Title

Systematically altering the lipophilicity of rhenium(I) tricarbonyl anticancer agents to tune the rate at which they induce cell death.

Journal

Dalton Transactions, 49(45)

Authors

Konkankit, Chilaluck

Vaughn, Brett

Huang, Zhouyang

et al.

Publication Date

2020-11-25

DOI

10.1039/d0dt01097a

Peer reviewed

HHS Public Access

Author manuscript

Dalton Trans. Author manuscript; available in PMC 2021 November 25.

Published in final edited form as:

Dalton Trans. 2020 November 25; 49(45): 16062–16066. doi:10.1039/d0dt01097a.

Systematically Altering the Lipophilicity of Rhenium(I) Tricarbonyl Anticancer Agents to Tune the Rate at Which They Induce Cell Death

Chilaluck C. Konkankit[†], **Brett A. Vaughn**[§], **Zhouyang Huang**[†], **Eszter Boros**[§], **Justin J. Wilson**[*,†]

[†]Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853, United States.

[§]Department of Chemistry, Stony Brook University, Stony Brook, New York 11794, United States.

Abstract

Rhenium-based anticancer agents have arisen as promising alternatives to conventional platinum-based drugs. Based on previous studies demonstrating how increasing lipophilicity improves drug uptake within the cell, we sought to investigate the effects of lipophilicity on the anticancer activity of a series of six rhenium(I) tricarbonyl complexes. These six rhenium(I) tricarbonyl structures, called **Re-Chains**, bear pyridyl imine ligands with different alkyl chains ranging in length from two to twelve carbons. The cytotoxicities of these compounds were measured in HeLa cells. At long timepoints (48 h), all compounds are equally cytotoxic. At shorter time points, however, the compounds with longer alkyl chains are significantly more active than those with smaller chains. Cellular uptake studies of these compounds show that they are taken up via both passive and active pathways. Collectively, these studies show how lipophilicity affects the rate at which these **Re-Chains** compounds induce their biological activities.

Developing new drugs is an iterative process and requires optimization of lead candidates to improve their biological efficacies. Several factors contribute to the success of potential drug candidates and are addressed during this optimization process. These characteristics include good solubility, stability, permeability, drug absorption, and pharmacokinetics.[1–5] The lipophilicity of a compound, often measured as an octanol-water partition coefficient (log *P*) value, can have large effects on all of these properties and is, therefore, often modified systematically during these efforts.[6–13] For example, Lipinski's rule of 5, an empirical set of guidelines for identifying molecules with "drug-like" properties, requires that drug candidates possess log *P* values of less than five.[14–16] The basis for this rule is likely a consequence of the fact that log *P* values affect the cellular uptake, cytotoxic potency, and protein-binding of drug candidates.[1,6,7,17–19] Log *P* values that exceed five may potentially lead to increased activity and enhanced liver and lung uptake, resulting in diminished selectivity and off-target side effects.[20]

[*]Corresponding Author: jjw275@cornell.edu.

Conflict of interest

The authors declare no competing financial interests.

Primarily motivated by the success of cisplatin and related platinum-containing drugs, a large number of efforts in recent years focused on developing new metal-based anticancer agents.[21] Similar to conventional organic drug candidates, these metal-containing compounds have biological activities that are modulated by their relative lipophilicities. The introduction of variable-size alkyl chains in metal complexes to systematically alter their lipophilicities, for example, has given rise to promising complexes of platinum[22–25] for anticancer therapy. Complexes of the third-row transition metal, rhenium, and its radioactive congener, technetium-99m (^{99m}Tc), have also been studied in this context, and in some cases their lipophilicities have been correlated to their cytotoxic activities.[26–28] Collectively, these studies highlight how transition metal compounds, like conventional organic drug candidates, can be modified to tune their biological properties.

Based on our group's prior investigations on the anticancer potential of rhenium(I) tricarbonyl ($Re(CO)_3$) complexes,[29–34] we sought to evaluate how systematically altering the lipophilicity of this class of compounds affects biological activity. To explore this hypothesis, a series of $Re(CO)_3$ complexes bearing pyridyl imine Schiff-base ligands with pendent alkyl chains ranging from two to twelve carbons was prepared. Our evaluation of their cytotoxic activity and cellular uptake in HeLa cells revealed that the more lipophilic compounds were able to trigger cell death more rapidly than their hydrophilic analogues. This study provides an unusual direct example of how compound lipophilicity can affect *the rate* at which a compound induces its biological activity and highlights how time-dependent measurements may give valuable insight on the investigation of new drug candidates.

Our efforts to prepare a series of $Re(CO)_3$ compounds with varying linear carbon chain lengths was motivated by a related previous study.[27] In this prior study, $Re(CO)_3$ complexes bearing axial alkylimidazole ligands were investigated in different biological models. Notably, it was observed that compounds with longer alkyl chains have increased cytotoxic activity in the anaerobically grown aerotolerant protistan fish parasite, *spironucleus vortens*, cells. To build upon these prior efforts, we sought to test the role of having long carbon chains on the equatorial diamine, rather than the axial, ligands of these complexes.

To prepare this class of compounds, we used highly modular chemistry, largely developed by the group of Ziegler,[35–44] to prepare $Re(CO)_3$ complexes bearing pyridyl imine complexes with variable length alkyl chains. Following this approach, we mixed $Re(CO)_5Cl$, picolinaldehyde, and variable chain length alkyl amines in refluxing methanol to afford the compounds shown in Scheme 1, collectively referred to as **Re-Chains**. These six compounds generally abide by Lipinski's rule of 5, which dictates that drug-like molecules have less than five hydrogen bond donors, less than ten hydrogen bond acceptors, less than 500 Da in molecular weight, and a log *P* value less than five.[14–16] **Re-C12**, has a molecular weight exceeding 500 Da, but we note that this aspect of Lipinski's rule most likely does not strictly apply to inorganic complexes for which certain metal atoms carry a significantly large portion of the whole molecular mass.

Following the one-pot syntheses of these six complexes, they were characterized by ^{1}H NMR spectroscopy (Fig. S1–S6, ESI), Fourier Transform infrared (FTIR) spectroscopy (Fig. S7–S12, ESI), UV–vis spectroscopy (Fig. S13, ESI), electrospray ionization mass

spectrometry (ESI-MS) (Fig. S14–19, ESI), and elemental analysis (EA). The ^{1}H NMR spectra display a diagnostic imine proton that resonates at 9.23–9.24 ppm, marking an upfield shift from the parent aldehyde at 9.98 ppm. The FTIR spectra reveal three intense C≡O stretching modes, consistent with complexes of C_1 symmetry, in which the two low-energy modes range in energy from 1880 to 1930 cm^{-1} and the high-energy modes range from 2019 to 2027 cm^{-1}. The UV–vis spectral data for the complexes in acetonitrile (MeCN) reveal two prominent electronic transitions: a high-energy peak at 290 nm assigned to the intraligand π–π^* transition and a lower-energy peak at 430 nm assigned to a metal-to-ligand charge transfer (MLCT) transition. The compounds were also characterized by ESI-MS, which predominantly displayed an *m/z* peak corresponding to the $[M–Cl]^+$ ion. Finally, the log *P* values of the $Re(CO)_3$ complexes were determined as water–octanol partition coefficients using the shake-flask method,[45] and the log *P* values were calculated for the free equatorial ligands using the ALOGPS 2.1 program (Table 1).[46,47] As expected, both the complex, **Re-C12**, and its free ligand, **C12**, are the most lipophilic compounds (log *P* = 2.95 and 6.79, respectively), whereas **Re-C2** and **C2** are the least lipophilic (log *P* = 1.59 and 1.45, respectively). We note that the experimentally measured log *P* values for our **Re-Chains** do not differ as greatly as the calculated values for the free ligands. We hypothesize that this discrepancy may arise from time-dependent aquation and hydrolysis processes at the Re centers, which will alter the measured lipophilicity values. Despite the small differences for the **Re-Chains**, these values demonstrate the increase in lipophilic character of the compounds as a consequence of incorporating longer alkyl chains.

Having synthesized and fully characterized the **Re-Chains** compounds, we sought to evaluate their in vitro anticancer activities via dose-escalation studies in HeLa cervical cancer cells. When HeLa cells were treated with these compounds for a 48-h incubation period, all rhenium complexes exhibited 50% growth inhibitory concentration (IC_{50}) values of approximately 15 μM (Fig. 1a), whereas cisplatin has an IC_{50} value of 9.8 μM in the same cell line.[34] This result, showing all six structures to possess equivalent cytotoxic activity, appeared to contrast our hypothesis regarding the role of lipophilicity in mediating the biological properties of this compound class. We reasoned, however, that the lack of differences in cytotoxic activities between these substantially different lipophilic complexes may lie in the rate at which they induce their cytotoxic effects. To test this hypothesis, we treated cells with the **Re-chains** (50 μM) for varying incubation times, allowing recovery time to keep the duration of the assay at 48 h (Fig. 1b). Our results indicate that there is a time-dependence on the cytotoxic activity of these compounds that depends on the alkyl chain length. Notably, more lipophilic compounds with long alkyl chains, like **Re-C12**, induce their cytotoxic effects on a much faster time scale than the less lipophilic analogues. For example, treatment for 6 h with 50 μM **Re-C12** kills >95% of the cells, whereas the other five compounds have no effect. By 48 h, all six compounds decrease cell viability below 30%, consistent with the similar IC_{50} values that we measured at this time point. Thus, these results indicate that lipophilicity does play a role in mediating the cytotoxic activities of these compounds; however, this effect is not readily observed at longer time points. Presumably, long incubation times allow the less lipophilic compounds to accumulate in the cells at equipotent concentrations as the more lipophilic species.

We next measured the cellular uptake of these compounds to explore the role of lipophilicity. HeLa cells were treated with 50 μM **Re-Chains** at both 37 °C and 4 °C for 3 h, after which the cells were harvested, digested and analyzed for rhenium content via inductively coupled plasma optical emission spectroscopy (ICP-OES). The low-temperature (4 °C) incubation was used as a means of shutting down active, or energy-dependent, transport pathways through the cell membrane. The measured cellular uptake of the **Re-Chains** compounds (at both temperatures) is shown in Fig. 2a and 2b. It is apparent from these data that cellular uptake scales proportionally with both the length of the carbon chain of the complex and the ligand log *P* values. The differences in cellular uptake is consistent with the different cytotoxic effects that we see in Fig. 1 for the 3-h time point, confirming that lipophilicity plays a mutually important role in uptake and cytotoxicity. Additionally, cell uptake at 4 °C is notably less than that for 37 °C. These findings suggest that the **Re-Chains** compounds are taken up, at least in part, by active transport. For related metal-based anticancer agents, like $[(\eta^6\text{-}p\text{-cymene})Os^{II}(N,N\text{-dimethylphenylazopyridine})X]^+$ in which X = Cl or I,[48] cellular uptake at 4 °C was diminished by factors of 20–30. In the case of **Re-Chains**, however, we only observe decreases ranging from 2.3–7-fold differences (Table S1, ESI). We interpret that moderate decreases in uptake of **Re-Chains** upon incubation at 4 °C, in comparison to related actively transported metal-based anticancer agents, reflects how passive uptake is their dominant mechanism of uptake. As an alternative explanation, lower uptake could be due to precipitation of this compound at this lower temperature. However, no visible precipitation was observed during these low temperature experiments, leading us to disfavor this hypothesis. Furthermore, even at 4 °C, the cellular uptake of **Re-Chains** still scales linearly with the carbon chain length and calculated ligand log *P* values, suggesting that lipophilicity is important for cellular uptake under both conditions.

In summary, we have prepared a small set of $Re(CO)_3$-diimine complexes bearing varying alkyl chain lengths using a three-component, one-pot reaction. In studying their cytotoxic effects and cellular uptake in HeLa cells, it was found that the more lipophilic compounds induce in vitro anticancer activities at much shorter time points. This result is most likely a consequence of faster cellular uptake kinetics for more lipophilic compounds. Although it has been more commonly noted that lipophilicity of drug candidates affects their biological activity, few studies to date have shown that many of these effects exhibit a time dependence. This observed time dependence on uptake and cytotoxicity, for example, could have important effects in the field of ^{99m}Tc-based radiopharmaceutical agents, for which their short half-lives require that cellular uptake and localization proceed rapidly.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This research was supported by the College of Arts and Sciences at Cornell University, the Cornell Technology Licensing Office Cornell Technology Acceleration and Maturation (CTAM) fund, and by the Office of the Assistant Secretary of Defense for Health Affairs through the Ovarian Cancer Research Program under award no. W81XWH-17-1-0097. This work made use of the NMR facility at Cornell University, which is in-part supported by the NSF under award number CHE-1531632. Prof. E. Boros received financial support for this work

provided by the National Institutes of Health (NIH) via a Pathway to Independence Award (NHLBI R00HL125728) and Stony Brook University for startup funds.

References

1. Arnott JA and Planey SL, Expert Opin. Drug Discov, 2012, 7, 863–875. [PubMed: 22992175]
2. Neidle S, Cancer Drug Design and Discovery, Elsevier, London, United Kingdom, 2008.
3. Veber DF, Johnson SR, Cheng H-Y, Smith BR, Ward KW and Kopple KD, J. Med. Chem, 2002, 45, 2615–2623. [PubMed: 12036371]
4. Van de Waterbeemd H, Smith DA, Beaumont K and Walker DK, J. Med. Chem, 2001, 44, 1313–1333. [PubMed: 11311053]
5. Pliška V, Testa B, van de Waterbeemd H and Mannhold R, Lipophilicity in Drug Action and Toxicology, Wiley, Weinheim, Germany, 1996.
6. Liu X, Testa B and Fahr A, Pharm. Res, 2011, 28, 962–977. [PubMed: 21052797]
7. Waring MJ, Bioorganic Med. Chem. Lett, 2009, 19, 2844–2851.
8. Gleeson MP, Hersey A, Montanari D and Overington J, Nat. Rev. Drug Discov, 2011, 10, 197–208. [PubMed: 21358739]
9. Hughes JD, Blagg J, Price DA, Bailey S, DeCrescenzo GA, Devraj RV, Ellsworth E, Fobian YM, Gibbs ME, Gilles RW, Greene N, Huang E, Krieger-Burke T, Loesel J, Wager T, Whiteley L and Zhang Y, Bioorganic Med. Chem. Lett, 2008, 18, 4872–4875.
10. Johnson TW, Gallego RA and Edwards MP, J. Med. Chem, 2018, 61, 6401–6420. [PubMed: 29589935]
11. Leeson PD and Springthorpe B, Nat. Rev. Drug Discov, 2007, 6, 881–890. [PubMed: 17971784]
12. Leeson PD and Davis AM, J. Med. Chem, 2004, 47, 6338–6348. [PubMed: 15566303]
13. Testa B, Crivori P, Reist M and Carrupt P-A, Perspect. Drug Discov. Des, 2000, 19, 179–211.
14. Benet LZ, Hosey CM, Ursu O and Oprea TI, Adv. Drug. Deliv. Rev, 2016, 101, 89–98. [PubMed: 27182629]
15. Lipinski CA, Lombardo F, Dominy BWand Feeney PJ, Adv. Drug Deliv. Rev, 2001, 46, 3–26. [PubMed: 11259830]
16. Goodwin RJA, Bunch J and McGinnity DF, in Advances in Cancer Research, ed. Tew KD, Elsevier Inc., 1st edn., 2017, vol. 134, pp. 133–171. [PubMed: 28110649]
17. Waring MJ, Expert Opin. Drug Discov, 2010, 5, 235–248. [PubMed: 22823020]
18. Moridani MY, in Prodrugs and Targeted Delivery: Towards Better ADME Properties, ed. Rautio J, Wiley-VCH Verlag GmbH & Co., Weinheim, Germany, 2011, pp. 79–109.
19. Freeman-Cook KD, Hoffman RL and Johnson TW, Future Med. Chem, 2013, 5, 113–115. [PubMed: 23360135]
20. Gao Y, Gesenberg C and Zheng W, in Developing Solid Oral Dosage Forms, ed. Jones Kristine, Academic Press Inc. (London) Ltd., 2nd edn., 2017, pp. 455–495.
21. Chellan P and Sadler PJ, Phil. Trans. R. Soc. A, 2015, 373, Article ID: 20140182.
22. Abu Ammar A, Raveendran R, Gibson D, Nassar T and Benita S, J. Med. Chem, 2016, 59, 9035–9046. [PubMed: 27603506]
23. Awuah SG, Zheng Y-R, Bruno PM, Hemann MT and Lippard SJ, J. Am. Chem. Soc, 2015, 137, 14854–14857. [PubMed: 26561720]
24. Zheng Y-R, Suntharalingam K, Johnstone TC, Yoo H, Lin W, Brooks JG and Lippard SJ, J. Am. Chem. Soc, 2014, 136, 8790–8798. [PubMed: 24902769]
25. Johnstone TC and Lippard SJ, Inorg. Chem, 2013, 52, 9915–9920. [PubMed: 23859129]
26. Balasingham RG, Thorp-Greenwood FL, Williams CF, Coogan MP and Pope SJA, Inorg. Chem, 2012, 51, 1419–1426. [PubMed: 22263612]
27. Hallett AJ, Placet E, Prieux R, McCafferty D, Platts JA, Lloyd D, Isaacs M, Hayes AJ, Coles SJ, Pitak MB, Marchant S, Marriott SN, Allemann RK, Dervisi A and Fallis IA, Dalton Trans, 2018, 47, 14241–14253. [PubMed: 29789819]

28. Boros E, Häfeli UO, Patrick BO, Adam MJ and Orvig C, Bioconjug. Chem, 2009, 20, 1002–1009. [PubMed: 19341277]
29. Knopf KM, Murphy BL, MacMillan SN, Baskin JM, Barr MP, Boros E and Wilson JJ, J. Am. Chem. Soc, 2017, 139, 14302–14314. [PubMed: 28948792]
30. Marker SC, MacMillan SN, Zipfel WR, Li Z, Ford PC and Wilson JJ, Inorg. Chem, 2018, 57, 1311–1331. [PubMed: 29323880]
31. Konkankit CC, King AP, Knopf KM, Southard TL and Wilson JJ, ACS Med. Chem. Lett, 2019, 10, 822–827. [PubMed: 31098006]
32. King AP, Marker SC, Swanda RV, Woods JJ, Qian S-B and Wilson JJ, Chem. Eur. J, 2019, 25, 9206–9210. [PubMed: 31090971]
33. Murphy BL, Marker SC, Lambert VJ, Woods JJ, MacMillan SN and Wilson JJ, J. Organomet. Chem, 2020, 907, 121064–12071.
34. Konkankit CC, Vaughn BA, MacMillan SN, Boros E and Wilson JJ, Inorg. Chem, 2019, 58, 3895–3909. [PubMed: 30793900]
35. Hasheminasab A, Wang L, Dawadi MB, Bass J, Herrick RS, Rack JJ and Ziegler CJ, Dalton Trans, 2015, 44, 15400–15403. [PubMed: 26252161]
36. Hasheminasab A, Engle JT, Bass J, Herrick RS and Ziegler CJ, Eur. J. Inorg. Chem, 2014, 2014, 2643–2652.
37. Hasheminasab A, Rhoda HM, Crandall LA, Ayers JT, Nemykin VN, Herrick RS and Ziegler CJ, Dalton Trans, 2015, 44, 17268–17277. [PubMed: 26374670]
38. Costa R, Chanawanno K, Engle JT, Baroody B, Herrick RS and Ziegler CJ, J. Organomet. Chem, 2013, 734, 25–31. [PubMed: 23976791]
39. Chanawanno K, Engle JT, Le KX, Herrick RS and Ziegler CJ, Dalton Trans, 2013, 42, 13679–13684. [PubMed: 23903568]
40. Qayyum H, Herrick RS and Ziegler CJ, Dalton Trans, 2011, 40, 7442–7445. [PubMed: 21691650]
41. Hasheminasab A, Dawadi MB, Mehr HS, Herrick RS and Ziegler CJ, Macromolecules, 2016, 49, 3016–3027.
42. Chanawanno K, Rhoda HM, Hasheminasab A, Crandall LA, King AJ, Herrick RS, Nemykin VN and Ziegler CJ, J. Organomet. Chem, 2016, 818, 145–153. [PubMed: 28496284]
43. Schrage BR, Herrick RS and Ziegler CJ, J. Organomet. Chem, 2019, 880, 170–174.
44. Chanawanno K, Kondeti V, Caporoso J, Paruchuri S, Leeper TC, Herrick RS and Ziegler CJ, Dalton Trans, 2016, 45, 4729–4735. [PubMed: 26863280]
45. Andrés A, Rosés M, Ràfols C, Bosch E, Espinosa S, Segarra V and Huerta JM, Eur. J. Pharm. Sci, 2015, 76, 181–191. [PubMed: 25968358]
46. Tetko IV and Tanchuk VY, J. Chem. Inf. Model, 2002, 42, 1136–1145.
47. Tetko IV and Bruneau P, J. Pharm. Sci, 2004, 93, 3103–3110. [PubMed: 15514985]
48. Ballesta A, Billy F, Coverdale JPC, Song J-I, Sanchez-Cano C, Romero-Canelón I and Sadler PJ, Metallomics, 2019, 11, 1648–1656. [PubMed: 31528927]

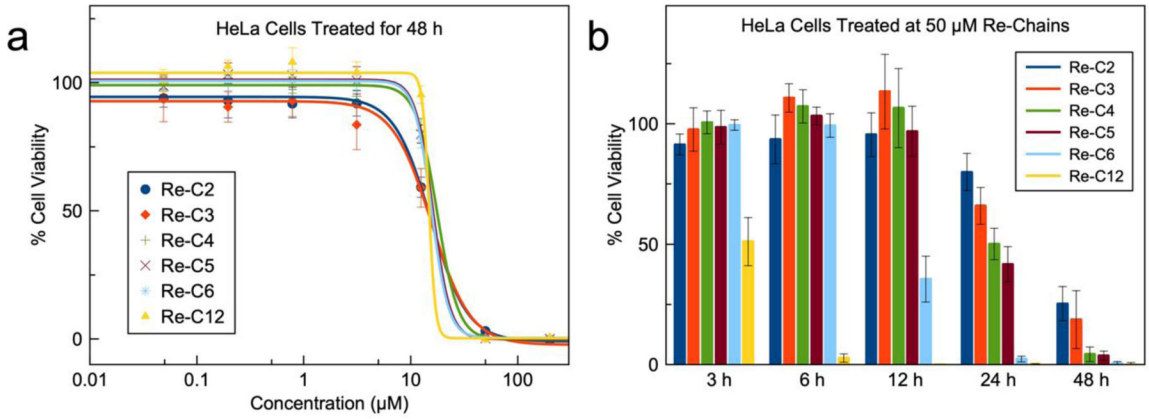


Fig. 1.

(a) Dose-response curves of HeLa cervical cancer cells and (b) time-dependent cell viability studies of HeLa cells treated with **Re-C2** (navy blue), **Re-C3** (red), **Re-C4** (green), **Re-C5** (maroon), **Re-C6** (light blue), **Re-C12** (yellow). The error bars represent the standard deviation from six replicates.

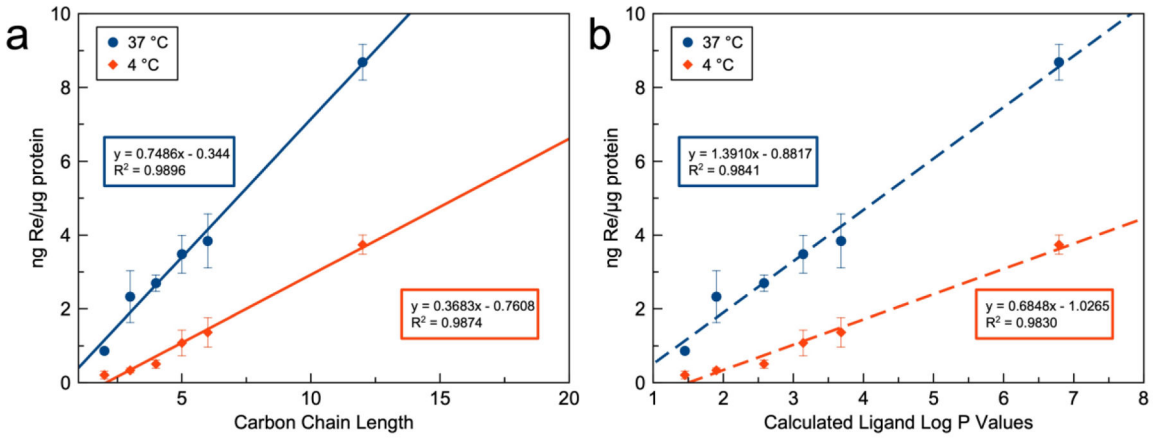
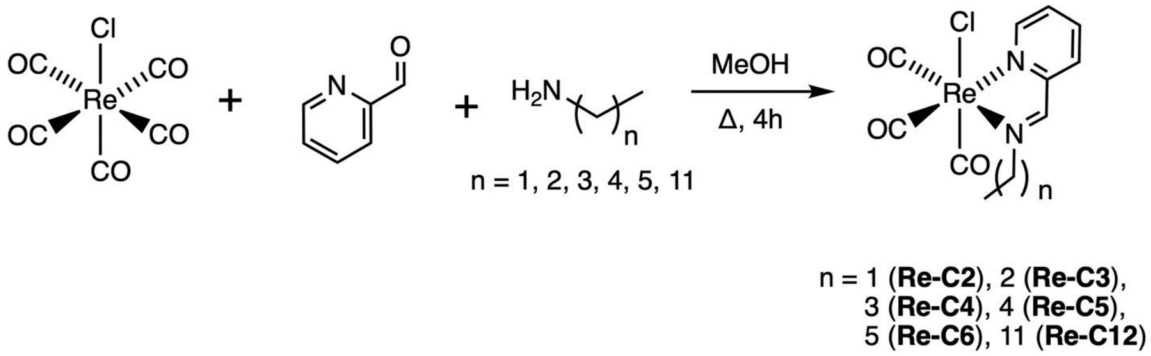


Fig. 2.

Cellular uptake of **Re-Chains** after incubating for 3 h at 37 °C (blue) and 4 °C (red) in relation to (a) carbon chain length and (b) calculated log *P* values for the free ligands. The error bars represent the standard deviation from three replicates.



Scheme 1.
General synthetic approach and structures of **Re-Chains** complexes.

Table 1

Log *P* values of **Re-Chains** and their free ligands.

	Log P^a		Calculated Log P^b
Re-C2	1.59	**C2**	1.45
Re-C3	2.16	**C3**	1.90
Re-C4	2.44	**C4**	2.58
Re-C5	2.80	**C5**	3.14
Re-C6	2.91	**C6**	3.68
Re-C12	2.95	**C12**	6.79

[a]Determined using the shake-flask method after 30 min of mixing octanol and water.

[b]Calculated using the ALOGPS 2.1 software.