	1100 ± 100
1b	370 ± 20	**3**	860 ± 30
2b	220 ± 10	cisplatin	30 ± 5

The 0.6 V more positive $Ru^{III/II}$ potential of **3** (i.e., favoring the lower oxidation state) possibly results from the π-acceptor ability of the metro ligands relative to the electron donor ability of the ma type ligands in **1** and **2**. Again, just a single broad wave is seen for **3**, and it is not clear how this relates to a possible mixture of **F**−**H**, although the all cis isomer **H** might be expected to have a higher potential than the other two isomers that have one set of trans ligands. The NO_2/NO_2^- couple (reduction potential) of metro on coordination to Ru^{II} is increased by ∼60 mV.

MTT Assay. The Ru complexes were examined on human breast cancer cells (MDA-MB-435S) using the MTT assay (Table 4), a colorimetric determination of cell viability during in vitro treatment with a drug.[36] The assay, developed as an initial stage of drug screening, measures the amount of MTT reduction by mitochondrial dehydrogenase and assumes that cell viability (corresponding to the reductive activity)

(35) (a) Siebald, H. G. L.; Fabre, P.-L.; Dartiguenave, M.; Dartiguenave, Y.; Simard, M.; Beauchamp, A. L. *Polyhedron* **1996**, *15*, 4221. (b) Queiroz, S. L.; Batista, A. A.; Oliva, G.; do P. Gambardella, M. T.; Santos, R. H. A.; MacFarlane, K. S.; Rettig, S. J.; James, B. R. *Inorg. Chim. Acta* **1998**, *267*, 209.

(36) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.

is proportional to the production of purple formazan that is measured spectrophotometrically.[37] A low IC_{50} is desired and implies cytotoxicity or antiproliferation at low drug concentrations.[38]

The MTT plots for **2a**,**b** (Figure 4) reveal the lowest IC_{50} values of ~200 μM, while the value for cisplatin was ~30 μM; **3** is not as potent as **2a**,**b**. The IC_{50} values for noncoordinated maltol and ethylmaltol are ~1600 and 1200 μM, respectively, while those of DMSO, TMSO, BESE, and metronidazole are >2000 μM; thus, the Ru complexes exhibit lower IC_{50} values than those of the corresponding free ligands. Of note, the etma complexes invariably have significantly lower IC_{50} values than those of the corresponding ma complexes. That the BESE species (**1c**, **2c**) are less active than the DMSO (**1a**, **2a**) and TMSO species (**1b**, **2b**) may reflect the presence of the chelating ligand that less readily dissociates than the monodentate sulfoxides, and this could inhibit subsequent binding to DNA. The nature of the Ru species present in the phosphate-buffered saline solutions is, of course, uncertain. The corresponding IC_{50} values for [RuCl(*p*-cymene)(BESE)]PF_6 and [$RuCl_2$(*p*-cymene)]$_2$(μ-BESE) against the same cell line are 55 and 360 μM, respectively.[17a]

Acknowledgment. We thank Dr. Elena Polishchuk and Ms. Candice Martins of the Biological Services in this department and Ms. Helen Wright for assistance with the MTT assay, Drs. Craig Pamplin and Chi-Wing Tsang for discussions, and the Natural Sciences and Engineering Research Council of Canada for financial support.

Supporting Information Available: Crystallographic data for **1c** and **3** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

IC030119J

(37) (a) Bellamy, W. T. *Drugs* **1992**, *44*, 690. (b) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.

(38) (a) Nikolova, A.; Ivanov, D.; Buyukliev, R.; Konstantinov, S.; Karaivanova, M. *Arzneim.-Forsch./Drug Res.* **2001**, *51*, 758. (b) Galeano, A.; Berger, M. R.; Keppler, B. K. *Arzneim.-Forsch./Drug Res.* **1992**, *42*, 821.