Organometallic 3-(1H-Benzimidazol-2-yl)-1H-pyrazolo-[3,4-b]pyridines as Potential Anticancer Agents

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Supporting Information

ABSTRACT: Six organometallic complexes of the general formula [M11Cl(η5-p-cymene)(L)]Cl, where M = Ru (11a, 12a, 13a) or Os (11b, 12b, 13b) and L = 3-(1H-benzimidazol-2-yl)-1H-pyrazolo-[3,4-b]pyridines (L1–L3) have been synthesized. The latter are known as potential cyclin-dependent kinase (Cdk) inhibitors. All compounds have been comprehensively characterized by elemental analysis, one- and two-dimensional NMR spectroscopy, UV–vis spectroscopy, ESI mass spectrometry, and X-ray crystallography (11b and 12b). The multistep synthesis of 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines (L1–L3), which was reported by other researchers, has been modified by us essentially (e.g., the synthesis of 5-bromo-1H-pyrazolo[3,4-b]pyridine-3-carboxylic acid (3) via 5-bromo-3-methyl-1H-pyrazolo[3,4-b]pyridine (2), the synthesis of 1-methoxymethyl-2,3-diaminobenzene (5) by avoiding the use of unstable 2,3-diaminobenzyl alcohol, and the activation of 1H-pyrazolo[3,4-b]pyridine-3-carboxylic acids (1, 3) through the use of an inexpensive coupling reagent, N,N′-carbonyldimidazole (CDI)). Stabilization of the 7b tautomer of methoxymethyl-substituted L3 by coordination to a metal(II) center, as well as the NMR spectroscopic characterization of two tautomers 7b-L3 and 4b-L3 in a metal-free state are described. Structure–activity relationships with regard to cytotoxicity and cell cycle effects in human cancer cells, as well as Cdk inhibitory activity, are also reported.

INTRODUCTION

Tumor-associated cell cycle defects, manifesting in unscheduled proliferation, and the associated genomic and chromosomal instabilities are mediated by misregulation of cyclin-dependent kinases (Cdks).1 Because of the main role in the division cycle, Cdks have been recognized as targets for anticancer therapy. Many small-molecule organic compounds, which have been identified as Cdk modulators, are currently in preclinical or clinical development.1–5 However, no Cdk inhibitors have gained marketing approval, despite 20 years of scientific investigation.6

Several studies have shown synergism when Cdk inhibitors were combined with organic (e.g., doxorubicin, paclitaxel)2,6 and inorganic (e.g., cisplatin, carboplatin) cytotoxic drugs.7–9 The reported effects inspired the design of metal complexes with biologically active ligands. The first publications have appeared recently and include Fe, Cu, and Pt complexes with Cdk inhibitors derived from 6-benzylaminopurine10–13 metal-based indolo-[3,2-d]benzazepines (paullones; Ga, Cu, Ru, and Os).14–19 and indolo-[3,2-c]quinolines (Ru, Os).20 Another class of compounds potentially suitable for targeted metal-based chemotherapy is that of 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines. These have been documented recently as potent Cdk1 inhibitors with antiproliferative activity in HeLa (cervical carcinoma), HCT116 (colon carcinoma), and A375 (melanoma) human cancer cell lines.21–23 Comparison of Cdk1 inhibitory activity with the inhibiting activity in four other protein kinases (VEGF-R2, HER2, Aurora-A, and RET) revealed selectivity for Cdk1. Structural modifications consisting of a replacement of both bicyclic rings in 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines by monocycles while retaining the imidazolyl pyrazole core have been proposed in order to obtain inhibitors with improved pharmacokinetic and solubility properties.24–26 The most promising were suggested to be 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines (see Chart 1). The inspection of substitution patterns on the benzimidazole moiety and structure–activity relationships revealed that a methoxymethyl group in position 7b (4b5) is favorable for Cdk1 inhibiting potency. The role of the pyrazole NH group is also of note, since its methylation led to a significant reduction of Cdk1 activity. The effect of various heteroaryl groups in position 5a was also remarkable for the development of more-effective Cdk inhibitors and antiproliferative agents.

Our previous experience with metal-based indolo-[3,2-d]benza- zepines prompted the use of the half-sandwich metal-arene moiety as a suitable scaffold to which 3-(1H-benzimidazol-2-yl)-1Hpyrazolo[3,4-b]pyridines may be attached. Organometallic compounds [M(η5-arene)(Y)X]n (where M = Ru, Os) exhibit

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promising anticancer activity and are the focus of attention for several groups. These compounds have shown activity toward classic (DNA) and nonclassic (e.g., Cdk) targets in anticancer chemotherapy.

Herein, we report (i) the modified synthetic approach to 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines (recall Chart 1: L1, X = H, Y = H; L2, X = Br; Y = H; L3, X = Br; Y = CH2OCH3); (ii) the synthesis and characterization of a new family of organoruthenium(II) (11a, 12a, 13a) and osmium(II) (11b, 12b, 13b) complexes of the general formula [MCl(η6-p-cymene)L]Cl, where L = 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines (L1 – L3) (Chart 1); (iii) stabilization of the 7b tautomer of methoxymethyl-substituted L3 by metal coordination as well as (iv) NMR spectroscopic characterization of two tautomers 7b-L3 and 4b-L3 in a metal-free state; and (v) cell cycle effects, as well as the antiproliferative and Cdk inhibitory activities of both metal-free ligands and organometallic complexes.

**EXPERIMENTAL SECTION**

Starting Materials. 3-Acetyl-2-chloropyridine and 3-methyl-1H-pyrazolo[3,4-b]pyridine were prepared according to literature protocols. 1H-Pyrazolo[3,4-b]pyridine-3-carboxylic acid (1) was obtained via the oxidation of 3-methyl-1H-pyrazolo[3,4-b]pyridine by KMnO4 in the presence of a base, followed by acidification with 37% HCl. 2-Aminobenzonitrile and dry dimethylformamide (DMF) were purchased from Acros Organics. Solvents [toluene, ethanol (EtOH), tetrahydrofuran (THF), diethyl ether (Et2O)] were dried using standard procedures. [RuCl2(μ-Cl)(η6-p-cymene)] and [OsCl2(μ-Cl)(η6-p-cymene)] were synthesized as described previously.

Synthesis of Ligands. 5-Bromo-3-methyl-1H-pyrazolo[3,4-b]pyridine (2). 3-Methyl-1H-pyrazolo[3,4-b]pyridine (10.9 g, 0.08 mol) and anhydrous sodium acetate (10.21 g, 0.13 mol) were suspended in glacial acetic acid (42 mL). Bromine (20.42 g, 0.13 mol) was added, and the resulting mixture was stirred at room temperature for 2–2.5 h and then at 110–115 °C for 2.5–3 h. Afterward, water (300–350 mL) was added and the mixture was stirred at room temperature. The formed light yellow precipitate was filtered off and dried in vacuo at 40–50 °C. Yield: 17 g. The raw product was used without further purification in the next step. Purification by column chromatography afforded a white powder (SiO2, EtOAc, Rf = 0.79; 12.5 g, 72.6% yield). M. (C7H6BrN3) = 212.05 g/mol. ESI-MS in MeOH (positive): m/z 213 [M+H]+, 235 [M+Na]+, 253 [M+K]+; (negative): m/z 211 [M–H]–. 1H NMR (500.32 MHz, MeOH-d4): δ 8.55 (d, 1H, J = 2.16 Hz, CH), 8.43 (d, 1H, J = 2.15 Hz, CH), 2.56 (s, 3H, CH3) ppm. 1H NMR (500.32 MHz, DMSO-d6): δ 13.42 (brs, 1H, NH), 8.54 (d, 1H, J = 2.19 Hz, CH), 8.53 (d, 1H, J = 2.18 Hz, CH), 2.49 (s, 3H, CH3) ppm. Colorless crystals of 2-MeOH suitable for X-ray diffraction (XRD) study were grown in EtOAc (see Figure S1 in the Supporting Information).

5-Bromo-1H-pyrazolo[3,4-b]pyridine-3-carboxylic acid (3). To sodium hydroxide (9.54 g, 0.24 mol) in water (150 mL) was added the raw product 2 (7.1 g, 0.03 mol). After a dropwise addition of K MnO4 (169.8 g, 0.11 mol) in water (300 mL) at 100 °C over 2 h, the reaction mixture was further heated for 1 h. MnO2 was filtered off and dried in vacuo at 60 °C for 2.5 h. The raw product was used without further purification in the next step. Purification by column chromatography afforded a yellow powder (SiO2, EtOAc, Rf = 0.79; 12.5 g, 72.6% yield). M. (C7H6BrN3) = 212.05 g/mol. ESI-MS in MeOH (positive): m/z 213 [M+H]+, 235 [M+Na]+, 253 [M+K]+; (negative): m/z 211 [M–H]–. 1H NMR (500.32 MHz, MeOH-d4): δ 8.55 (d, 1H, J = 2.16 Hz, CH), 8.43 (d, 1H, J = 2.15 Hz, CH), 2.56 (s, 3H, CH3) ppm. 1H NMR (500.32 MHz, DMSO-d6): δ 13.42 (brs, 1H, NH), 8.54 (d, 1H, J = 2.19 Hz, CH), 8.53 (d, 1H, J = 2.18 Hz, CH), 2.49 (s, 3H, CH3) ppm. Colorless crystals of 2-MeOH suitable for X-ray diffraction (XRD) study were grown in EtOAc (see Figure S1 in the Supporting Information).
temperature for 3 h. A saturated aqueous solution of NaHCO₃ (300 mL) and MeOH (300 mL) then added. The mixture was filtered, and the white precipitate was washed with EtOAc. The filtrates were combined, and organic solvents were evaporated under reduced pressure. The remaining aqueous solution was extracted with EtOAc (2 × 300 mL). The organic phase was dried over Na₂SO₄, filtered, and evaporated to yield a red oil (4.85 g). The raw product was purified by column chromatography (SiO₂, EtOAc, or EtOAc/hexane 1:1, first fraction, a red-orange oil crystallized to form a red solid at 4 °C; yield: 3.68 g, 65%). M₄(C₂H₂O₄H₂O) = 182.18 g/mol. ¹H NMR (500.32 MHz, DMSO-d₆): δ 8.00 (d, 1H, J = 8.71 Hz, C₁H₃), 7.48 (d, 1H, J = 7.11 Hz, C₃H₄), 7.09 (br, 2H, NH₂), 6.67 (t, 1H, J = 8.45 Hz, C₂H₂), 4.48 (s, 2H, CH₂), 3.31 (s, 3H, CH₃) ppm.

1-Methoxymethyl-2,3-diaminobenzene (5). A mixture of 4 (1.4 g, 0.008 mol) and 10% Pd/C (0.18 g) in dry EtOH (55 mL) was stirred under hydrogen atmosphere at room temperature for 18–24 h. The catalyst was removed by filtration through GF-3-filter under argon and washed with dry EtOH (50–70 mL) in dry EtOH (55 mL) was stirred under hydrogen atmosphere at room temperature for 18–24 h. Water (5 mL) then added and the suspension stirred until all CO₂ was ceased. The white precipitate was filtered off, washed with water (3–5 mL), and dried in a sublimator in vacuo at 60 °C to remove the imidazole as a contaminant. Yield: 1.13 g; 25–29%, based on 3-methyl-1H-pyrazolo[3,4-b]pyridine. M₄(C₆H₄N₂O₂) = 213.19 g/mol. ESI-MS in methanol (positive): m/z 214 [M⁺], 237 [M⁺N⁺]; (negative): m/z 212 [M−H−]. ¹H NMR (500.32 MHz, DMSO-d₆): δ 14.95 (bs, 1H, NH), 8.89 (s, 1H, CH₃), 8.74 (dd, 1H, J = 1.61 Hz, J = 4.51 Hz, CH₃), 8.63 (dd, 1H, J = 1.59 Hz, J = 8.12 Hz, CH₃), 8.14 (t, 1H, J = 1.23 Hz, CH₃), 7.51 (dd, 1H, J = 4.46 Hz, J = 8.06 Hz, CH₃), 7.20 (s, 1H, CH₃) ppm.

1H 1H 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H]/C0 1H 1H 1H}/
additional amount of the product (0.3 g). Yield: 1.2 g, 47%, based on the remaining solid was purified by column chromatography to give an M

$^1$H NMR (500.32 MHz, DMSO-$d_6$): $δ$ 1.34 (brs, 1H, $H_1$), 13.25 (brs, 1H, $H_9$), 8.99 or 8.98 (d+$d$, (1 + 1.3)H, $J$ = 2 Hz, $H_7a$+$H_7b$), 8.75 (d, (1 + 1.3)H, $J$ = 2 Hz, $H_8a$+$H_8b$), 7.72 (dd, 1H, $J$ = 1.8 Hz, 6.6 Hz, $H_3b$+$H_3c$), 7.28–7.21 (m, (2 + 2.6)H, $H_5b$+$H_6a$+$H_5a$+$H_6c$), 4.96 (s, 2.6H, $H_11b$), 3.44 (s, 3.9H, $H_11c$), 2.18 (s, 3H, $H_10c$), 0.94 (d, 1H, $J$ = 6.9 Hz, $H_9c$+$H_9d$), 0.89 (d, 3H, $J$ = 6.9 Hz, $H_9e$+$H_9f$) ppm. $^{13}$C NMR (125.81 MHz, DMSO-$d_6$): $δ$ 167.5 ($C_1$), 121.3 ($N_1$) ppm.

$^{5}$-Bromo-3-(4-methoxymethyl-1H-benzimidazol-2-yl)-1H-pyrazolo-[3,4-b]pyridine (13, L3). The raw product 10 (2.3 g) was heated in a glacial acetic acid (40 mL) at 125°C for 2 h. The solvent was evaporated under reduced pressure, and the residue dried in vacuo at 50°C. After washing with CHCl$_3$ (30 mL), CH$_2$Cl$_2$/MeOH (2:1), 5–7 mL the gray product was purified by column chromatography (SiO$_2$, EtOAc: R$_f$ = 0.68) to give a white powder (0.9 g). The filtrates were evaporated and the remaining solid was purified by column chromatography to give an additional amount of the product (0.3 g). Yield: 1.2 g, 47%, based on 7.

$M_{c_{1}H_{1}BrN_{3}O_{2}} = 358.19$ g/mol. Anal. Calcld for 13: C, 32.9%; H, 3.8%; N, 19.55. Found: C, 32.00; H, 3.19; N, 19.19. ESI-MS in methanol (positive): $m/z$ 381 [M$^+$Na]$^+$; (negative): $m/z$ 357 [M$^-$H$^-$$]^-.

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Inorganic chemistry (negative): $m/z$ 313 [M$^-$H$^-$$]$. UV–vis (methanol), $λ_{max}$ nm (ε, $M^{-1} cm^{-1}$): 234 (28154), 282 (17628), 335 (17198). $^1$H NMR (500.32 MHz, DMSO-$d_6$): $δ$ 14.43 (brs, 1H, $H_1$), 13.19 (brs, 1H, $H_9$), 8.99 (d, 1H, $J$ = 2.2 Hz, $H_7a$+$H_7b$), 8.75 (d, 1H, $J$ = 2.3 Hz, $H_8a$+$H_8b$), 7.77 (d, 1H, $J$ = 7.9 Hz, $H_3b$+$H_3c$), 7.54 (d, 1H, $J$ = 7.9 Hz, $H_4a$+$H_4b$), 7.26 (m, 2H, $H_5b$+$H_6a$+$H_5a$+$H_6c$) ppm. $^{13}$C NMR (125.81 MHz, DMSO-$d_6$): $δ$ 151.49 ($C_8$), 150.66 ($C_{12b}$), 146.42 ($C_{2b}$), 144.24 ($C_{8b}$ or $C_{9b}$), 135.61 ($C_3a$), 134.67 ($C_{8b}$ or $C_{9b}$), 133.35 ($C_{5b}$ or $C_{6b}$), 122.27 ($C_{5b}$ or $C_{6b}$), 119.55 ($C_{10}$ or $C_{17}$), 114.87 ($C_{7a}$ or $C_{7b}$), 114.93 ($C_{4a}$ or $C_{4b}$), 112.09 ($C_{4b}$ or $C_{4c}$) ppm. $^{13}$N NMR (50.70 MHz, DMSO-$d_6$): $δ$ 167.5 ($N_1$), 121.3 ($N_1$) ppm.

**Synthesis of Organometallic Complexes.** ($^{5}$-$p$-Cymene)$\cdot$3-(1H-benzimidazol-$k$-$N$-$2$-$yl$)-1H-pyrazolo-$k$($3,4$-$b$)pyridine chlorido(II)$ chloride, [R$^2Cl_2$(p-$p$-cymene)]$L_1 jealous$ (11a). A mixture of L1 (54.7 mg, 0.23 mmol) and [RuCl$_3$(p-$p$-cymene)$\cdot$L$_2$Cl]$_2$ (70 mg, 0.11 mmol) in dry ethanol (25 mL) was stirred at room temperature for 1 h. Ethanol then was removed under reduced pressure up to ca. 2 mL and dry Et$_2$O (40 mL) was added. The yellow precipitate was filtered off and dried in vacuo at 40°C. Yield: 106 mg, 73% as 12a$\cdot$H$_2$O$\cdot$M$_c_{(C_{23}H_{22}BrCl_2N_5Ru)} = 620.33$ g/mol. Anal. Calcld for 12a$\cdot$H$_2$O$\cdot$M$_c$: C, 31.85; H, 3.97; N, 11.84. Found: C, 31.78; H, 3.85; N, 11.79.

$H^1$ NMR (500.32 MHz, DMSO-$d_6$): $δ$ 14.34 (brs, 1H, $H_1$), 9.21 (s, 1H, $H_9$), 8.73 (s, 1H, $H_8$), 8.06 (d, 1H, $J$ = 7.6 Hz, $H_7$), 7.79 (d, 1H, $J$ = 8.5 Hz, $H_6$), 7.52 (m, 2H, $H_4a$+$H_4b$), 6.32 (d, 2H, $J$ = 5.8 Hz, $H_3a$+$H_3b$), 6.22 (d, 1H, $J$ = 6.2 Hz, $H_3c$+$H_3d$), 6.03 (d, 1H, $J$ = 6.1 Hz, $H_2c$+$H_2d$), 2.52 (sep, 1H, $H_2c$ under DMSO-$d_6$ peak), 2.18 (s, 3H, $H_10$), 0.94 (d, 3H, $J$ = 6.8 Hz, $H_9c$+$H_9d$), 0.89 (d, 3H, $J$ = 6.9 Hz, $H_9e$+$H_9f$) ppm. $^{13}$C NMR (125.81 MHz, DMSO-$d_6$): $δ$ 154.04 ($C_1$), 151.24 ($C_2$), 146.57 ($C_3$), 141.37 ($C_4$), 134.63 ($C_5$), 133.43 ($C_6$), 131.82 ($C_7$), 125.33 ($C_8$ or $C_9$), 124.88 ($C_8$ or $C_9$), 117.78 ($C_{10}$), 114.72 ($C_{11}$ or $C_{17}$), 114.72 ($C_{12}$ or $C_{17}$), 114.72 ($C_{13}$ or $C_{17}$), 114.72 ($C_{14}$ or $C_{17}$), 114.72 ($C_{15}$ or $C_{17}$), 114.72 ($C_{16}$ or $C_{17}$), 114.72 ($C_{17}$ or $C_{17}$).
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[\text{M}^n_\text{Cl}_x]^{(\text{X}^\text{II})}\text{[CuCl}(\text{η}_6\text{C}_6\text{H}_5\text{BrCl}_2\text{N}_2\text{O}_4)\text{]} = 664.39 \text{ g/mol. Anal. Calcd for } \text{C}_{26}\text{H}_{32}\text{BrCl}_2\text{N}_2\text{O}_4; (\text{X}^\text{II}) = \text{C} = 62.59, \text{H} = 6.17, \text{Br} = 9.23, 15\text{N} = 3.20, (\text{negative}): m/z 568 (M–2HCl–Cl), 704 [M–2HCl–Cl]+; (negative): m/z 680 (M–2HCl–H), 716 [M–2HCl–H]+.

UV–vis–IR spectra were recorded on a Lambda 20 UV–vis spectrophotometer (Perkin–Elmer), using samples dissolved in methanol (1.0 cm). 1H and 13C NMR spectra were measured at 500.32 (1H), 125.81 (13C) and 50.70 (15N) MHz. 1H Signals are referenced relative to the solvent signals (DMSO–d_6: δ 2.51, MeOH–d_4: δ 3.31). The one-dimensional (1D, \text{C}^\text{13}, \text{N}) and two-dimensional spectra (\text{C}^\text{13}N, \text{H} HSQC, \text{C}^\text{13}C, \text{H} HMBC, \text{H}–\text{H COSY, H}–\text{H TOCSY, H}–\text{H NOESY (L3, 12a, 12b, 13a, 12b) were recorded} with a Bruker Model DXP500 (Ultrashield Magnet) system in DMSO–d_6 (L1–L3, 11a, 12a, 13a). 1H, \text{H ROESY (L3, 11a, 12a, 13a, 13b) were recorded with a Bruker Model DXP500 (Ultrashield Magnet) system in DMSO–d_6 (L1–L3, 11a, 12a, 13a). MeOH–d_4 (11b, 12b, 13b) and D_2O/MeOH–d_4 (\text{H} NMR, 11a, 12a, 13a) were used with standard pulse programs at 500.32 (1H) and 125.81 (13C) and 50.70 (15N) MHz. 1H Signals are referenced relative to the solvent signals (DMSO–d_6 at 2.51, MeOH–d_4 at 3.33 ppm).

Physical Measurements. Elemental analyses (C, H, N, Cl) were performed by the Microanalytical Service of the Institute of Physical Chemistry, University of Vienna. Electrospray ionization mass spectrometry (ESI–MS) was carried out with an Esquire 3000 instrument (Bruker Daltonics, Bremen, Germany), using solutions of compounds in methanol. The expected and measured isotope distributions were compared. UV–vis spectra were recorded on a Lambda 20 UV–vis spectrophotometer (Perkin–Elmer), using samples dissolved in methanol (L1–L3, 11a, 12a, 13a, 11b, 12b, and 13b) and water (11a) over 4 or 48 h, respectively. The one-dimensional (1D, \text{C}^\text{13}, \text{N}) and two-dimensional spectra (\text{C}^\text{13}N, \text{H} HSQC, \text{C}^\text{13}C, \text{H} HMBC, \text{H}–\text{H COSY, H}–\text{H TOCSY, H}–\text{H NOESY (L3, 12a, 12b, 13a, 12b) were recorded} with a Bruker Model DXP500 (Ultrashield Magnet) system in DMSO–d_6 (L1–L3, 11a, 12a, 13a). MeOH–d_4 (11b, 12b, 13b) and D_2O/MeOH–d_4 (\text{H} NMR, 11a, 12a, 13a) were used with standard pulse programs at 500.32 (1H) and 125.81 (13C) and 50.70 (15N) MHz. 1H Signals are referenced relative to the solvent signals (DMSO–d_6 at 2.51, MeOH–d_4 at 3.33 ppm).

Crystallographic Structure Determination. XRD measurements were performed on a Bruker Model X8 APEXII CCD diffractometer. Single crystals were positioned at distances of 35, 40, 40, 40, 40, and 35 mm from the detector, and 1556, 1131, 518, 1911, 1176, 1978, and 1039 frames were measured, each for 30, 30, 30, 20, 70, 60, and 30 s over 1° scan width for 2.0.9H_2O, 11b–4H_2O, 12b, 12b–2CH_3OH.2H_2O, 13c, 13d–CH_3OH, and 13e–0.75CH_3OH–0.25H_2O, respectively. The data were processed using SAINT software. Crystal data, data collection parameters, and structure refinement details are given in Table 1. The structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-H atoms were

113.85 (C_6), 112.48 (C_4 or C_6), 103.96 (C_6), 103.74 (C_6), 89.44 (C_4 or C_6), 83.69 (C_6), 82.16 (C_6 or C_4), 80.76 (C_6 or C_4), 31.09 (C_6), 22.20 (C_6 or C_4), 22.17 (C_6 or C_4), 19.16 (C_6) ppm. 1^H NMR (500.32 MHz, DMSO–d_6): δ 13.43 (3H, 1H), 9.18 (1H, J = 2.1 Hz), 8.48 (1H, J = 2.2 Hz), 7.94 (1H, J = 8.2 Hz), 7.45 (1H, J = 7.8 Hz), 7.39 (1H, J = 7.2 Hz), 6.16 (1H, J = 6.0 Hz), 6.13 (1H, J = 6.1 Hz), 6.07 (1H, J = 5.9 Hz), 5.89 (1H, J = 6.4 Hz), 4.85 (2H, 4.04 (3H, 2.14 (3H, 0.93 (3H, 6.9 Hz), 0.86 (3H, 6.9 Hz). 11719 dx.doi.org/10.1021/ic201704u | Inorg. Chem. 2011, 50, 11715–11728
Table 1. Crystal Data and Details of Data Collection for 2·0.5H₂O, 11b·4H₂O, 12b·2CH₃OH·H₂O, 13c·13d·CH₃OH·0.25H₂O, and 13e·0.75CH₃OH·0.25H₂O

| Formula wt, Fw | β/C176 | γ/C176 | V[Å³] | Z | λ[Å] |  Fcalcd [g cm⁻³] | Crystal size [mm³] | temp, [K] | μ[mm¹] | wR | GOF |
|----------------|--------|--------|-------|---|-----|----------------|-------------------|---------|------|----|-----|
| 221.06         | 98.267(3) | 97.046(3) | 104.380(5) | 104.233(2) | 0.71073 | 1.790 | 0.20 | 120(2) | 4.954 | 1.092 | 1.092 |
| 702.63         | 81.446(4) | 99.334(4) | 108.744(5) | 93.139(5) | 0.71073 | 1.851 | 0.05 | 100(2) | 5.309 | 1.011 | 1.011 |
| 709.47         | 104.380(5) | 91.531(5) | 76.887(5) | 98.681(5) | 0.71073 | 0.970 | 0.04 | 100(2) | 7.245 | 0.939 | 0.939 |
| 809.58         | 104.233(2) | 81.191(5) | 5.962 | 108.744(5) | 0.71073 | 6.587 | 0.02 | 100(2) | 2.414 | 1.087 | 1.087 |
| 627.93         | 108.744(5) | 93.139(5) | 2.414 | 93.139(5) | 0.71073 | 0.986 | 0.02 | 100(2) | 2.310 | 1.048 | 1.048 |
| 1324.36        | 93.029(2) | 81.191(5) | 6.587 | 98.681(5) | 0.71073 | 1.048 | 0.02 | 100(2) | 1.903 | 1.054 | 1.054 |
| 1499.11        | 93.029(2) | 93.139(5) | 1.903 | 98.681(5) | 0.71073 | 1.054 | 0.02 | 100(2) | 1.903 | 1.054 | 1.054 |

Cell Lines and Culture Conditions. A549 (non-small cell lung carcinoma) and SW480 (colon carcinoma, human) cells were kindly provided by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria). CH1 cells (ovarian cancer, human) were a gift from Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, U.K.). All cell culture media and supplements were purchased from Sigma–Aldrich. Cells were grown in 75-cm² culture flasks (Iwaki) in a complete medium (i.e., Minimum Essential Medium supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 4 mM L-glutamine, and 1% nonessential amino acids from 100× stock) as adherent monolayer cultures. Cultures were grown at 37 °C under a humidified atmosphere containing 5% CO₂ and 95% air.

Inhibition of Cancer Cell Growth. Antiproliferative activity in vitro was determined by the colorimetric MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, Fluka). For this purpose, cells were harvested from culture flasks by the use of trypsin and seeded in complete medium (100 μL/well) into 96-well plates (Iwaki) in densities of 4 × 10⁴ (A549), 1.5 × 10⁵ (CH1), and 2.5 × 10⁴ (SW480) viable cells per well. Cells were allowed for 24 h to settle and resume proliferation. Test compounds were dissolved in DMSO first, appropriately dilutet in complete medium, and instantly added to the plates (100 μL/well), where the DMSO content did not exceed 0.4% and 1% for the ligands and complexes, respectively. After exposure for 96 h, the medium was replaced with 100 μL/well RPMI 1640 medium (supplemented with 10% heat-inactivated fetal bovine serum and 4 mM L-glutamine) plus 20 μL/well MTT solution in phosphate-buffered saline (5 mg/mL), followed by incubation for 4 h. Subsequently, the medium/MTT mixture was removed, and the formazan product formed by viable cells was dissolved in DMSO (150 μL/well). Optical densities were measured with a microplate reader (Tecan Spectra Classic) at 550 nm (and a reference wavelength of 690 nm) to yield relative quantities of viable cells as percentages of untreated controls, and 50% inhibitory concentrations (IC₅₀) were interpolated from concentration–effect curves. Calculations are based on at least three independent experiments with triplicates for each concentration level.

Cell Cycle Analyses. To study the effects on the cell cycle of exponentially growing CH1 cells by flow cytometric analysis of their relative DNA content, cells were harvested from culture flasks, seeded in complete medium into 90-mm Petri dishes (1×10⁵ cells/dish) and, after recovery for 24 h, exposed to various concentrations of the test compounds for 24 h. For this purpose, test compounds were diluted from DMSO stocks with complete medium (see above) such that the effective DMSO content did not exceed 0.5%. After exposure, treated and control cells were collected by scratching, washed with PBS, and stained with 5 μg/mL propidium iodide overnight. Their fluorescence was measured with a FACS Calibur instrument (Becton Dickinson), and the obtained histograms were analyzed with Cell Quest Pro software (Becton Dickinson).
At least two independent experiments were performed for each setting, and 2.5 or 3.0 × 10^4 cells were measured per sample.

**Kinase Assay.** The Cdk-inhibitory capacities of test compounds were studied by a radiochemical assay using recombinant Cdk1/cyclin B and Cdk2/cyclin E isolated from SF21 insect cells and histone H1 as the substrate for phosphorylation, as described by Marko et al. Briefly, MOPS-buffered assay mixtures containing the test compound (with a maximum of 1% DMSO), the respective kinase/cyclin complex, histone H1, and 0.4 μCi (γ32P)ATP per sample were incubated for 10 min at 30 °C. Aliquots of the solution were spotted onto phosphocellulose squares, which had been washed three times with 0.75% phosphoric acid, followed by acetone. The dried squares were measured in scintillation vials by β-counting (Perkin–Elmer Tri-Carb 2800TR; Quanta Smart software). Results were obtained in duplicate in at least two independent experiments, and IC50 values were calculated by interpolation.

### RESULTS AND DISCUSSION

#### Synthesis of Ligands and Complexes.

Several routes to 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines have been proposed by Johnson & Johnson Pharmaceutical Research & Development L.L.C. The first one was developed for 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines with an unsubstituted benzimidazole moiety and involved sulfur-induced benzimidazole ring formation via the treatment of 5-bromo-1H-pyrazolo[3,4-b]pyridine-3-carboxaldehyde with 1,2-diaminobenzene. The poor reproducibility of this synthesis prompted the exploration of an alternative way, via 5-bromo-1H-pyrazolo[3,4-b]pyridine-3-carboxylic acid, which was used for the preparation of 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines with a substituted benzimidazole moiety.

The patented route to 5-bromo-1H-pyrazolo[3,4-b]pyridine-3-carboxylic acid (3) consists of four steps (see Scheme 1, steps i−iv): oxidation of 3-methyl-1H-pyrazolo[3,4-b]pyridine by KMnO4 in the presence of a base with subsequent acidification with H2SO4 esterification of 1H-pyrazolo[3,4-b]pyridine-3-carboxylic acid (1) in the presence of H2SO4 in methanol, bromination of 1H-pyrazolo[3,4-b]pyridine-3-carboxylic acid methyl ester in an AcOH/ AcONa mixture, and hydrolysis of 5-bromo-1H-pyrazolo[3,4-b]pyridine-3-carboxylic acid methyl ester in the presence of NaOH, followed by acidification with HCl.

The overall yield of 5-bromo-1H-pyrazolo[3,4-b]pyridine-3-carboxylic acid (3) was 18%.

We performed the synthesis of 3 in two steps (see Scheme 1, steps v and vi): bromination of 3-methyl-1H-pyrazolo[3,4-b]pyridine in AcOH/ AcONa mixture and oxidation of crude 5-bromo-3-methyl-1H-pyrazolo[3,4-b]pyridine (2) by KMnO4 in a basic medium, followed by acidification with 37% HCl, with an overall yield of 24−35%. 5-Bromo-3-methyl-1H-pyrazolo[3,4-b]pyridine (2) is a known compound, the synthesis of which is well-documented.

For the benzimidazole ring formation, 1,2-diaminobenzene and 1-methoxymethyl-2,3-diaminobenzene have been used. The reported synthesis of 1-methoxymethyl-2,3-diaminobenzene (5) from 2,3-diaminobenzyl alcohol afforded the desired product in 34% yield (see Scheme 2, step i). However, the instability and low yield of diamines, as well as the necessity to purify the desired ether using column chromatography, stimulated the search for a more-convenient procedure that was subsequently proposed: etherification of 2-amino-3-nitrobenzyl alcohol, followed by the reduction of 1-methoxymethyl-2-amino-3-nitrobenzene (4) with 10% Pd/C in ethanol under a hydrogen atmosphere afforded 5 in 65−75% yield (see Scheme 2, steps ii and iii). Patented benzimidazole ring formation by cyclization of the 3-carboxyl group of 5-bromo-1H-pyrazolo[3,4-b]pyridine-3-carboxylic acid (3) with substituted 1,2-diaminobenzenes was realized via amide formation using coupling reagents, followed by treatment with glacial acetic acid. HATU (N,N,N′,N′-tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate) was used as a coupling reagent.

Three 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines (L1−L3) (where X = H, Br, and Y = H, CH2OCH3) have been synthesized in this work (see Chart 1): 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridine (L1) is a new compound that we have prepared as a model ligand for coordination to metals; 3-(1H-benzimidazol-2-yl)-5-bromo-1H-pyrazolo[3,4-b]pyridine (L2) was previously known as BOC-protected compound prepared via 5-bromo-1H-pyrazolo[3,4-b]pyridine-3-carboxaldehyde; S-bromo-3-(4-methoxymethyl-1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridine (L3) was synthesized via 5-bromo-1H-pyrazolo[3,4-b]pyridine-3-carboxylic acid (3) and patented as a potential Cdk inhibitor and antiproliferative agent.
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Scheme 3. Synthesis of 3-(1H-Benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines L1–L3

![Scheme 3](image)

Reagents and conditions: (i) CDI, dry DMF, room temperature, 20–24 h; (ii) 1,2-diaminobenzene or S, dry DMF, 80–85 °C; S—20 h; and (iii) glacial AcOH, 120–125 °C, 2.5–4.5 h, chromatographic purification.

Figure 1. Part of the ¹H,¹H ROESY plot of L3.

For the synthesis of L1–L3, we used N₂N’-carbonyldiimidazole (CDI) as an amide-coupling reagent, because it is relatively inexpensive and the side products, carbon dioxide and imidazole, could be easily removed from the reaction mixture.

CDI-mediated amidation of acids 1 and 3 was performed as shown in Scheme 3 (steps i and ii). In the first step, the acyl-imidazolides 6 and 7 were obtained in dry DMF at room temperature and isolated as white solids. The yields based on imidazolides shown in Scheme 3 (steps i and ii). In the first step, the acyl-imidazolides 6 and 7 were obtained in dry DMF at room temperature and isolated as white solids. The yields based on imidazolides shown in Scheme 3 (steps i and ii).

Amides 8–10 were used without further purification in ring closure reactions in a glacial acetic acid at 120–125 °C and afforded the desired 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]-pyridines: L1 (55–60%), L2 (58–61%), and L3 (44–51%), based on 6 and 7 (see Scheme 3, step iii). The reported synthesis of L3 is a one-pot procedure for amide formation using HATU with 57% yield, followed by cyclization under acidic conditions (acetic acid) with 87% yield.

Thus, the ligands L1, L2, and L3 have been prepared in 7, 8, and 11 steps, correspondingly.

Finally, the ligands L1–L3 were reacted with [MºCl₂(η²-p-cymene)]₂ (where M = Ru, Os) in a 2:1 molar ratio in dry ethanol at room temperature to give [MCl(η²-arene)(L)]Cl complexes (11a, 11b, 12a, 12b, 13a, 13b) in quantitative yields. Crystalization of [RuºCl(η²-p-cymene)(L)]Cl (13a) in EtOH or EtOH/Et₂O resulted in XRD-quality crystals of [RuºCl(η²-p-cymene)(L₃—H)] (13c), while the crystallization of 13a in methanol afforded crystals of composition [RuºCl(η²-p-cymene)(L₃—H)]Cl·[RuºCl(η²-p-cymene)(L₃—H)]·CH₃OH (13d·CH₃OH). The osmium(II) analogue 13e·0.75CH₃OH·0.25H₂O was obtained via the crystallization of 13b in methanol.

NMR Evidence of Ligand Coordination. The full assignment of proton, carbon, and nitrogen resonances for L1–L3, 11a, 11b, 12a, 12b, 13a, and 13b is quoted in Tables S1–S3 in the Supporting Information.

3-(1H-Benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines with an unsubstituted benzimidazole moiety (L1, L2) display one set of signals. L1 is characterized by three doublets of doublets for H₄a and H₆a (8.85, 7.39, 8.66 ppm, correspondingly) of pyrazolopyridine moiety, two doublets for H₄a and H₆a (7.54 and 7.75 ppm), whereas the benzimidazole moiety and two singlet resonances for H₁a and H₁b (14.19 ppm). A singlet at 12a (14.91 ppm) assigned to N₁b (see Table S3 in the Supporting Information).

Coordination of L1 and L2 to ruthenium(II) made it possible to assign the two doublets to proton resonances of H₄a and H₆a of the benzimidazole moiety. In the ¹H,¹H ROESY plots, one of them has cross-peaks with a CH cymene ring and the nearest to them is H₄a (e.g., at 8.11 (11a), 8.06 (12a) ppm). A singlet at 14.91 (11a), 14.34 (12a) ppm was assigned to NH proton and showed no couplings with other atoms. The nitrogen resonance shift at 128.7 (11a), 127.3 (12a) ppm is closer to the benzimidazole NH chemical shift in metal-free ligands (121.3 (L1, L2) ppm) and, therefore, was assigned as N₁b (see Table S3 in the Supporting Information). The H₁b resonance shows a significant shift by 1.8 and 1.15 ppm for 11a and 12a, respectively, upon ligand coordination (L1 and L2) to the metal(II)-arene moiety.

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The nearest to the metal binding site pyrazolopyridine proton H_{1a} was not detected for 11a, 11b, 12a, and 12b, and the proton resonance of H_{1b} was also not seen for 11b or 12b.

L3 displays two sets of signals originated from 7b-L3 and 4b-L3 tautomers (see Chart 1). The signals of pyrazolopyridine moieties are partially overlapped (e.g., H_{1a} (H_{1a}^0) at 14.47 and 14.43 ppm, H_{2a} (H_{2a}^0) at 8.99 and 8.98 ppm, H_{3a} (H_{3a}^0) at 8.75 ppm), whereas the signals of the benzimidazole moieties are better resolved (see Table S1 in the Supporting Information).

According to the $^1$H,$^1$H ROESY plot, one of the C atoms of the benzimidazole moiety (H_{1b}) displays two sets of signals originated from 7b-L3 and 4b-L3 tautomers (Figure 1).

Tautomers 7b-L3 and 4b-L3 are present in solution in 1:1.3 molar ratio and give, for the substituted benzimidazole moiety, two singlets (H_{1b} at 13.22 ppm) and two doublets (H_{11b} at 13.25 ppm), two C atoms at 13.22 ppm gives a cross-peak with H_{4b} at 7.47 ppm and belongs to 4b-L3 tautomer (Figure 1).

The binding site in 4b’-L3 tautomer is sterically shielded by a methoxymethyl group. Consistent with this NMR spectra display, only one set of signals is shown for 13a and 13b with the coordination of the 7b-L3 tautomer to ruthenium(II) and osmium(II) via N_{2a} and N_{3b} (see Scheme 4).

The preference for coordination of the 7b-L3 tautomer is also confirmed by $^1$H,$^1$H ROESY plots: H_{4b} at 7.99 (13a), 7.91 (13b) ppm) has couplings with CH protons of cymene ring. The cross-peak between H_{6b} at 4.88 ppm (13a) and NH at 13.88 ppm - (13a) enabled the assignment of this singlet to a benzimidazole moiety (H_{1a}). In addition, the chemical shifts for the C atoms of the benzimidazole moiety (C_{4,6}H_{5,5b}C_{6b}C_{6b}C_{6b}) of the coordinated ligand and metal-free 7b-L3 tautomer are very similar (see Table S2 in the Supporting Information). The nitrogen resonance at 122.9 ppm (13a) compares well to the benzimidazole NH chemical shift in metal-free L3 at 121.4 ppm. As for 11a, 11b, 12a, and 12b, the nearest to the coordination place pyrazolopyridine proton H_{1a} resonance was not detected.

According to the $^1$H,$^1$H ROESY plots of 11a, 12a, 13a, and 13b only CH cymene ring protons have couplings with the nearest H_{4b} benzene ring proton, suggesting that the isopropyl or methyl groups are further away from the H_{4b} proton.

Crystal Structures. The results of the XRD studies of [OsCl(η^6-p-cymene)(L3)]Cl[4H_2O (11b·4H_2O), [OsCl(η^6-p-cymene)-(L3)]Cl (12b), [RuCl(η^6-p-cymene)(L3)]Cl·2CH_3OH·2H_2O (12b·2CH_3OH·2H_2O), [RuCl(η^6-p-cymene)(L3)]Cl·0.75CH_3OH·0.25H_2O (13e·0.75CH_3OH·0.25H_2O) are shown in Figures 2, Figure S2 in the Supporting Information, and Figures 3–6, respectively. All complexes have a typical “three-legged piano-stool” geometry of ruthenium(II) and osmium(II) arene complexes, with an η^6-π-bound p-cymene ring forming the seat and three other donor atoms (two nitrogens N1 and N4, which act as proton donors in intramolecular hydrogen bonding interactions N3–H...Cl2 [N3...Cl2 3.067(7) Å, N3–H...Cl2 177.3°] and N4–H...O3 [N4...O3 2.671(9) Å, N4–H...O3 170.9°]; thermal ellipsoids have been drawn at 50% probability level. Selected bond lengths and angles: Os–Cl1, 2.421(2) Å; Os–N1, 2.072(7) Å; Os–N5, 2.096(7) Å; Os–C(arene)av, 1.349(11) Å; N2–N1, 1.366(10) Å; N1–C6, 1.357(11) Å; C6–C7, 1.428(12) Å; C7–N5, 1.345(11) Å; N5–C13, 1.391(11) Å; N1–Os–N5, 76.6(3)°; N1–Os–Cl1, 85.5(2)°; N5–Os–Cl1, 83.4(2)°.

Figure 2. Fragment of the crystal structure of 11b·4H_2O showing nitrogen atoms N3 and N4, which act as proton donors in intramolecular hydrogen bonding interactions N3–H...Cl2 [N3...Cl2 3.067(7) Å, N3–H...Cl2 177.3°] and N4–H...O3 [N4...O3 2.671(9) Å, N4–H...O3 170.9°]; thermal ellipsoids have been drawn at 50% probability level. Selected bond lengths and angles: Os–Cl1, 2.421(2) Å; Os–N1, 2.072(7) Å; Os–N5, 2.096(7) Å; Os–C(arene)av, 1.349(11) Å; N2–N1, 1.366(10) Å; N1–C6, 1.357(11) Å; C6–C7, 1.428(12) Å; C7–N5, 1.345(11) Å; N5–C13, 1.391(11) Å; N1–Os–N5, 76.6(3)°; N1–Os–Cl1, 85.5(2)°; N5–Os–Cl1, 83.4(2)°.

Figure 3. ORTEP plot of the structure of the cation [OsCl(η^6-p-cymene)(L2)]^+ in 12b·2CH_3OH·2H_2O. Thermal ellipsoids have been drawn at the 50% probability level. Selected bond lengths and angles: Os–Cl1, 2.4043(1) Å; Os–N1, 2.083(3) Å; Os–N5, 2.097(3) Å; Os–C(arene)av, 2.197(26) Å; C1–N2, 1.361(5) Å; N2–N1, 1.344(4) Å; N1–C6, 1.336(5) Å; C6–C7, 1.447(6) Å; C7–N5, 1.335(5) Å; N5–C13, 1.380(5) Å; N1–Os–N5, 74.3(13)°; N1–Os–Cl1, 84.29(10)°; and N5–Os–Cl1, 82.5(10)°.
N5 of the corresponding 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridine and one chlorido ligand as the legs of the stool. Selected bond distances and angles are quoted in the figure captions. All complexes crystallize as racemates, because of the presence of the stereogenic metal center.

The bidentate 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]-pyridines, which can have different substituents in positions 5a and 7b, reveal different acid–base properties. It can act as a neutral organic ligand, with nitrogen atoms N2 and N4 as proton donors involved in intermolecular hydrogen bonding interactions N4–H···O1(−x + 1, −y + 1, −z + 2) [N4···O1, 2.730(4) Å; N4–H···O1, 176.1(9)°] and N2–H···O3(−x + 2, −y + 1, −z + 1) [N2···O3, 2.697(4) Å; N4–H···O1, 172.3(8)°] with one methanol and one water molecule, respectively, as is the case for 12b·2CH3OH·2H2O (Figure 3) or N2–H···Cl2·Cl2X and N4–H···Cl1(−x, −y, −z + 2) [N4···Cl1, 3.213(9) Å; N4–H···O1, 162.91(8)°] in 12b (see Figure S2 in the Supporting Information), correspondingly. The ligand can be protonated at N3 (N3–H···Cl2) and deprotonated at N2 [N2···H–O4(−x + 1, −y + 1, −z + 1) with N2···O4, 2.923(9) Å; N2···H–O4, 138.7(8)° (overall charge zero)] with atom N4 as a proton donor to one of the four co-crystallized water molecules N4–H···O3, as occurs in 11b·4H2O (see Figure 2).

In 13c, the ligand was found to be deprotonated at N2, acting as a proton acceptor in the intermolecular hydrogen bonding interaction N2···H–N4 (i designates symmetry transformations x, −y + 1, z + 0.5) used to generate equivalent atoms) [N2···N4, 2.896(7) Å; N2···H–N4, 155.6(6)°] (see Figure 4). Complexes 13d·CH3OH and 13e·0.75CH3OH·0.25H2O crystallized both in the centrosymmetric triclinic space group P1. The asymmetric unit in both consists of a neutral complex [MIII(Cl(η6-p-cymene)(L3–H))] and a complex cation [MIVCl(η6-p-cymene)(L3)] (M = Ru or Os), a chloride counterion and co-crystallized solvent (methanol or methanol/water). Deprotonation of the organic ligand in [MIVCl(η6-p-cymene)(L3–H)] is corroborated by the presence of hydrogen bonding of the type N2a···H–N2b(−x + 1, −y + 1, −z + 1) in the crystal structure of the ruthenium complex 13d·CH3OH (Figure 5) and a similar interaction N2b···H–N2a(−x + 1, −y + 1, −z + 1) in the crystal structure of the osmium analogue 13e·0.75CH3OH·0.25H2O. In Figure 6 the structure of [OsvIII(Cl(η6-p-cymene)(L3–H))] is shown. In both structures,
### Table 2. Antiproliferative Activity of Metal-Free Ligands (L1–L3), and Their Ruthenium(II) (11a–13a) and Osmium(II) (11b–13b) Arene Complexes, in Three Human Cancer Cell Lines (CH1, SW480, and A549)

| compound | metal | CH1 [μM] | SW480 [μM] | A549 [μM] |
|----------|-------|----------|------------|------------|
| L1       |       | 11 ± 3   | 23 ± 6     | 29 ± 7     |
| 11a      | Ru    | 96 ± 18  | >320        | 525 ± 102  |
| 11b      | Os    | 64 ± 19  | 223 ± 29   | >640       |
| L2       |       | 1.5 ± 0.6| 5.1 ± 1.0  | 6.7 ± 0.3  |
| 12a      | Ru    | 21 ± 3   | 70 ± 8     | 268 ± 35   |
| 12b      | Os    | 22 ± 3   | 29 ± 2     | 123 ± 21   |
| L3       |       | 0.63 ± 0.09| 0.74 ± 0.26| 52.5 ± 0.5 |
| 13a      | Ru    | 11 ± 1   | 11 ± 2     | 68 ± 12    |
| 13b      | Os    | 7.9 ± 2.2| 12 ± 2     | 89 ± 11    |

*CH1 denotes ovarian cancer, human; SW480 denotes colon carcinoma, human; and A549 denotes non-small-cell lung carcinoma, human.

*IC50 values listed were obtained by the MTT assay (96 h exposure).

The metal-free ligands L1–L3 turned out to exert only modest effects on cell cycle distribution, with a slight increase of the G2/M fraction from 32% in the untreated control to 53% by 20 μM L2 as the strongest effect observed in this setting (higher concentrations of this compound led to disintegration of cells already after 24 h). However, the less cytotoxic ruthenium complexes 12a and 13a, both bearing substituted ligands (L2 and L3 correspondingly), cause a more pronounced G2/M phase arrest, as reflected by an increase of this cell fraction to 65% at 80 μM and 59% at 40 μM, respectively, and a concomitant decrease of the G0/G1 fraction to 21% and 24% (compared to 42% in controls), whereas ruthenium complex 11a bearing an unsubstituted ligand L1 is devoid of activity on the cell cycle. On the other hand, the osmium complexes do not generally show stronger effects on the cell cycle than the metal-free ligands, perhaps with the exception of 13b, which induces an increase of the G2/M fraction up to 53% at 80 μM, accompanied by a decline of the G0/G1 fraction to 31% (see Figure 8).

**Cdk-Inhibitory Activity.** Although the lack of generally pronounced cell cycle effects does not argue for a strong role of Cdk inhibition in the mechanism of action of the investigated compounds, inhibitory potencies were studied in cell-free experiments with two recombinant Cdk/cyclin complexes, by means of the histone H1 kinase assay. Results reveal that all compounds are capable of inhibiting kinase activities in a concentration-dependent manner, being more effective on Cdk2/cyclin E than

![Figure 7. Concentration-effect curves of each ligand, compared to the corresponding ruthenium and osmium complexes, in CH1 ovarian cancer cells (MTT assay, 96 h exposure): (A) L1, 11a, 11b; (B) L2, 12a, 12b; and (C) L3, 13a, 13b. Both the presence of substituents in the ligands and complexation result in a marked shift of antiproliferative activity.]
on Cdk1/cyclin B (see Figure 9). In contrast to the observed cell cycle effects, but in accordance with antiproliferative activities, Cdk-inhibitory potency of the metal-free ligands is consistently higher than that of the metal complexes. In particular, only L1–L3 effectively inhibit Cdk2/cyclin E in low concentrations (1 μM or 10 μM), whereas all complexes require higher concentrations to exert >50% inhibitory effects. As in the MTT assay, differences between the effects of corresponding ruthenium and osmium complexes are minor, compared to the differences from those of the metal-free ligands.

**CONCLUSION**

The known multistep synthesis of 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines, which we have modified, essentially afforded three organic compounds (L1–L3) that possess Cdk-inhibiting properties. These were used as bidentate ligands, and six novel organometallic compounds of the general formula [M₄Cl(η⁶-p-cymene)(L)]Cl, where M = Ru (11a, 12a, 13a) or Os (11b, 12b, 13b) and L = L1–L3, have been synthesized and comprehensively characterized using spectroscopic and X-ray diffraction methods. Complexation of L1–L3 with ruthenium or osmium yielded compounds with increased solubility in the biological medium, yet lowered antiproliferative activity in human cancer cell lines. Modulation of biological activities by substitution at the ligands can be accomplished in the metal-free molecules and their metal complexes in a similar way. The known Cdk-inhibitory activity of the ligands could be confirmed in cell-
free experiments, in particular in Cdk2/cyclin E, and their
stronger effects on Cdk activity parallel their higher capacity of
inhibiting cancer cell growth in vitro, compared to their metal
complexes. Nevertheless, the lack of pronounced effects on the
cell cycle of chemosensitive ovarian cancer cells argues against a
major role for inhibition of cell growth, at least in this setting.

ASSOCIATED CONTENT

Supporting Information. Assigned NMR (1H, 13C, 15N)
signals for L1–L3, 11a–13a, 11b–13b (Tables S1–S3): ORTEP
plot of C(s)-br-cyclopentadienyl (L2)2 (Figure S2): stability of complexes in solution:
time-dependent UV–vis spectra of L1, 11a, and 11b in MeOH
(Figure S3): time-dependent UV–vis spectra of L3, 13a, and
13b in MeOH (Figure S4): time-dependent UV–vis spectra of
11a in H2O for 48 h (Figure S5); crystallographic data for
2·0·5H2O, 11b·4H2O, 12b, 12b·2CH3OH·2H2O, 13c, 13d·
CH3OH, and 13e·0·75CH3OH·0·25H2O (in CIF format). This
material is available free of charge via the Internet at http://pubs.
acs.org.

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