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Supporting Information

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Tumor-Targeting of EGFR Inhibitors by Hypoxia-Mediated Activation**

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Supporting Information

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Materials and Methods.

Chemicals. All solvents and reagents were obtained from commercial suppliers and, unless specified differently, used without further purification. HOAc was freshly distilled. Anhydrous MeOH and THF were bought from Sigma-Aldrich over molecular sieves. Erlotinib and Erlotinib hydrochlorid were purchased from LC Laboratories®, 2,2'-bipyridine-5-carboxylic acid from SYNTHON Chemicals GmbH, which was further purified using preparative RP-HPLC (H_2O/MeOH 70:30). Compound **A** was synthesized according to literature methods[1]. $[Co(acac)_2en]$ (acac = acetylacetonato, en = ethylenediamine) was prepared from $[Co(acac)_2(H_2O)_2]$ according to Sankhla *et al.*[2], which was synthesized from $Co(NO_3)_2{\cdot}6H_2O$ as described by Chaudhari *et al.*[3]. Preparative RP-HPLC was performed on an Agilent 1200 Series system controlled by Chemstation software. The experimental conditions were as follows: stationary phase: ethylene bridged hybrid C18; column: XBridge BEH C18 OBD Prep Column, 130Å, 5 µm, 19 mm x 250 mm (Waters Corp., Massachusetts, USA); flow rate: 17.06 ml/min, injection volume: 5–7 ml; column temperature: 25°C. Elemental analyses were performed by the Microanalytical Laboratory of the University of Vienna. ESI-MS spectrometry was carried out with a Bruker $Esquire_{3000}$ ion trap spectrometer (Bruker Daltonic, Bremen, Germany). Expected and experimental isotope distributions were compared. ^1H and ^{13}C NMR spectra were recorded in d_6-DMSO or D_2O referring to the respective solubility of the synthesized compounds, with a Bruker FT-NMR Avance III 500 MHz spectrometer at 500.10 (^1H) and 125.75 (^{13}C) MHz at 298 K. Chemical shifts (ppm) were referenced internal to the solvent residual peaks. For the description of the spin multiplicities the following abbreviations were used: s = singlet, d = duplet, t = triplet, q = quartet, m = multiplet. Fluorescence measurements were performed on a Horiba FluoroMax®-4 spectrofluorometer and processed using the FluorEssence v3.5 software package. All tested solutions had a concentration of 3×10^{-5} M and were prepared immediately prior to analysis. Scans were run at room temperature with excitation and emission slit widths of 4 nm for measurements in phosphate-buffered saline (PBS) and 3 nm in DMEM, respectively. Emission scans were run from 380–600 nm using an excitation wavelength of 370 nm for PBS, and 420–600 nm with an

excitation wavelength of 410 nm for DMEM. Between the stability measurements in DMEM, the solutions were stored at 37°C.

Crystallographic Structure Determination. X-ray diffraction measurements were performed on a Bruker D8 VENTURE system equipped with a multilayer monochromator and a Mo K/a INCOATEC microfocus sealed tube (λ = 0.71073 Å). A single crystal of approximate dimensions, 0.040 mm x 0.049 mm x 0.312 mm, was coated with Paratone-N oil, mounted at room temperature on a MiTiGen LD 200 MicroLoop and cooled to 100K under a stream of N_2 maintained by a KRYOFLEXII low-temperature apparatus. The crystal was positioned at 35 mm from the detector, and 2240 frames were collected, each for 8 s over 0.4° scan width. The data were processed using SAINT software[4]. The structures were solved by direct methods and refined by full-matrix least-squares techniques. Data were corrected for absorption effects using the multi-scan method (SADABS [5]) Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were placed at calculated positions and refined as riding atoms in the subsequent least squares model refinements. The isotropic thermal parameters were estimated to be 1.2 times the values of the equivalent isotropic thermal parameters of the atoms to which hydrogens were bound. The following computer programs were used: structure solution, SHELXS-97[6]; refinement, SHELXL- Version 2013/3[7], OLEX2[8], and ShelXle[9], molecular diagrams, ORTEP[10]; Processor: Intel Xeon CPU E3-1270 V2 @ 3.50GHz; scattering factors [11]. (CCDC 994541)

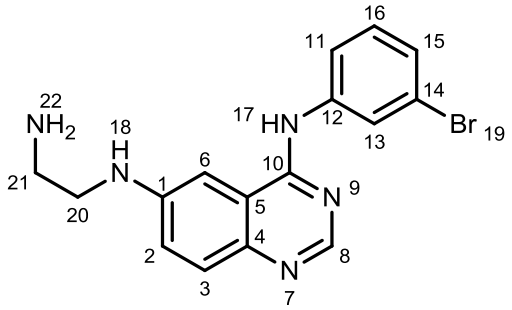
Synthesis of Ligands and Complexes

***N*^{4}-(3-bromophenyl)-*N*^{6}-(pyridin-2-ylmethylene) quinazoline-4,6-diamine (L1).** To a solution of **A** (469 mg, 1.49 mmol) in a minimum of hot MeOH was added drop-wise pyridin-2-carbaldehyde (710 µl, 7.43 mmol). The reaction mixture was refluxed for 3 h and stored over night at room temperature. A yellow precipitate was formed which was filtered, washed with MeOH and dried *in vacuo*. Yield: 563 mg (94%). Anal. Calcd for $C_{20}H_{14}BrN_5 \cdot 0.5H_2O$ (M_r = 413.27 g/mol): C, 58.13; H, 3.66; N, 16.95; O, 1.94. Found: C, 58.17; H, 3.42; N, 16.59; O, 1.98. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 7.32 (d, J = 7.9 Hz, 1H), 7.39 (dd, J = 8.0 Hz, J = 8.0 Hz, 1H), 7.60 (ddd, J = 1.2 Hz, J = 4.8 Hz, J = 7.5 Hz, 1H), 7.89 (d, J = 8.8 Hz, 1H), 7.95–7.97 (m, 2H), 8.02–8.05 (m, 1H), 8.25–8.28 (m, 2H), 8.55 (d, J = 2.0 Hz, 1H), 8.68 (s, 1H), 8.79 (d, J = 4.8 Hz, 1H), 8.86 (s, 1H), 9.93 (s, 1H) ppm (data according to literature[12]).

***tert*-Butyl {2-[(4-((3-bromophenyl)amino)quinazolin-6-yl)amino]ethyl}carbamate (B).** To a solution of **A** (500 mg, 1.6 mmol) and HOAc (90.8 µl, 1.6 mmol) in anhydrous MeOH (16 ml, stored over a 3-4 Å molecular sieve) was added *N*-boc-2-aminoacetaldehyde (303 mg, 1.9 mmol) and the resulting mixture was stirred under Ar at room temperature for 1 h. Sodium cyanoborohydride (120 mg, 1.9 mmol) was added and the resulting suspension was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (200 ml), washed with 1 M HCl (150 ml), sat. $NaHCO_3$ (150 ml), and brine (150 ml). The organic layer was dried over $MgSO_4$, filtered, concentrated under reduced pressure and dried *in vacuo* to give a bright yellow solid. Yield: 615 mg (85%). ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 1.40 (s, 9H), 3.26 (s, 4H), 6.22 (s, 1H), 6.97 (s, 1H), 7.23 (s, 1H), 7.27–7.29 (m, 2H), 7.36 (dd, J = 8.0 Hz, J = 8.0 Hz, 1H), 7.57 (d, J = 9.0 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 8.17 (s, 1H), 8.41 (s, 1H), 9.36 (s, 1H) ppm.

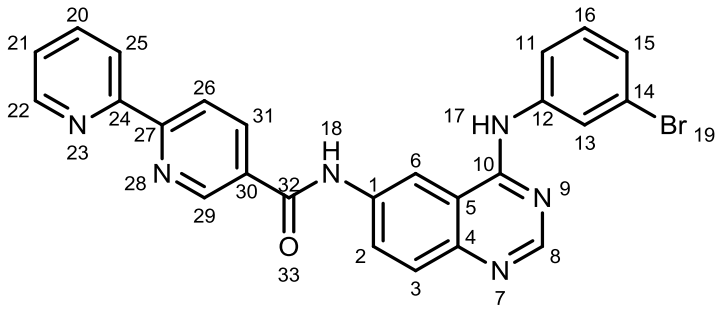
***N*^{6}-(2-aminoethyl)-*N*^{4}-(3-bromophenyl)quinazoline-4,6-diamine dihydrochloride (L2).** To a solution of **B** (614 mg, 1.3 mmol) in EtOH (13.5 ml) was added conc. HCl at elevated temperature and the reaction mixture was refluxed for 4 h. The solution was cooled to room temperature and stored for 10 min at 4°C. The deep yellow precipitate was filtered, washed with EtOH and dried *in vacuo*. Yield: 462 mg (82%). Anal. Calcd for $C_{16}H_{16}BrN_5 \cdot 2HCl$ (M_r = 431.16 g/mol): C, 44.57; H, 4.21; N, 16.24. Found: C, 44.54; H, 4.35; N, 16.25. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 3.05–3.08 (m,

2H, H21), 3.63 (t, ^3J = 5.8 Hz, 2H, H20), 7.18 (s, 1H, H18), 7.45 (dd, ^3J = 8.0 Hz, ^3J = 8.0 Hz, 1H, H16), 7.50–7.53 (m, 2H, H2, H15), 7.78 (d, ^3J = 9.0 Hz, 1H, H3), 7.88 (d, ^3J = 8.0 Hz, 1H, H11), 8.02 (s, 1H, H6), 8.13 (s, 1H, H13), 8.27 (s, 3H, H22), 8.77 (s, 1H, H8), 11.54 (s, 1H, H17) ppm. ^{13}C NMR (125.75 MHz, DMSO-d_6): δ 37.6 (C21), 40.6 (C20), 99.1 (C6), 115.9 (C5), 121.1 (C3), 121.5 (C14), 124.3 (C11), 127.0 (C2), 127.8 (C13), 129.2 (C15), 130.9 (C16), 131.2 (C4), 139.2 (C12), 146.6 (C8), 149.2 (C1), 158.8 (C10) ppm.



***N*-{4-[(3-bromophenyl)amino]quinazolin-6-yl}-[2,2'-bipyridine]-5-carboxamide (L3).** Compound **A** (128.8 mg, 0.41 mmol) was dissolved in DMF (8 ml) and 2,2'-bipyridine-5-carboxylic acid (90.0 mg, 0.45 mmol), 1-Hydroxybenzotriazol hydrate (HOBt · H_2O; 125.2 mg, 0.82 mmol), *O*-(Benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium tetrafluoroborat (TBTU; 262.5 mg, 0.82 mmol) and DIPEA (157 µl, 0.90 mmol) were added. The reaction mixture was stirred for 17 h at room temperature. DMF was removed under reduced pressure and the residue diluted with EtOAc (40 ml) and sat. $NaHCO_3$ (20 ml). The suspension was sonicated for 5 min and the precipitate filtered and washed with H_2O and EtOAc. The crude product was dissolved in a mixture of hot THF (15 ml) and H_2O (10 ml) and refluxed for 2 h, subsequently cooled to room temperature and stored at 4°C over night. The off-white precipitate was filtered, washed with H_2O and EtOAc and dried *in vacuo*. After several hours more product precipitated out of the filtrate, which was filtered again, washed with H_2O and EtOAc and dried *in vacuo*. Yield: 130.7 mg (60%). Anal. Calcd for $C_{25}H_{17}BrN_6O$ · $2H_2O$ (M_r = 533.38 g/mol): C, 56.30; H, 3.97; N, 15.76. Found: C, 56.16; H, 3.63; N, 15.59. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 7.31 (d, ^3J = 8.0 Hz, 1H, H15), 7.37 (dd, ^3J = 8.0 Hz, ^3J = 8.0 Hz, 1H, H16), 7.55 (ddd, ^4J = 0.9 Hz, ^3J = 4.8 Hz, ^3J = 7.4 Hz, 1H, H21), 7.88 (d, ^3J = 8.9 Hz, 1H, H3), 7.91 (d, ^3J = 8.0 Hz, 1H, H11), 8.02–8.07 (m, 2H, H2, H20), 8.22 (s, 1H, H13), 8.50 (d, ^3J = 7.9 Hz, 1H, H25), 8.56–8.61 (m, 2H, H26, H31), 8.64 (s, 1H, H8),

8.77 (d, ^{3}J = 4.8 Hz, 1H, H22), 8.96 (s, 1H, H6), 9.32 (s, 1H, H29), 9.99 (s, 1H, H17), 10.90 (s, 1H, H18) ppm. ^{13}C NMR (125.75 MHz, DMSO-d_6): δ 114.3 (C6), 115.8 (C5), 120.6 (C26), 121.3 (C11), 121.6 (C14), 121.7 (C25), 124.8 (C13), 125.4 (C21), 126.4 (C15), 128.8 (C2), 128.9 (C3), 130.4 (C30), 130.8 (C16), 136.9 (C1), 137.2 (C31), 138.0 (C20), 141.6 (C12), 147.6 (C4), 149.2 (C29), 150.1 (C22), 153.9 (C8), 154.8 (C24), 157.9 (C10), 158.2 (C27), 164.4 (C32) ppm.



Sodium hexanitrocobaltate(III) (C). Sodium nitrite (60 g, 0.87 mol) was dissolved in H_2O (60 ml) at 70°C and cobalt(II) nitrate hexahydrate (20 g, 69 mmol) was added in portions. Afterwards HOAc (50%, 20 ml) was added drop-wise and air was bubbled through the reaction mixture for 30 min. At 0°C EtOH (100 ml) was added to the mixture which yielded in an orange precipitate which was filtered, washed with EtOH and Et_2O and dried *in vacuo*. Yield: 25.4 g (91%).

***cis*-Sodium bis(2,4-pentanedionato)dinitrocobaltate(III) (D).** To a stirred solution of **C** (2.0 g, 5.0 mmol) in H_2O (6.5 ml) was added a solution of NaOH (416 mg, 10.4 mmol) and acetylacetone (1.06 ml, 10.4 mmol) in H_2O (6.5 ml). The mixture was stirred for 5 min and filtered. The filtrate was allowed to stand overnight and the formed precipitate was filtered and washed with acetone and Et_2O. The crude product was dissolved in a minimum amount of H_2O and filtered into a sat. sodium nitrite solution of the same volume. A red precipitate was formed, which was filtered, washed with EtOH and Et_2O and dried *in vacuo*. Yield: 801 mg (44%). Anal. Calcd for $C_{10}H_{14}CoN_2O_8Na$ (M_r = 372.15 g/mol): C, 32.27; H, 3.79; N, 7.53. Found: C, 32.37; H, 3.76; N, 7.26. ^{1}H NMR (500.1 MHz, D_2O): δ 2.11 (s, 6H), 2.20 (s, 6H), 5.66 (s, 1H), 5.77 (s, 1H) ppm.

Bis(2,4-pentanedionato) N^6-(2-aminoethyl)- N^4-(3-bromophenyl) quinazoline-4,6-diamine cobalt(III) hexafluorophosphate (1a). Complex **D** (164 mg, 0.44 mmol) was dissolved in H_2O (2.5 ml) and MeOH (2.5 ml). **L2** (200 mg, 0.46 mmol) in H_2O (1.7 ml) and MeOH (4 ml) was neutralized with NaOH (37 mg, 0.93 mmol) and added with activated charcoal (108 mg) to the cobalt complex solution. The resulting mixture was stirred for 20 min at room temperature, filtered through celite which was washed with small amounts of MeOH. NH_4PF_6 (400 mg, 2.5 mmol) in H_2O (500 µl) was added to the filtrate and the solution was stored at 0°C for 1 h and overnight at 4°C. Crude product was precipitated by the addition of H_2O (10 ml). The formed dark green residue was filtered off and washed with ice-cold H_2O and Et_2O. Further purification was performed using RP-HPLC (H_2O/MeOH, 5–95% MeOH, without formic acid or TFA to avoid counter ion exchange). Yield: 79 mg (24%). Anal. Calcd for $C_{26}H_{30}BrCoF_6N_5O_4P$ (M_r = 760.35 g/mol): C, 41.07; H, 3.98; N, 9.21. Found: C, 40.76; H, 4.04; N, 9.07. ESI-MS in MeOH (positive): *m/z* 614, ($[Co(acac)_2(\mathbf{L2})]^+$); 514, ($[Co(acac)(\mathbf{L2}) - H]^+$). ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 1.63 (s, $3H_a$, CH_3), 1.74 (s, $3H_b$, CH_3), 1.96 (s, $3H_a$, CH_3), 1.99 (s, $3H_a$, CH_3 and s, $3H_b$, CH_3), 2.09 (s, $3H_b$, CH_3), 2.25 (s, $3H_a$, $3H_b$, CH_3), 2.67–2.83 (m, $2H_a$, $2H_b$), 2.84–2.98 (m, $1H_a$, $1H_b$), 3.59–3.68 (m, $1H_a$), 3.69–3.79 (m, $1H_b$), 4.77 (s, $1H_a$, CH_{acac}), 5.54 (s, $1H_b$, CH_{acac}), 5.56–5.64 (m, $1H_a$, $1H_b$, NH_2), 5.68 (s, $1H_a$, CH_{acac}), 5.74 (s, $1H_b$, CH_{acac}), 5.82–5.94 (m, $1H_a$, $1H_b$, NH_2), 7.28 (d, $1H_b$), 7.35–7.44 (m, $2H_a$, $2H_b$), 7.49 (d, $1H_a$), 7.66 (d, $1H_b$), 7.70–7.76 (m, $1H_a$, $1H_b$), 7.77–7.84 (m, $1H_a$, $1H_b$), 8.04–8.17 (m, $3H_a$, $1H_b$), 8.61–8.76 (m, $1H_a$, $2H_b$), 9.89 (s, $1H_a$), 10.04 (s, $1H_b$) ppm ($_a$ and $_b$ refer to the two observed signal sets, a distinct assignment was not possible). ^{13}C NMR (125.75 MHz, DMSO-d_6): δ 26.2, 26.4 (3C), 26.7 (2C), 26.9, 27.1, 42.4, 42.5, 51.7, 52.7, 96.4, 98.2, 98.3, 98.5, 113.8, 115.7, 116.0, 121.7 121.8, 121.9, 129.4, 125.3 (2C), 125.4, 127.0 (2C), 127.7 (2C), 130.7, 130.8, 131.1 (2C), 141.0, 141.2, 142.6, 143.2, 148.5, 157.4, 157.7, 189.1, 189.5, 189.6 (2C), 189.8 (2C), 190.3 (2C) ppm.

Bis(2,4-pentanedionato) N^6-(2-aminoethyl)- N^4-(3-bromophenyl) quinazoline-4,6-diamine cobalt(III) chloride (1b). Complex **D** (329 mg, 0.884 mmol) was dissolved in H_2O (6.4 ml) and MeOH (4.8 ml). **L2** (400 mg, 0.928 mmol) in H_2O (4 ml) was neutralized with 0.28 M NaOH in MeOH (6.7 ml) and added with activated charcoal (216 mg) to the cobalt complex solution. The resulting mixture was stirred for 1.5 h

at room temperature, filtered through celite which was washed with small amounts of MeOH and H_2O. Brine (30 ml) was added to the filtrate and it was stored at 4°C overnight. The supernatant was decanted and the resulting precipitate was dried *in vacuo*. Another product fraction was collected from the supernatant, obtained by adding another 30 ml of brine and cooling to 4°C for about 2 h. Further purification was performed using RP-HPLC (H_2O/MeOH, 45–95% MeOH, without formic acid or TFA to avoid counter ion exchange). Yield: 344 mg (59%). Anal. Calcd for $C_{26}H_{30}BrClCoN_5O_4 \cdot 0.5H_2O$ (M_r = 659.85 g/mol): C, 47.33; H, 4.74; N, 10.61. Found: C, 47.48; H, 4.63; N, 10.59. ESI-MS in H_2O (positive): *m/z* 614, $([Co(acac)_2(\mathbf{L2})]^+$); 514, $([Co(acac)(\mathbf{L2}) - H]^+$). ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 1.63 (s, $3H_a$, CH_3), 1.73 (s, $3H_b$, CH_3), 1.96 (s, $3H_a$, CH_3), 1.99 (s, $3H_a$, CH_3 and s, $3H_b$, CH_3), 2.09 (s, $3H_b$, CH_3), 2.25 (s, $3H_a$, $3H_b$, $2{\cdot}CH_3$), 2.68–2.83 (m, $2H_a$, $2H_b$), 2.85–2.92 (m, $1H_a$), 2.92–3.00 (m, $1H_b$), 3.66–3.75 (m, $1H_a$), 3.76–3.84 (m, $1H_b$), 4.78 (s, $1H_a$, CH_{acac}), 5.53 (s, $1H_b$, CH_{acac}), 5.55–5.63 (m, $1H_a$, $1H_b$, NH_2), 5.67 (s, $1H_a$, CH_{acac}), 5.73 (s, $1H_b$, CH_{acac}), 5.80–5.90 (m, $1H_a$, $1H_b$, NH_2), 7.31 (dd, $1H_b$), 7.35–7.43 (m, $2H_a$, $2H_b$), 7.49 (dd, $1H_a$), 7.70 (d, $1H_a$, $1H_b$), 7.78 (d, $1H_b$), 7.84–7.89 (m, $1H_a$, $1H_b$), 8.11 (dd, $1H_a$), 8.12–8.17 (m, $1H_a$, $1H_b$), 8.23 (s, $1H_a$), 8.64–8.70 (m, $1H_a$, $2H_b$), 10.06 (s, $1H_a$), 10.16 (s, $1H_b$) ppm ($_a$ and $_b$ refer to the two observed signal sets, a distinct assignment was not possible).

Bis(2,4-pentanedionato) ethylenediamine cobalt(III) hexafluorophosphate ($[Co(acac)_2en]PF_6$). Complex **D** (500 mg, 1.34 mmol) was dissolved in H_2O (7.5 ml) and MeOH (5 ml) and ethylenediamine (94.2 µl, 1.41 mmol) was added with activated charcoal (325 mg). The resulting mixture was stirred for 1 h at room temperature and filtered through celite which was washed with small amounts of H_2O. NH_4PF_6 (759 mg, 4.66 mmol) was added to the filtrate and the solution was stored at 0°C for 1 h and overnight at 4°C. The formed violet crystals were filtered and washed with ice-cold H_2O. Yield: 240 mg (39%). Anal. Calcd for $C_{12}H_{22}CoF_6N_2O_4P$ (M_r = 462.21 g/mol): C, 31.18; H, 4.80; N, 6.06. Found: C, 30.88; H, 4.73; N, 6.19. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 2.05 (s, 6H), 2.08 (s, 6H), 2.36–2.54 (m, 4H), 5.03 (s, 1H), 5.37 (s, 1H), 5.61 (s, 1H) ppm.

Biological Methods

Chemicals for cell culture tests. Erlotinib hydrochloride and all other investigated compounds were dissolved in DMSO. These stock solutions were further diluted into culture media at the indicated concentrations. The final DMSO concentrations were always less than 1%.

Cell culture. The human NSCLC cell lines A431 (wt EGFR-overexpressing and erlotinib-sensitive), Calu3 (wt EGFR-expressing and erlotinib-sensitive), and H1975 (EGFR mutation at L858R as well as T790M and erlotinib-resistant) were purchased from American Type Culture Collection (ATCC), Rockville, MD, USA and cultivated unless otherwise indicated in humidified incubators (37°C, 21% O_2, 5% CO_2) in RPMI 1640 medium (Calu3 in MNP medium) containing 10% fetal calf serum (PAA, Linz Austria). Cell cultures were periodically checked for *mycoplasma* contamination.

Cytotoxicity assays. Cells were plated (2×10^3 cells/well) in 96-well plates and allowed to recover for 24 h. Subsequently, the dissolved drugs were added. After 72 h drug exposure, the proportion of viable cells was determined by MTT assay following the manufacturer's recommendations (EZ4U, Biomedica, Vienna, Austria). Cytotoxicity was expressed as IC_{50} values calculated from full dose-response curves using Graph Pad Prism software.

Hypoxic conditions. Cells were plated in 96- or 6-well plates, respectively, in an HERA cell 150i (Thermo Scientific) with at 1% O_2/ 5% CO_2 level for indicated time points (72 h and 4 h for MTT assays and protein isolation, respectively) before analysis/harvesting.

Fluorescence microscopy. A431 cells were incubated with 10 µM **L2** and **1a** under normoxic and hypoxic conditions, respectively, for 6 h. After incubation, the medium was removed, the cells washed with PBS and microphotographs were taken using UV fluorescence microscopy (Nikon Eclipse Ti microscope with a DAPI filter and a high pressure mercury lamp) and a 20x objective (manual settings for all pictures taken: 300 ms exposure time, 2.4x enhanced).

Kinase Screen. The EGFR wt kinase inhibitory potential of the ligands **L1**, **L2**, and **L3** was evaluated using the SelectScreen® Biochemical Kinase Profiling Service at Life Technologies (Thermo Fisher Scientific, Madison, USA). The test compounds were screened in a final concentration of 1% DMSO using the Z'-LYTE® Assay.

Western blot. To assess the impact of our new drugs on the EGFR signaling pathway, cells were starved by serum deprivation for 24 h before treatment to reduce the impact of other tryosine kinase receptors like PDGFR or IGFR. Then cells were incubated for 4 h with the drugs. To assure EGF-dependent activation of the signaling pathway, EGFR stimulation was induced 15 min before protein isolation with 50 ng/ml EGF. Then, cells were harvested, proteins were isolated, resolved by SDS/PAGE, and transferred onto a polyvinylidene difluoride membrane for Western blotting as previously described (26). The following antibodies were used: EGFR, pEGFR (Tyr1068), ERK1/2 (p44/42 MAPK), and pERK (Thr202/Tyr204) (all polyclonal rabbit - Cell Signaling Technology, Beverly, MA, USA). β-actin was monoclonal mouse AC-15 (Sigma). All primary antibodies were used in 1:1000 dilutions. Additionally, horseradish peroxidase-labeled secondary antibodies from Santa Cruz Biotechnology were used at working dilutions of 1:10000.

Animals. Six- to 8-week-old female CB-17 scid/scid (SCID) mice were purchased from Harlan Laboratories (San Pietro al Natisone, Italy). The animals were kept in a pathogen-free environment and every procedure was done in a laminar airflow cabinet. The experiments were done according to the regulations of the Ethics Committee for the Care and Use of Laboratory Animals at the Medical University Vienna (proposal number BMWF-66.009/0084-II/3b/2013), The U.S. Public Health Service Policy on Human Care and Use of Laboratory Animals as well as the United Kingdom Coordinating Committee on Cancer Prevention Research's Guidelines for the Welfare of Animals in Experimental Neoplasia.

Xenograft experiments. For the local tumor growth experiments, A431 and Calu3 cells (1×10^6) were injected subcutaneously into the right flank. Animals were randomly assigned to treatment groups and therapy was started when tumor nodules were palpable. Animals were treated with erlotinib (orally 25 mg/kg dissolved in Cremophor EL diluted 1:1 in 96% ethanol and then diluted 1:10 with deionized water right before administration; A431: five times a week for two weeks day 6–10 and 13–17; Calu3: five times a week for three weeks day 10–14, 17–21, and 24–28) or **1b** (either intravenously 5 mg/kg on day 6, 8, 10, 13, 15, and 17 or intraperitoneally 25 mg/kg dissolved in 0.5% NaCl; A431: five times a week for two weeks, day 6–10 and 13–17; ; Calu3: five times a week for three weeks day 10–14,

17–21, and 24–28). Animals in the control group received the Cremophor EL solvent orally and NaCl intraperitoneally. Animals were controlled for distress development every day and tumor size was assessed regularly by caliper measurement. Tumor volume was calculated using the formula: (length × width^2) / 2.

Statistics. All data are expressed as mean ± S.D.. Results were analyzed and illustrated with GraphPad Prism (version 5; GraphPad Software, San Diego, CA). Statistical analyses were performed using one and two-way ANOVA with drug treatment and time as independent variables, and conducted with Bonferroni post-tests to examine the differences between the different drug treatment regimens and the diverse responses. The statistical significance is either described in the respective figure legends, or indicated with asterisks (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Supplementary Figures

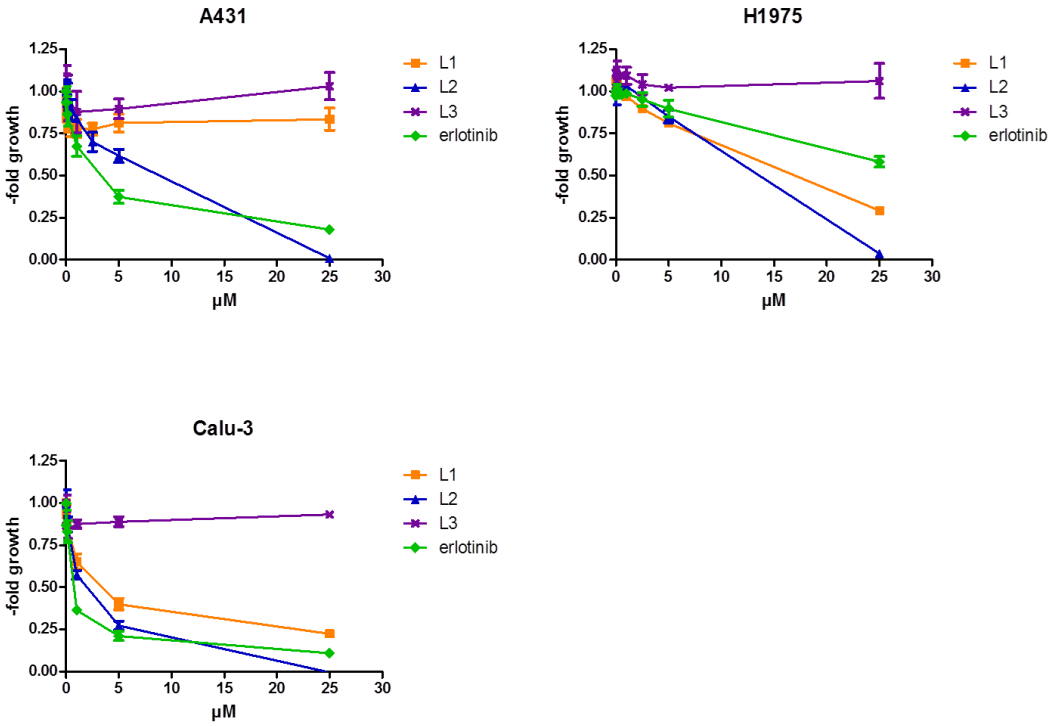


Figure S1. Viability of A431, H1975, and Calu3 cells measured with MTT assay after 72 h treatment with **L1**, **L2**, **L3,** and erlotinib. Values given are means ± standard deviations of one representative experiment (out of at least three experiments delivering comparable results) performed in triplicates.

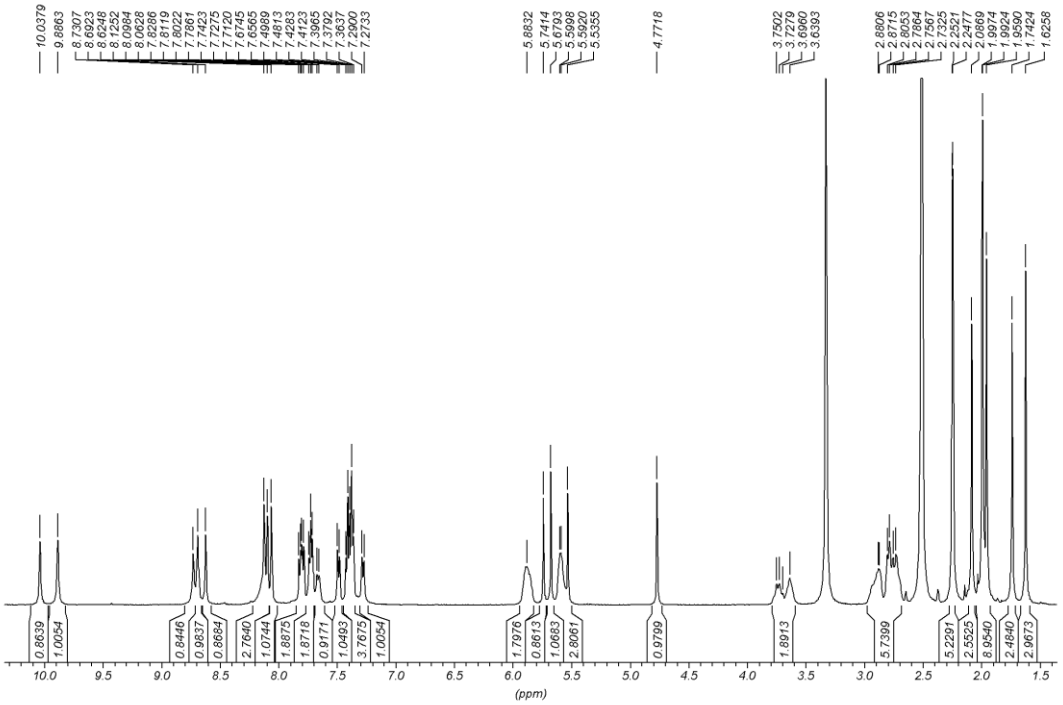


Figure S2. ^{1}H-NMR spectrum of complex **1a** in d_6-DMSO.

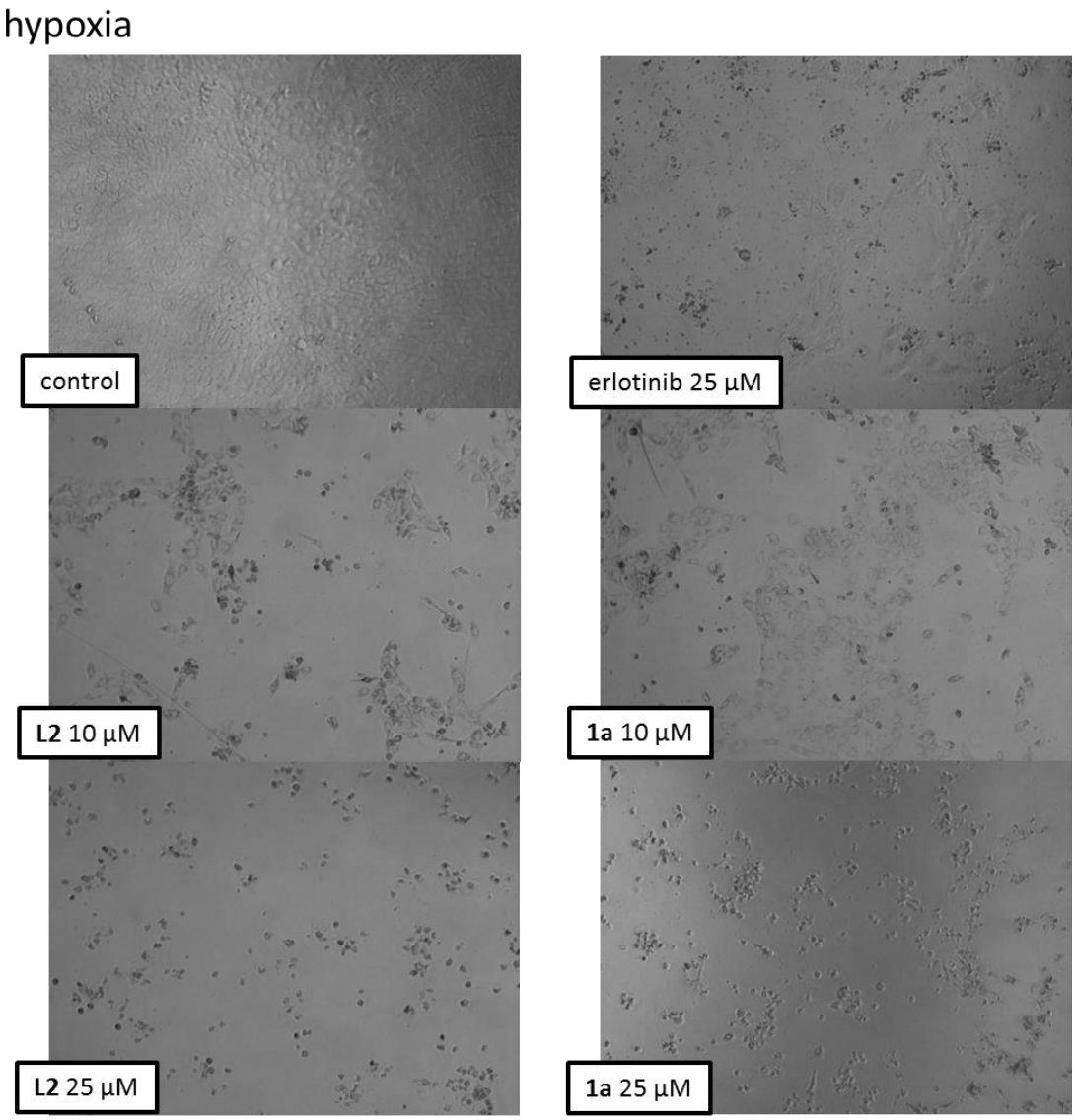


Figure S3. Impact of hypoxia on anticancer activity of erlotinib, **L2**, and **1a**. A431 cells were treated with **L2**, **1a**, and erlotinib under hypoxic conditions for 72 h. Photomicrographs were taken with a 20x objective with phase contrast settings on a Nikon Eclipse Ti microscope.

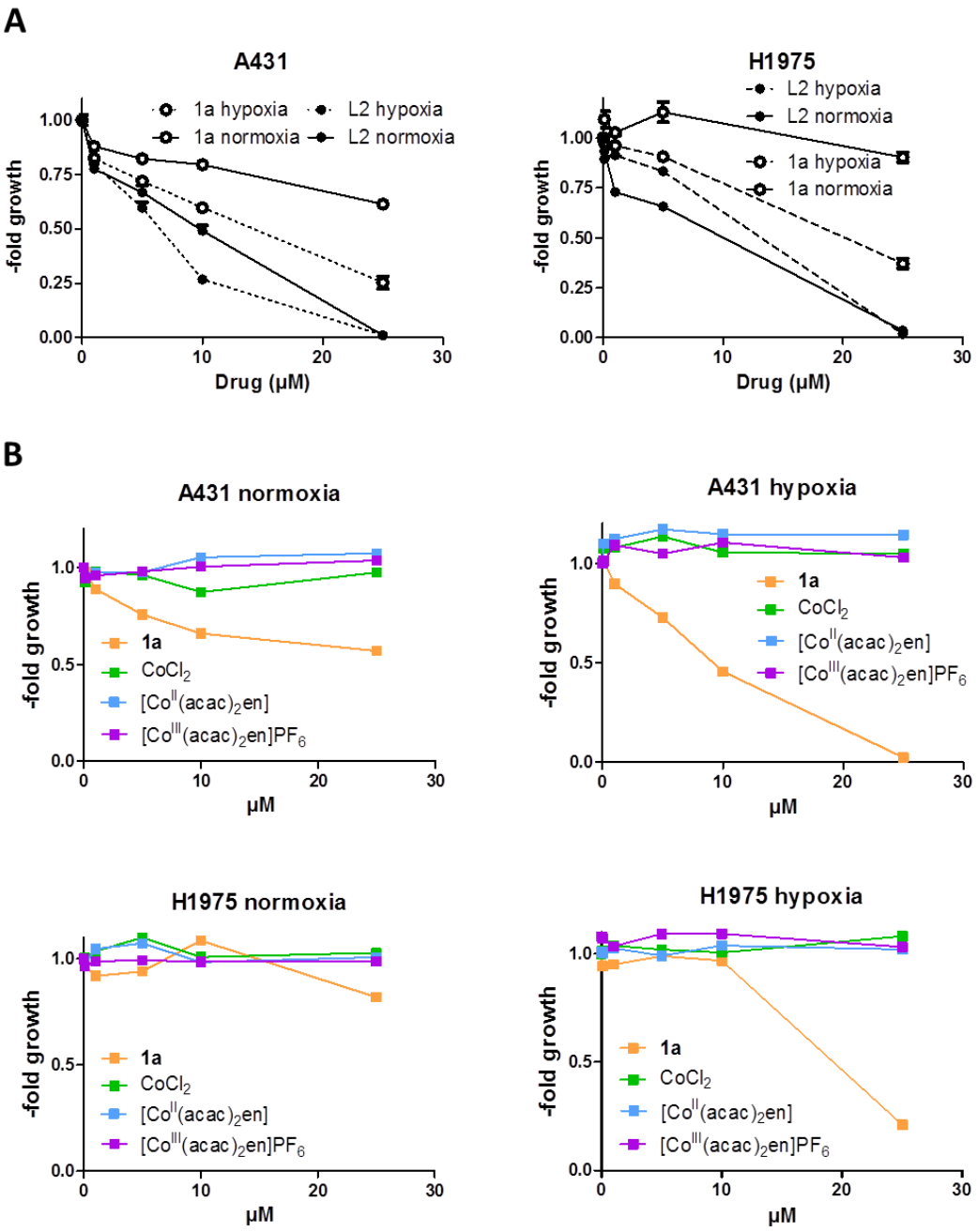


Figure S4. Impact of hypoxic and normoxic conditions on the anticancer activity of A) **L2** vs. **1a** and B) $CoCl_2$, $[Co^{II}(acac)_2en]$ and $[Co^{III}(acac)_2en]PF_6$ vs. **1a**. A431 (EGFR-overexpressing) and H1975 (overexpression of EGFR with a T790M mutation) cells were incubated with the indicated drugs under hypoxia or normoxia for 72 h. Cell viability was measured using a MTT-based system. Values given are means ± standard deviation (SD) of one representative experiment performed in triplicates.

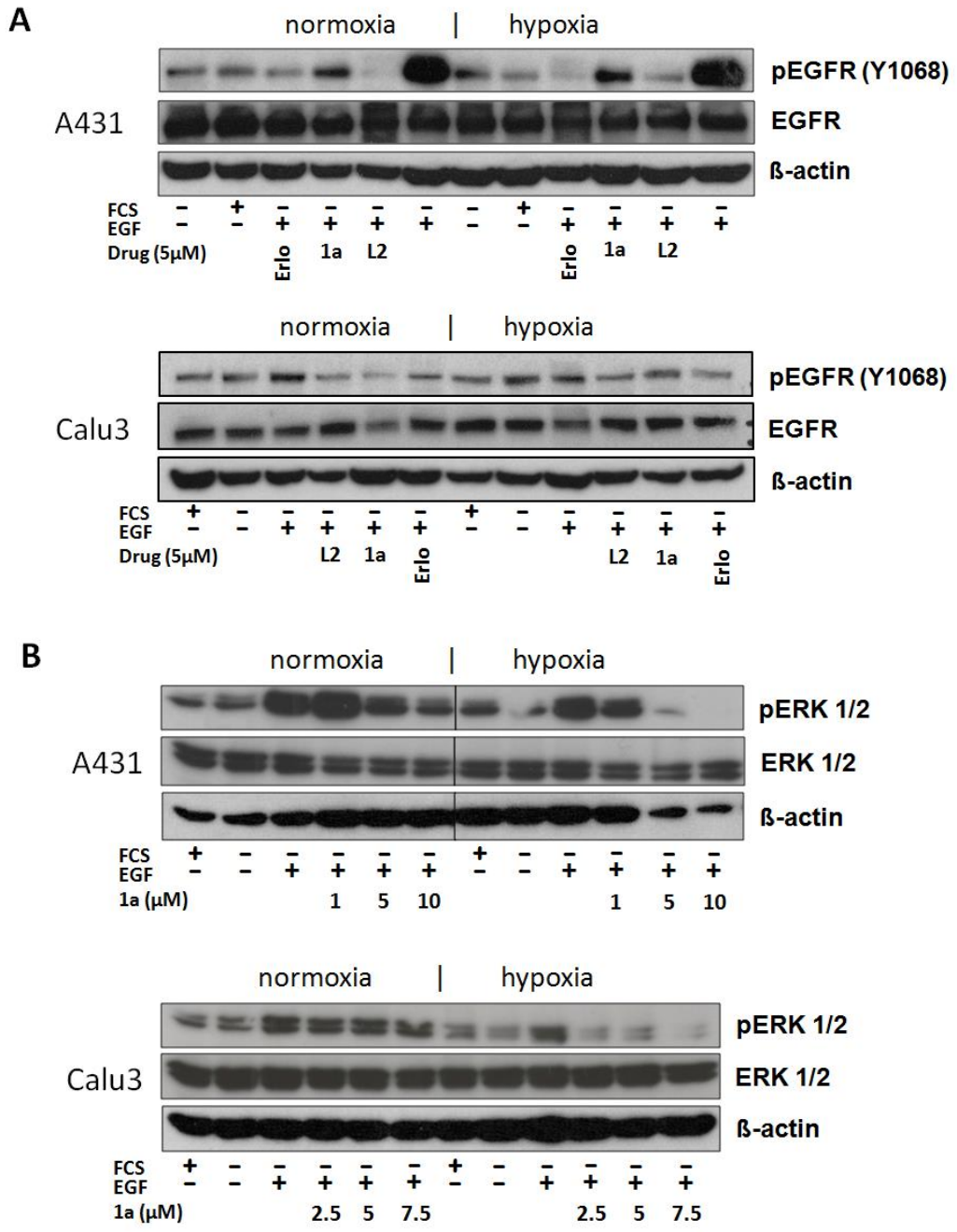


Figure S5. Impact of hypoxia on the EGFR-inhibitory potential of **L2**, **1a**, and erlotinib. A) A431 and Calu3 cells were grown in medium with (+) or without (-) FCS and treated with 5 µM of the indicated drugs for 4 h. After EGFR stimulation with 50 ng/ml EGF for 15 min, cells were harvested, lysed, and phosphorylation levels of EGFR (on tyrosine 1068) as well as total EGFR determined by Western blotting. B) A431 and Calu3 were treated for 4 h with the indicated concentrations of **1a**, while being starved and stimulated as described under A). Phosphorylation levels of ERK1/2 as well as total ERK 1/2 were determined by Western blotting.

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