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Leonidova, Anna ; Gasser, Gilles

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The Underestimated Potential of Organometallic Rhenium Complexes as Anticancer Agents

Anna Leonidova[a] and Gilles Gasser[a] *

[a] Department of Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich;

email: gilles.gasser@chem.uzh.ch; WWW: www.gassergroup.com

ABSTRACT: In the recent years, organometallic compounds have become recognized as promising anti-cancer drug candidates. While radioactive $^{186/188}$Re compounds are already used in clinics for cancer treatment, cold Re organometallic compounds have mostly been explored as luminescent probes and photosensitizers in photo-catalysis. However, a growing number of studies have recently revealed the potential of Re organometallic complexes as anticancer agents. Several compounds have displayed cytotoxicity equaling or exceeding that of the well-established anti-cancer drug cisplatin. In this review, we present the currently known Re organometallic complexes that have shown anti-proliferative activity on cancer cell lines. A particular emphasis is placed on their cellular uptake and localization as well as their potential mechanism of action.

INTRODUCTION

Despite the growing body of research, cancer remains among the leading causes of death in the world, in particular in high-income countries (although cancer rates are increasing in lower-income countries which already account for 70% of world cancer deaths).[1] The survival rates hugely vary with the type and stage of tumor, as well as age, sex and socio-economic situation of each patient.[1, 2] For example, about 50% of adult (15-99 years old) patients diagnosed with cancer in England and Wales in 2010-2011 are expected to survive for another 10 years or more.[2]

However, while patients with testicular cancer, melanoma, prostate cancer and Hodgkin lymphoma have a ten-year survival rate of over 80%, less than 15% of patients with pancreas, lung, brain, esophagus and stomach cancers survive that long.[2] Cancer treatment depends on the type, stage and location of tumor, but most patients undergo chemotherapy either as the main treatment or in combination with surgery and/or radiotherapy. Over 50% of tumors are treated with platinum-based drugs (cisplatin, carboplatin, oxaliplatin) alone or co-administered with other chemotherapeutic agents.[3] Nevertheless, new anti-cancer drugs are still being actively sought due to the emergence of platinium resistance and the severe side effects associated with the chemotherapic treatments.[4] In the last decades, many organometallic compounds (i.e. compounds containing at least one metal-carbon bond) have proven themselves as extremely promising anti-cancer drug candidates.[5-14] Organometallic complexes offer structural and stereochemical variety,[15, 16] the possibility of rational ligand design, and diverse mechanisms of action (e.g. *via* redox activity, ligand exchange, catalytic activity and/or photo-activity).[17] [18-21]

Compared with some intensively investigated organometallic complexes such as those based on ruthenium,[12, 13] relatively few examples of cytotoxic rhenium organometallic compounds are found in the literature. However, numerous studies have been recently reported describing the potential of such compounds.[22-47] Indeed, Re organometallic compounds possess several intrinsic properties advantageous for the development of novel anti-cancer drug candidates. For instance, many Re organometallic complexes are excellent luminescent probes with long-lived emission states, large Stokes shifts, high quantum yields and emission that can be tuned by varying the ligands.[37, 48] They can thus be easily followed inside cells by conventional and/or time-resolved (e.g. FLIMS) emission microscopy to elucidate their cellular distribution and mechanism of action. Furthermore, all reported cytotoxic Re organometallic compounds are based on the $Re(CO)_3$ core, which is chemically robust and easily accessible, e.g. from $[Re(CO)_3(H_2O)_3]^+$/$[ReBr_3(CO)_3]^{2-}$,[29, 34] $Re(CO)_5Cl$/$Re(CO)_5Br$[26-28, 31-33, 41, 42, 49] or $Re_2(CO)_{10}$[24, 30, 40] in 1-3 step synthesis. The introduced ligands can be designed to incorporate other functional moieties such as targeting biomolecules.[28, 29, 34, 35, 39, 41, 44, 46, 47, 50, 51] However, the key property of the Re organometallic complexes is the possibility to prepare "hot" analogues that can be applied

for radio-imaging as well as for therapy. Besides ^{186}Re and ^{188}Re isotopes, ^{99m}Tc can often be used with the same set of ligands as the cold Re due to the similarity in the coordination chemistry of these group 7 congeners.[52-55] Thus, a Re complex has the potential to be a multi-modal molecule, which acts as a luminescent probe, a radio-imaging and a pharmaceutical agent. Importantly, "hot" analogues can be used to run *in vivo* biodistribution and pharmacokinetics studies, which are crucial for the development of new drugs.

In this review, we present the cytotoxic Re organometallic complexes that have been reported in the literature to date. Their modes of cytotoxic action, when known, are discussed in detail. The compounds presented in this review are categorized into two main groups according to the nature of their anti-proliferative activity, namely "traditional" cytotoxicity and photo-toxicity. Anti-cancer and other therapeutic applications of radio-pharmaceuticals based on $^{186/188}$Re have been extensively described in several excellent reviews.[54, 56-59] Since some of them are extremely recent, this topic is not discussed herein. Of note, two relatively recent and comprehensive reviews by Lo *et al*.[37] and Coogan *et al.*[48] have discussed the cellular uptake, localization and cytotoxicity of luminescent Re(I) organometallic complexes, but both are more focused on imaging applications and do not cover non luminescent organometallic Re compounds. As can been seen in these reviews, several Re complexes have been reported to interact with DNA and/or proteins, yet not all of them have been screened for cytotoxicity. Here we discuss only the Re organometallic compounds that have been studied in cell culture.

CYTOTOXIC Re ORGANOMETALLIC COMPLEXES

Re(I) tricarbonyl bisimine complexes. A large percentage of cytotoxic Re organometallic compounds have been initially developed as luminescent probes and their anti-cancer effect has only been discovered during biocompatibility screenings. Unsurprisingly, the largest family of anti-proliferative Re organometallic compounds is that of [2+1] Re(I) tricarbonyl bisimine complexes $[Re(CO)_3(bisimine)L]$ where L is a monodentate pyridine derivative or a halide (Cl, Br) ligand. These compounds normally possess outstanding photochemical properties and their bisimine or pyridine ligands can be easily derivatized. Typically used bisimine ligands are 2,2'-

bipyridine or phenantroline-based (Figure 1) and, despite apparent similarity, cytotoxicity varies considerably from one derivative to another.

The cytotoxicity of several bipyridine and phenantroline based Re(I) complexes (Figure 1A and B, **1**-**14**) have been reported. Their anti-proliferative activity reached or even exceeded the one of cisplatin on various human cancer cell lines.[28, 30, 32, 39, 40, 45, 49, 51] The cytotoxicity of the compounds generally increased with lipophilicity,[32, 33, 39, 49, 51] most probably due to an improved cellular uptake. Re(I) complexes **9** (Figure 1A) derivatized with a fluorous pendant were the only exception to this trend. Their cytotoxicity diminished with rising lipophilicity.[39]

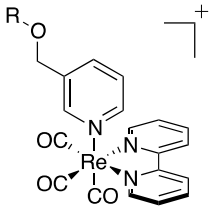
However, these compounds are exceptionally lipophilic ($logP_{o/w}$ in the range of 5 to 9) and could form self-aggregates due to favorable F-F interactions. The uptake of anti-proliferative Re(I) tricarbonyl bisimine complexes is often very fast and efficient, yet little is known about their mechanism. Some cytotoxic cationic complexes such as **1a-c** appear to accumulate in cells *via* passive diffusion.[49] Re luminescent probes used to sense Cd(II) and Zn(II) (**5** on Figure 1A) are, however, taken up *via* an energy-dependent pathway such as endocytosis as demonstrated by temperature and ATP production inhibition uptake assays.[32] Interestingly, fluorescence microscopy cellular localization studies have never – to the best of our knowledge – identified a cytotoxic Re(I) tricarbonyl bisimine complex located inside the nucleus. All compounds screened for cytotoxicity were indeed imaged in the cytoplasm.[28, 32, 33, 39, 51] When co-localization experiments were performed on Re organometallic complexes **7i** and **9f** (Figure 1A), predominately mitochondrial (up to 80%) localization was reported.[39] A non-negligible percentage of endosomal accumulation (about 20%) has also been detected for compound **7i**[39] As a note of caution, although fluorescent microscopy imaging is an important tool in the investigation of the cellular behavior of Re organometallic complexes, their luminescence can sometimes be quenched in cells.[39, 44, 47] Due to this problem, direct measurements of Re content by inductively coupled plasma mass spectrometry (ICP-MS) or atomic absorption spectroscopy (AAS)[60] can be a more accurate method to quantify the cellular uptake and distribution of Re complexes.

The first cytotoxic bisimine Re complex (**15** on Figure 1B) with anti-proliferative properties, however, was in some ways an exception to the typical photo-physical and biological properties of these compounds. Based on a pyridine-triazine ligand (2-amino-4-phenylamino-6-(2-pyridyl)-1,3,5-triazine), compound **15** emitted only in the solid state and was designed in a crescent shape to fit the major or minor grooves of DNA.[27] Experimental evaluation of its interactions with DNA has indeed demonstrated that **15** was non-intercalatively binding to DNA with a preference for AT-rich sequences. Modeling studies suggested the minor groove as binding site. The resulting cytotoxicity was only moderate (IC_{50} of 30-50 μM compared with 10-20 μM IC_{50} for cisplatin) on KB-3-1 (human epidermoid carcinoma), HepG2 (human liver hepatocellular carcinoma) and HeLa (human cervical cancer) and low (195 μM compared with 40 μM for cisplatin) on human multi-drug resistant epidermal carcinoma KB-V-1 cell line. Since the compound was not fluorescent in solution, its localization in cell could not be determined by fluorescence microscopy. Therefore it is unknown whether it reaches cell nucleus or/and mitochondria where it could interact with DNA.

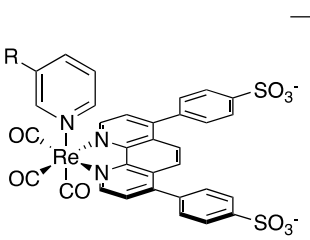
Another Re(I) tricarbonyl diimine compound using neither bipyridine nor phenathroline, but asymmetric azines (**16** on Figure 1B) as bidentate ligands have been reported to be active on human neuroblastoma NB69, glioblastoma astrocytoma U373, breast cancer MRC-7 (hormone-responsive) and EVSA-T (hormone-independent) with IC_{50} in the 4–10 μM range.[31] Some azines are known to possess some anti-cancer activity on their own, but their cytotoxicity was increased by coordination to the Re center. Curiously, $Re(CO)_5Cl$ displayed the same activity as Re(I) azines, while $Re(CO)_5Br$ failed to produce a strong anti-proliferative effect on U2OS (osteosarcoma, >100 μM), HepG2 (>100 μM) and MCF-7 (57 μM) in a different study.[44]

Although a substantial number of Re(I) tricarbonyl bisimine complexes $[Re(CO)_3(bisimine)L]$ have been reported, their mechanism of cytotoxicity has not yet been fully elucidated. In particular, studies on the compounds' stability in biological conditions (e.g. in blood plasma) are lacking. Evaluation of $[Re(CO)_3(bisimine)L]$ complexes' stability could shed some light on their behavior in cells, since, for some of these compounds, the monodentate ligand

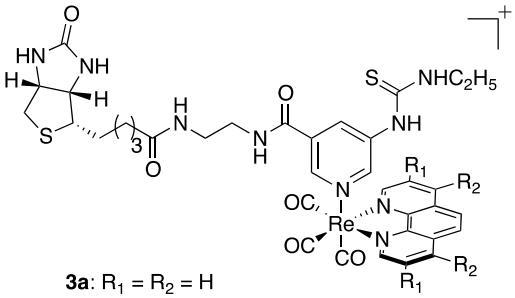
can be easily substituted[51, 61-64] or modified[40] under physiological conditions. For instance, the monodentate pyridine ligand can sometimes be substituted by stronger donor groups (e.g. histidine or cysteine),[61] while alkylcarbonato ligands (compounds **11** and **12** on Figure 1B) are transformed into alkyloxo *via* CO_2 loss in cell culture medium.[40] In fact, [2+1] ^{99m}Tc tricarbonyl pyridine-based complexes have also been prepared, but have not been extensively developed due to concerns about their stability.[61-63]



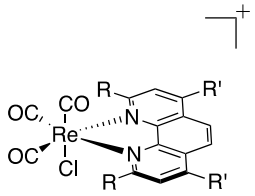
1a-c: R = $C(O)(CH_2)_nCH_3$
n = 6, 12, 16
1d: R = H



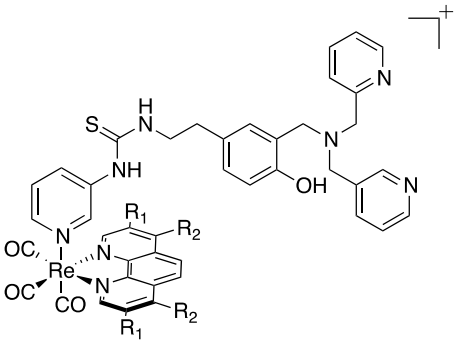
2a: R = H
2b: R = CH_2OH



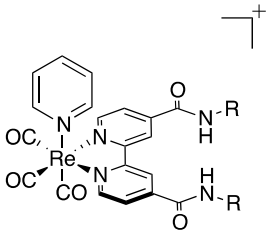
3a: $R_1 = R_2 = H$
3b: $R_1 = R_2 = Me$
3c: $R_1 = H$ $R_2 = Ph$



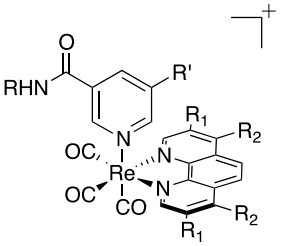
4a: R = R' = H
4b: R = Me R' = H
4c: R = Me R' = Ph



5a: $R_1 = R_2 = H$
5b: $R_1 = R_2 = Me$
5c: $R_1 = H$ $R_2 = Ph$



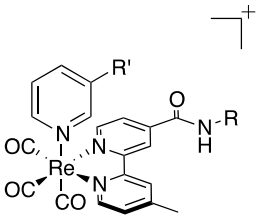
6a: R = C_4H_9
6b: R = C_2H_4-biotin
6c: R = C_2H_4-NHC(O)-C_5H_{10}-biotin



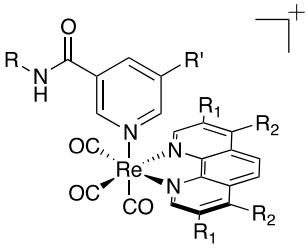
7a-c: $R_1 = R_2 = H$, R = PEG5kDa,
R' = NH_2, NCS, NHC(S)NH-C_4H_9
7d: $R_1 = R_2 = H$, R = C_2H_5 R' = NH_2

7e-g: $R_1 = R_2$ =Me R = PEG5kDa,
R' = NH_2, NCS, NHC(S)NH-C_4H_9
7h: $R_1 = R_2 = H$, R = C_2H_5 R' = NH_2

7i-k: R_1= H, R_2 = Ph, R = PEG5kDa,
R' = NH_2, NCS, NHC(S)NH-C_4H_9
7l: R_1= H, R_2 = Ph, R = C_2H_5 R' = NH_2



8a-c: R = PEG5kDa,
R' = NH_2, NCS, NHC(S)NH-C_4H_9
8d: R = C_2H_5 R' = NH_2



9a-i: R = $(CH_2)_3(CF_2)_7CH_3$

9a-c: R' = NH_2
9a: $R_1 = R_2 = H$
9b: $R_1 = R_2 = Me$
9c: $R_1 = H$ $R_2 = Ph$

9d-f: R' = SCN
9d: $R_1 = R_2 = H$
9e: $R_1 = R_2 = Me$
9f: $R_1 = H$ $R_2 = Ph$

9g-i: R' = NHC(S)NHEt
9g: $R_1 = R_2 = H$
9h: $R_1 = R_2 = Me$
9i: $R_1 = H$ $R_2 = Ph$

9j-l: R = Et R' =NH_2
9j: $R_1 = R_2 = H$
9k: $R_1 = R_2 = Me$
9l: $R_1 = H$ $R_2 = Ph$

Figure 1A. Cytotoxic Re(I) tricarbonyl bisimine complexes.

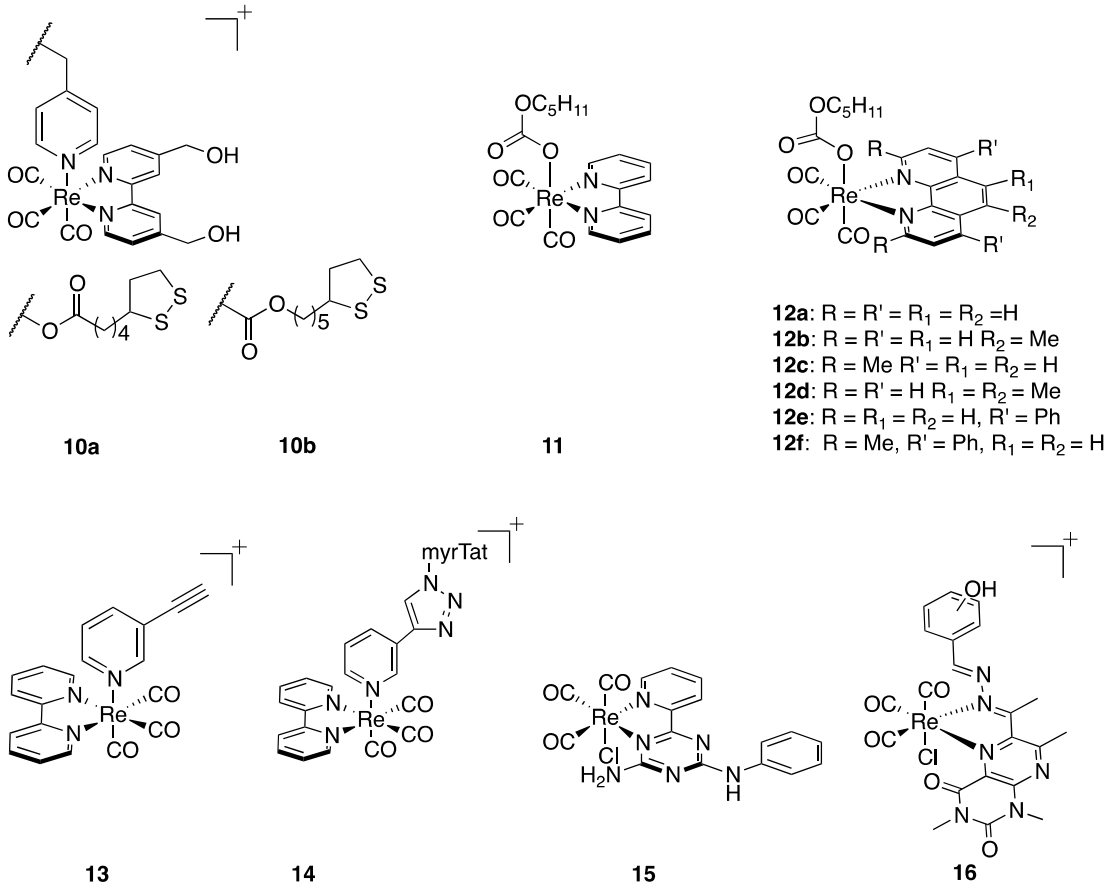


Figure 1B. Cytotoxic Re(I) tricarbonyl bisimine complexes (continued).

Table 1. Cytotoxicity of Re organometallic compounds towards human cancer cell lines.

Compound	IC_{50} (μM)	Known drug[a] IC_{50} (μM)	Cell line	Incubation time	Reference
3a	22.7	26.7	HeLa	48 h	[28]
3b	17.5	26.7	HeLa	48 h	[28]
3c	28.5	26.7	HeLa	48 h	[28]
5a	4.0 ± 0.5	19 ± 0.4	HeLa	48 h	[32]
5b	2.3 ± 0.2	19 ± 0.4	HeLa	48 h	[32]
5c	0.7 ± 0.1	19 ± 0.4	HeLa	48 h	[32]
6a	7.7 ± 1.0	25.6 ± 2.3	HeLa	48 h	[33]
7a	26.0 ± 1.6	11.9 ± 0.3	HeLa	48 h	[39]
7d	15.0 ± 4.8	11.9 ± 0.3	HeLa	48 h	[39]
7e	11.9 ± 1.6	11.9 ± 0.3	HeLa	48 h	[39]
7h	5.0 ± 0.4	11.9 ± 0.3	HeLa	48 h	[39]
7i	6.6 ± 0.4	11.9 ± 0.3	HeLa	48 h	[39]
7l	3.6 ± 0.4	11.9 ± 0.3	HeLa	48 h	[39]
8a	>1151.7	11.9 ± 0.3	HeLa	48 h	[39]
8d	159.1 ± 8.0	11.9 ± 0.3	HeLa	48 h	[39]
9a	4.01 ± 0.14	34.0 ± 3.3	HeLa	48 h	[65]
9b	6.10 ± 0.37	34.0 ± 3.3	HeLa	48 h	[65]
9c	10.7 ± 0.53	34.0 ± 3.3	HeLa	48 h	[65]
9g	12.9 ± 0.23	34.0 ± 3.3	HeLa	48 h	[65]
9h	16.6 ± 0.36	34.0 ± 3.3	HeLa	48 h	[65]
9i	39.9 ± 1.76	34.0 ± 3.3	HeLa	48 h	[65]
9j	15.0 ± 4.8	34.0 ± 3.3	HeLa	48 h	[65]
9k	5.0 ± 0.4	34.0 ± 3.3	HeLa	48 h	[65]
9l	3.6 ± 3.3	34.0 ± 3.3	HeLa	48 h	[65]
11	3.0 ± 2.5	5.0 ± 2.5	MDA-MB-468	48 h	[45]
12a	2.0 ± 1.5	5.0 ± 2.5	MDA-MB-468	48 h	[45]
12b	3.0 ± 1.5	5.0 ± 2.5	MDA-MB-468	48 h	[45]
12c	2.0 ± 2.5	5.0 ± 2.5	MDA-MB-468	48 h	[45]
12d	3.0 ± 4.5	5.0 ± 2.5	MDA-MB-468	48 h	[45]
12e	4.0 ± 6.5	5.0 ± 2.5	MDA-MB-468	48 h	[45]
13	29.9 ± 6.1	9.1 ± 2.8	HeLa	48 h	[51]
14	13.0 ± 2.8	9.1 ± 2.8	HeLa	48 h	[45]
15	43 ± 2.2	22.1 ± 3.6	KB-3-1	48 h	[27]
15	30.9 ± 1.1	10.5 ± 0.5	HepG2	48 h	[27]
15	50.3 ± 0.2	11.6 ± 0.2	HeLa	48 h	[27]
16	about 4	N/A	U373	72 h	[31]
16	about 4	N/A	MCF-7	72 h	[31]
19	78.2 ± 0.2	84	A2780/AD	24 h	[29]
23c	6.6 ± 3.0	42 ± 7 (5-FdU)	A549	48 h	[35]
26d	27 ± 11	42 ± 7 (5-FdU)	A549	48 h	[50]
26e	46 ± 22	42 ± 7 (5-FdU)	A549	48 h	[50]

28	3.4 ± 1.6	42 ± 7 (5-FdU)	A549	48 h	[50]
29	8.6 ± 0.2	2.7 ± 0.1	MCF-7	48 h	[44]
29	16.9 ± 1.5	8.2 ± 1.5	U2OS	48 h	[44]
29	29.8 ± 1.0	3.9 ± 1.0	HepG2	48 h	[44]
30a	13.6	19.5 (Ara-C)	HeLa	N/A	[22]
30b	11.9	19.5 (Ara-C)	HeLa	N/A	[22]
30c	11.4	19.5 (Ara-C)	HeLa	N/A	[22]
30d	8.6	19.5 (Ara-C)	HeLa	N/A	[22]
30e	9.9	19.5 (Ara-C)	HeLa	N/A	[22]
31a	6.4	19.5 (Ara-C)	HeLa	N/A	[22]
31b	5.7	19.5 (Ara-C)	HeLa	N/A	[22]
31c	6.5	19.5 (Ara-C)	HeLa	N/A	[22]
32	7.8	19.5 (Ara-C)	HeLa	N/A	[22]
33	5.2	51.2 (Ara-C)	MCF-7	N/A	[24]
34	3.4	51.2 (Ara-C)	MCF-7	N/A	[24]
35, 36	4-10	N/A	MCF-7	72 h	[26]
35, 36	4-10	N/A	EVSA-T	72 h	[26]
35, 36	4-10	N/A	NB69	72 h	[26]
35, 36	4-10	N/A	H4	72 h	[26]
35, 36	4-10	N/A	ECV	72 h	[26]
37c	7.3 ± 0.4	18 ± 1	MOLT-4	N/A	[42]
37c	24 ± 4	71 ± 8	MCF-7	N/A	[42]
38a	4.4	51.2 (Ara-C)	MCF-7	N/A	[23]
38b	2.6	51.2 (Ara-C)	MCF-7	N/A	[23]
38c	2.4	51.2 (Ara-C)	MCF-7	N/A	[23]
38d	2.6	51.2 (Ara-C)	MCF-7	N/A	[23]
39	1.0	51.2 (Ara-C)	MCF-7	N/A	[23]
40b	4.75	N/A	MCF-7	N/A	[38]
40b	75.1	N/A	HeLa	N/A	[38]
17	9.3 ± 2.2	9.2 ± 0.6	HeLa	48 h 300–400 nm 2.6 J cm^{-2}	[66]
18	17.3 ± 2.9	9.2 ± 0.6	HeLa	48 h 300–400 nm 2.6 J cm^{-2}	[66]
47	18.3 ± 1.4	9.2 ± 0.6	HeLa	48 h 300–400 nm 2.6 J cm^{-2}	[66]
48	5.4 ± 1.0	9.2 ± 0.6	HeLa	48 h 300–400 nm 2.6 J cm^{-2}	[66]
48	13.6 ± 1.7	N/A	PC-3	48 h 300–400 nm 2.6 J cm^{-2}	[66]
49	0.9 ± 0.1	N/A	HeLa	24 h 590–700 nm	[46]

				5 J cm^{-2}	
50	3.3 ± 2.3	N/A	HeLa	24 h 590–700 nm 5 J cm^{-2}	46
51c	0.1	N/A	HeLa	24 h >505 nm 105.1 J cm^{-2}	43
52	9.3 ± 0.8	9.2 ± 0.6	HeLa	48 h 300–400 nm 2.6 J cm^{-2}	47
53	9.7 ± 4.4	9.2 ± 0.6	HeLa	48 h 300–400 nm 2.6 J cm^{-2}	47
53	19.2 ± 2.4	74.8 ± 14.8	PC-3	48 h 300–400 nm 2.6 J cm^{-2}	47

a – cisplatin unless otherwise indicated (5-FdU – 5-fluorodeoxyuridine, Ara-C – cytarabine), *N/A* – not available.

Re(I) tricarbonyl complexes with *N, N, N*-tridentate ligands. The second type of Re organometallic complexes that yielded several cytotoxic compounds is Re(I) tricarbonyl complexes with a tridentate ligand coordinated to the Re center *via* three N atoms (Figure 2). Dipicolylamine (Dpa) and *N, N*-bisquinoline (BQ) based ligands have been initially used with the $Re(CO)_3$ core for ^{99m}Tc/Re(I) studies and luminescent labeling. Specific targeting of the folate and cublin receptors overexpressed in certain types of cancer has been achieved by coupling the $[ReBQ(CO)_3]^+$ complexes **17** and **18** (Figure 2) to folate[29] and vitamin B_{12},[34] respectively. The resulting Re bioconjugates **19** and **20** (Figure 2) were rapidly taken up *via* the corresponding receptor-mediated pathways. The specificity of **19** and **20** was controlled by comparing the uptake on receptor overexpressing cell lines (human adramycin and cisplatin-resistant ovarian carcinoma A2780/AD for folate and human placental choriocarcinoma BeWo for B_{12}) with that on a receptor-negative cell line (Chinese hamster ovary, CHO). In the case of the Re-folate conjugate **19**, fluorescence microscopy imaging revealed a rapid internalization of **19** by A2780/AD cells and a non-specific surface binding of **19** to CHO cells. Folate conjugation greatly increased the cytotoxicity of **17** from around 0.6 mM to 80 μM.[29] However, the non-specific binding of **19** to CHO cells resulted into the same anti-proliferative effect as on a folate receptor-positive A2780/AD cell line. Of note, a DNA-mediated mode of action for the Re-folate conjugate **19** could be ruled out. The biological activity of **19** has been attributed to the intact complex since 48 h incubation of **19** in PBS (pH 7.4) with a 100-fold excess of histidine or glutathione did not result in any change in LC-MS chromatogram. Fluorescence microscopy showed that the Re-B_{12} conjugate **20**, on the other hand, displayed no emission in CHO cells and a strong signal in BeWo cells.[34] These results were reflected in the observed cytotoxicity of the Re-B_{12} conjugate that was more active against the cubilin-positive BeWo cell line. Yet, compound **20** only produced its anti-proliferative effect at high concentration (IC_{50} 2 mM).

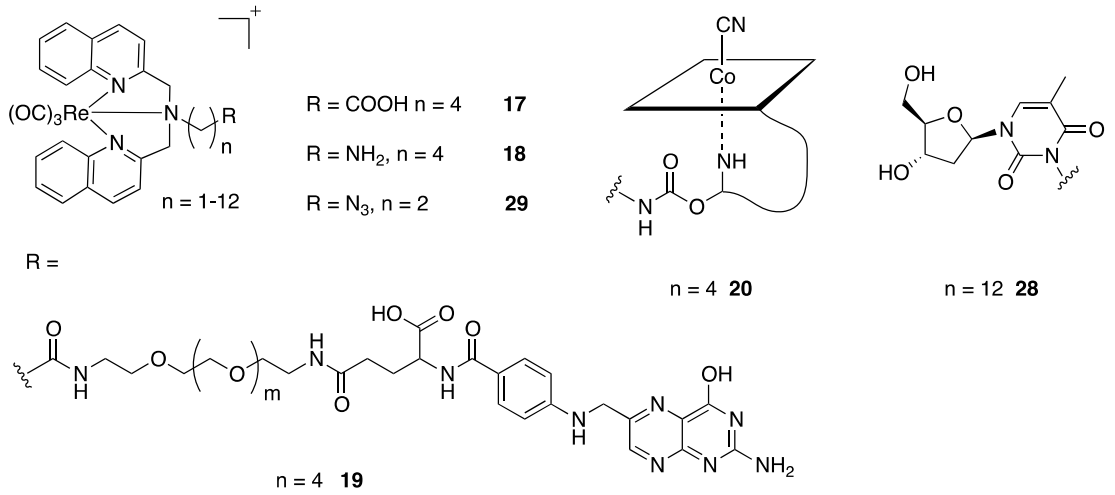


Figure 2. Cytotoxic Re(I) tricarbonyl complexes with bisquinoline-based ligands.

Some of the most cytotoxic Re organometallic compounds have been prepared by conjugation of $[Re(CO)_3Dpa]^+$ and $[ReBQ(CO)_3]^+$ to thymidine and uridine at the C5', C2', N3 and C5 positions with spacers of different lengths (**28** on Figure 2 and **21–27** on Figure 3).[35, 50] The activity of complexes **21–28** varies greatly with the derivatization position and the length and nature of the spacer. In fact, C2' (**21**) and C5 (**22**) were not active against A549 lung cancer cell line. Derivatization *via* positions C5' (**23–25**) and N3 (**26**–**28**) proved to be the most advantageous in the view of cytotoxicity. However, the anti-proliferative effect of C5' and N3 derivatives strongly depended on the spacer used. While C5' conjugates **24** and **25** and N3 derivatives **27** have IC_{50} values in the mM range, compounds **23**, **26** and **28** possess much higher cytotoxicity in μM range. Elongation of the spacer and use of $[ReBQ(CO)_3]^+$ resulted into an increase in the cytotoxicity, reaching an IC_{50} value of about 3.5 μM (**28**).[50] Interestingly, while the above discussed folate and B_{12} conjugation allowed the complex to act *via* the corresponding receptors, thymidine-derivatized Re complexes were not taken up *via* the thymidine pathway and only weakly affected hTk-1, which is the primary enzyme involved in thymidine salvage pathway. The cellular localization of the $[ReBQ(CO)_3]^+$ bioconjugates vary considerably. While the Re-folate bioconjugate **19** seemed uniformly spread all over A2780/AD cells,[29] the Re-B_{12} conjugate **20** had a more pronounced emission in the nucleoli, besides cytoplasm.[34] The thymidine derivative **28** was only observed outside the nucleus and co-localized with endosome

staining by fluorescence microscopy.[50] While no exact ^{99m}Tc analogues of these complexes exist, several chemically robust ^{99m}Tc-labeled compounds have been prepared with dipicolylamine and *N, N*-bisquinoline ligands.[67-71] One of such derivatives has been used to image transplanted neural stem and progenitor cells in a SPECT/CT study on mice.[68]

As already discussed for Re bisimine compounds above, despite the favorable photophysical properties of certain Re organometallic complexes, emission microscopy localization is not always possible or accurate due to luminescence quenching. Complexes of type $[ReBQ(CO)_3]^+$ sometimes share the same problem. For instance, a $[ReBQ(CO)_3]^+$ complex with an azide group (**29** on Figure 2) could not be localized by fluorescence microscopy in HeLa cells, although the cytotoxicity profile indicates that it is taken up by cells. In fact, **29** approaches the cytotoxicity of cisplatin on U2OS, HepG2 and MCF-7 (IC_{50} 6–39 μM, cisplatin 1–9 μM).[44] Although **29** could not be imaged in cells by fluorescent microscopy, the major mode of action of this $[ReBQ(CO)_3]^+$ compound has been studied in detail using MCF-7 cells grown on a biosensor chip micro-bioreactor and isolated mitochondria. The complex had a strong effect on cellular metabolism by acting directly on mitochondria respiration. This activity was most likely due to the intact **29** as no decomposition was detected by LC-MS when **29** was incubated in human blood plasma for 72 h.

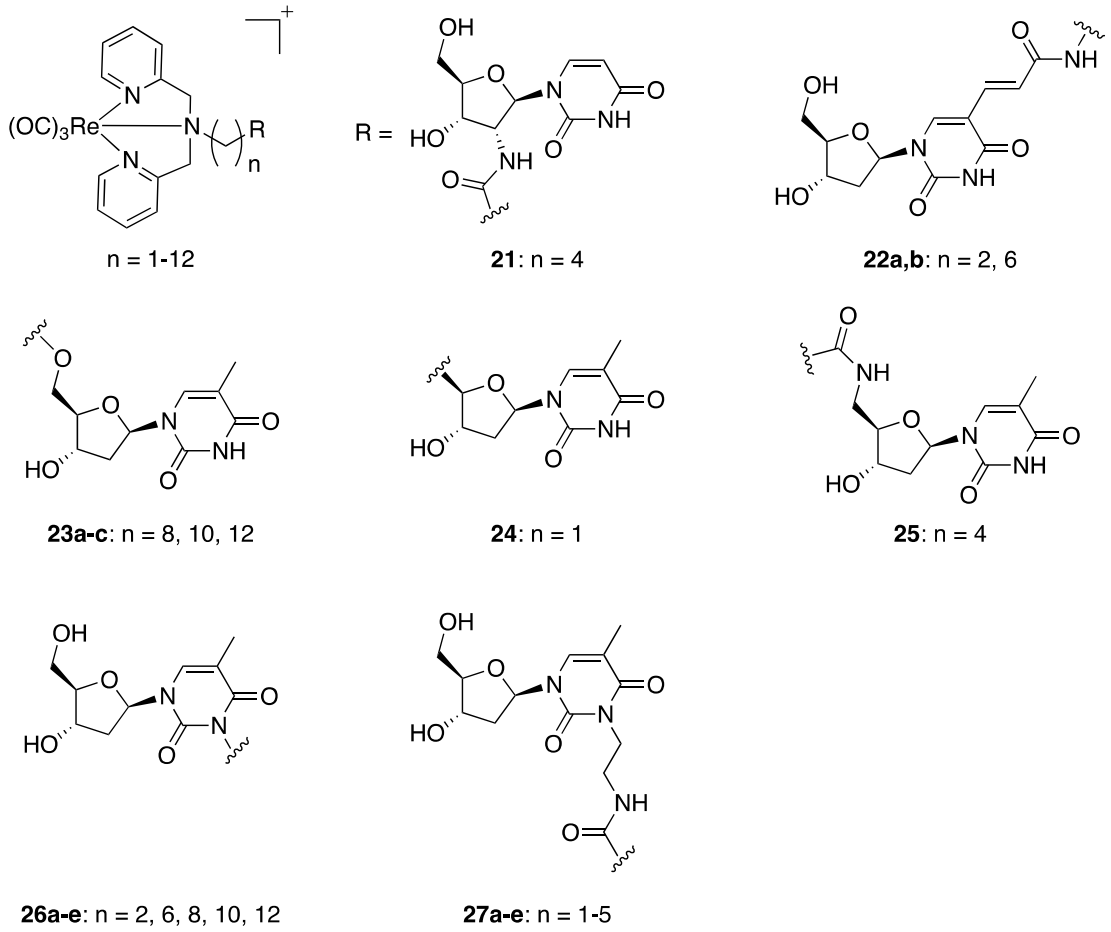


Figure 3. Cytotoxic Re(I) tricarbonyl complexes with dipicolylamine-based ligands.

Alkoxo/hydroxo Re(I) carbonyl complexes. Re(I) alkoxo/hydroxo carbonyl complexes (Figure 4) were the first Re organometallic compounds that were shown to possess an anti-cancer activity.[22, 24] Designed with the cisplatin ligand exchange-based mechanism of action in mind, this type of complexes has labile alkoxy/hydroxyl ligands. The dppf ligand of the Re compounds **31** and **32** (Figure 4) can also be released/exchanged and contributes to the overall cytotoxic effect, as dppf has been reported to be toxic on its own.[72, 73] Re(I) alkoxo/hydroxo compounds have been screened on over ten different human and murine tumor cell lines. These complexes were generally very active against suspended cancer cell cultures. However, on solid tumor cells, their cytotoxicity significantly varied with each particular cell line. The mechanism of toxicity has been described in considerable detail for compounds **30**–**31**.[22] The alkoxo/hydroxo Re(I)

carbonyl compounds are able to cause DNA strand scission and severely affect the nucleic acid metabolism *via* enzymes such as dihydrofolate reductase, PRPP-amido transferase and thymidine kinase.[22] Similarly to **30**–**32**, complexes **33** and **34** (Figure 4) are more cytotoxic towards suspended cancer cells than solid tumor cell lines.[24] They, however, show improved activity on MCF-7 breast cancer. Their mechanism of action has not been studied, but their labile hydroxide and aminoethanol ligands are likely to be displaced in cells.

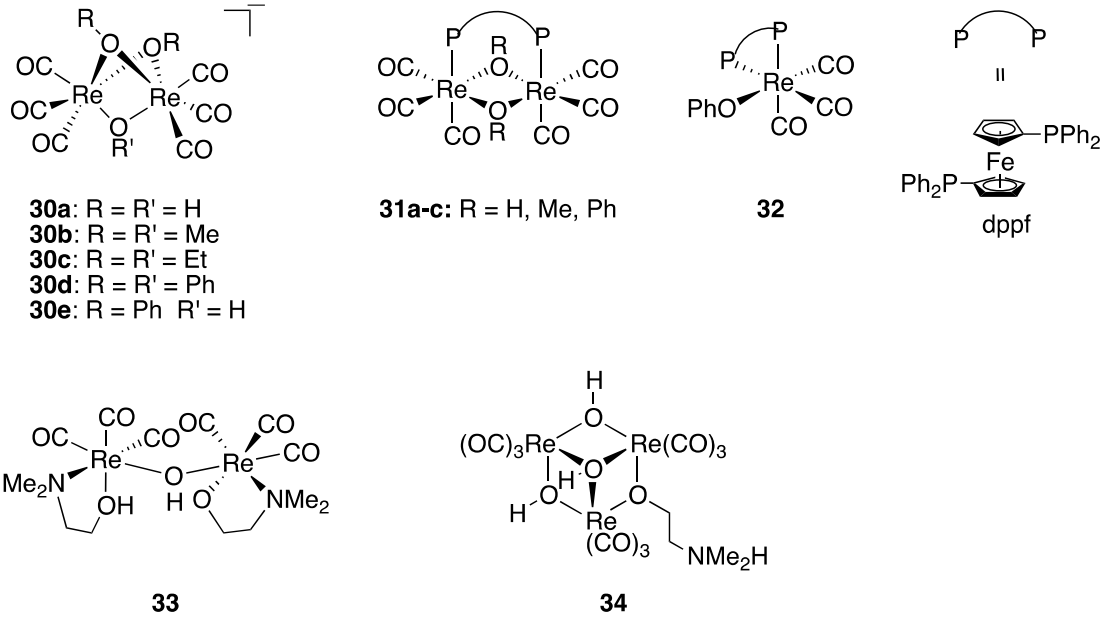
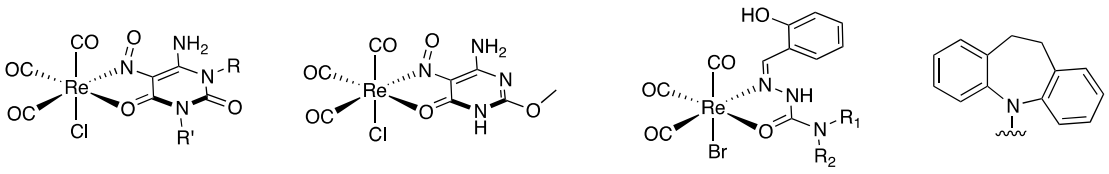


Figure 4. Cytotoxic Re(I) alcoxo/hydroxo complexes.

Re(I) tricarbonyl complexes with NO bidentate ligand. Two types of cytotoxic Re(I) carbonyl complexes coordinating a bidentate ligand *via* N and O atoms $[Re(CO)_3(N\text{-}O)X]$ (X =Cl, Br) have been reported to date (Figure 5).[26, 42] Various 5-nitrosopyrimidine Re complexes (**35**, **36**) induced a non-negligible cell growth at low concentration (up to 2 μM), but reduced cellular proliferation at higher concentration (IC_{50} 4–10 μM) on five human tumor cell lines (breast cancer MCF-7 and EVSA-T, neuroblastoma NB69, glioma H4 and bladder carcinoma ECV).[26] Their mode of action was not explored, but enhanced cellular growth at lower concentration was explained in terms of the "heavy atom" effect on the estrogen receptor that has been previously described for Cd.[74, 75]

Semicarbazones were used as ligands in Re compounds **37**[42] since they are known to have their own pharmacological activity. The resulting complexes achieved cytotoxicity comparable to cisplatin towards acute lymphoblastic leukemia MOLT-4 cells (IC_{50} 1–24 μM, cisplatin 18 μM) and did not strongly affect healthy fibroblast cells (IC_{50} > 125 μM). The activity was correlated with lipophilicity and was generally similar or higher compared to non-coordinated semicarbazone ligands. However, most Re semicarbazone complexes displayed very low toxicity toward the MCF-7 cells. The "hit" Re semicarbazone compound **37c** that combined strong cytotoxicity (7.3 and 24 μM) against both MOLT-4 and MCF-7 cancer cell lines with low toxicity towards healthy fibroblasts might have actually inherited its properties from the free ligand that has very similar IC_{50} values. Interestingly, NMR studies in DMSO showed that **37c** had a more acid phenolic proton than the corresponding ligand and tended to dimerize *via* loss of Br ligands. The authors judged dimerization in cell unlikely due to low concentrations, but hypothesized that good donor groups from biomolecules could easily substitute the Br ligand.



35a R' = R = CH_3
35b: R' = H, R = CH_3
35c: R' = R = H

36

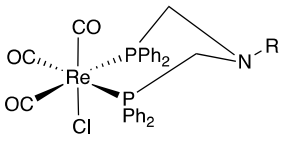
37a: R_1= R_2 = Bu
37b: R_1= R_2 = Hx
37c: R_1= R_2 = Bn
37d: R_1= Me R_2 = Ph
37e: R_1= R_2 = Ph

37f: NR_1R_2 =

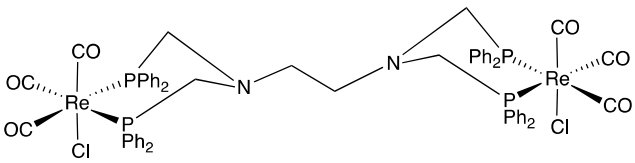
Figure 5. Cytotoxic Re(I) tricarbonyl complexes with N-O bidentate ligand.

Re(I) tricarbonyl complexes with P-P and Se-Se bidentate ligands. Tricarbonyl Re(I) bidentate phosphine complexes [$Re(CO)_3$(P-P)Br] **38** and **39** (Figure 6) have also been among the first Re organometallic compounds screened for cytotoxicity.[23] Tested on over 10 various cancer cell lines, these compounds were found to have IC_{50} values of about 5–10 μM, showing particular activity against MCF-7 breast cancer and HeLa-S^3 suspended uterine carcinoma. More recently, Re(I) tricarbonyl complexes with diseleno ligands $Re(CO)_3$(Se-Se)Cl (**40**, **41** on Figure

6) have been tested on HT 29 (colorectal cancer), MCF-7 (hormone-dependent breast cancer), A549S (lung adenocarcinoma) and HeLa (solid uterine carcinoma).[38] Curiously, these compounds also possessed a particularly high activity (4 μM) against MCF-7 breast cancer cells, while being only moderately or essentially non-toxic to other cell lines. This property has been further explored in the course of an *in vivo* study on MCF-7 tumor bearing mice.[41] In this work, the treatment with Re organometallic complex **40b** was combined with cisplatin and Gd(III) administration, since both inorganic Re/cisplatin and Gd/cisplatin co-administration studies had previously shown synergetic action between cisplatin/Re and cisplatin/Gd(III) compounds, respectively. The intraperitoneal cisplatin administration (2 per week, 3 mg/kg) combined with oral daily (5/7 days/week) administration of a Gd complex ((tetrakis(1-octanol) tris(5-aminosalicylate) Gd(III)) and $Re(CO)_3(Se\text{-}Se)Cl$ (**40b**) led to 50% tumor volume reduction after 3 weeks treatment. By comparison, only 25% tumor volume reduction was observed for mice treated with cisplatin only. However, no control groups were treated with Re or Gd compound only, so the extent of complex **40b** contribution to tumor reduction has not been directly assessed. The largest effect on tumor volume was achieved using the maxim dose of Gd (100 mg/kg) and the minimal dose of Re (10 mg/kg). In the mice treated with higher doses of Re, more deaths were observed.



38a-e: R = Ph, CH_2CH_2OH, CH_2COOCH_2Ph, $CH_2CO\text{-}NHCH_2COOCH_2Ph$, CH_2COOH



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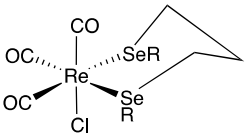
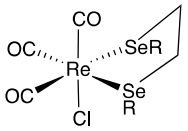


40a,b: R = Ph, CH_2COOH



41a,b: R = Ph, CH_2COOH

Figure 6. Cytotoxic Re(I) tricarbonyl complexes with P-P and Se-Se bidentate ligands.

Re-containing enzyme and receptor inhibitors. While a couple of cytotoxic Re complex discussed above might act *via* enzyme/receptor inhibition, none of them have been initially intended as such. In contrast, certain Re organometallic compounds, cyclopentadienyl (Cp) Re complexes in particular, have been purposely designed to bind a particular enzyme or receptor.[25, 76, 77] Specific interaction between Re complexes and their biological targets has been achieved by incorporating a $Re(CO)_3$ core into a known organic enzyme inhibitor or receptor ligand. The earliest examples of such Re compounds with anti-proliferative activity are Jaouen's Re-Tamoxifen (**42**, Figure 7) derivatives where a phenyl group is replaced by a Cp ring of $[Re(CO)_3Cp]$.[25] Tamoxifen inhibits the estrogen receptor (ER) and is used for the treatment of hormone-responsive breast tumors. The derivatization with Re slightly decreased the affinity of Tamoxifen for ER. However, cytotoxicity assays on MCF-7 ER(+) breast cancer cells showed that Re-Tamoxifen conjugates were even slightly more active than Tamoxifen by itself. A small effect (about 10% reduction in cell viability at 1 μM) was also observed on Tamoxifen-resistant MDA-MB231 breast cancer cell line. *In vivo* distribution study in rats has been realized with a related compound where $^{99m}Tc(CO)_3Cp$ moiety was conjugated to atriaryl butane skeleton of tamoxifen *via* an amide bond.[78]

Recently, the $Re(CO)_3$ core has been conjugated to sulfonamide moieties to target human carbonic anhydrases (hCAs), which have emerged as an interesting target for therapy and diagnosis due to their overexpression induced by hypoxia in cancer cells.[79-81] High concentration of carbonic acid resulting from this excessive activity of hCAs leads to a pronounced decrease in extracellular pH (down to ca. 6 instead of 7.4).[76, 79] Re complexes (**43**–**46**, Figure 7) have been shown to be excellent hCA inhibitors with submicromolar affinities and interesting selectivity profiles for various hCA isoforms.[76, 82] No IC_{50} values were reported, but efficient inhibition of hCA in HeLa and HT29 cells has been demonstrated by monitoring the rise in extracellular pH upon incubation with Re inhibitors. The X-ray structure has shown that Re hCA inhibitors bound to the enzyme in a similar manner as organic inhibitors and the Re core only participated in

hydrophobic interactions with Phe, Leu and Pro amino acid residues. ^{99m}Tc analogues of compounds **46a** and **b** have also been synthesized in an excellent yield,[82] while the ^{99m}Tc version of inhibitor **43** has been already tested on xenograft mice with CAIX expressing tumors.[76] However, these *in vivo* biodistribution studies have shown that, despite excellent *in vitro* results, Re complex **43** failed to significantly accumulate in tumors.

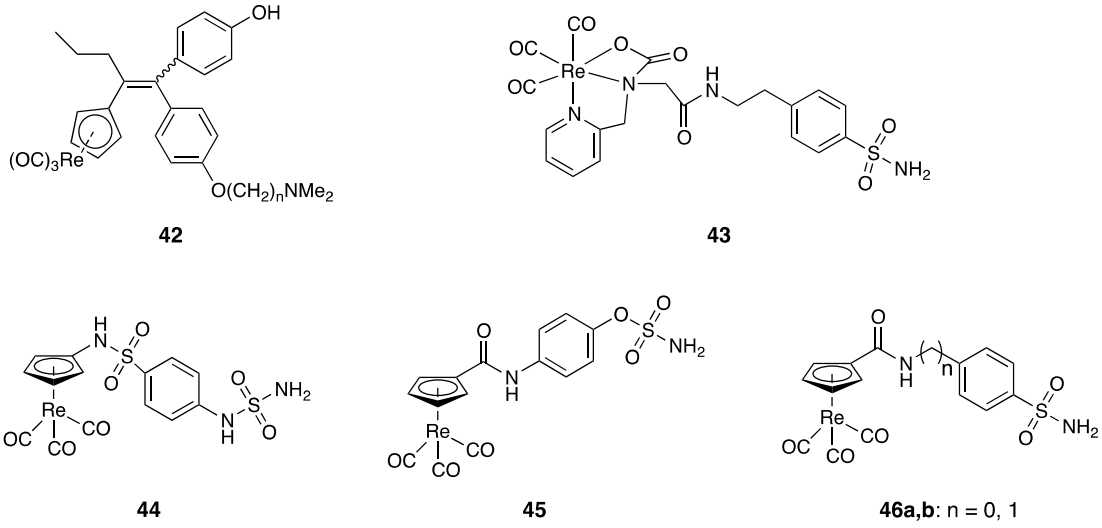


Figure 7. Re organometallic enzyme and receptor inhibitors.

PHOTO-TOXIC Re ORGANOMETALLIC COMPLEXES

Very few photo-toxic Re organometallic compounds have been identified to date (Figure 8).[43, 44, 46, 47] Most of them act as photodynamic therapy (PDT) photosensitizers (PS) – i.e. they transfer the energy received from light to oxygen (present in tissues) to generate reactive oxygen species (ROS). A PS excited by irradiation undergoes two types of reactions.[83] In the so-called type I reaction, the PS directly interacts with a substrate (e.g. cell membrane, protein) to form radicals or radical ions, which can in turn interact with oxygen to produce the highly reactive singlet oxygen. The type II reaction is defined as generation of singlet oxygen *via* PS-oxygen interaction.[83] Although many more Re compounds have been identified as efficient triplet

photosensitizers,[39, 84-88] this property has been more applied in photo-catalysis than in anti-cancer drug development.

The Re(I) tricarbonyl *N,N*-bisquinoline complexes $[ReBQ(CO)_3]^+$ **17** and **18** already discussed above are capable of producing singlet oxygen when irradiated at 355 nm.[44, 47] Singlet oxygen generation of these Re complexes decreases with solvent polarity and therefore depends on their cellular localization. UV-A irradiation of the essentially non-toxic Re(I) tricarbonyl *N,N*-bisquinoline complexes **17** and **18** used in folate[29] and B_{12}[34] conjugation studies has revealed that the anti-proliferative activity of these compounds on HeLa cells could be increased by a 10-fold to 9.3 and 17.3 μM (HeLa) respectively by using a relatively low light dose (2.6 J/cm^2).[44] This success, however, could not be reproduced on PC-3 cell line[47] without conjugation of the Re complex to a bombesin derivative (**48** on Figure 8) to target the GRP receptors overexpressed in certain types of cancer (5.4 μM on HeLa and 13.6 μM on PC-3).[44, 47] Addition of a nuclear localization signal peptide (NLS) (**47** on Figure 8) led to a strong increase of the dark cytotoxicity (35.1 μM HeLa). This could be the result of change in cellular localization upon NLS conjugation from cytoplasm to nucleoli, as observed by fluorescent microscopy. The bombesin derivative **48** could not be visualized on HeLa cells due to luminescence quenching.

Some photosensitizing porphyrin-Re conjugates (**49**, **50** on Figure 8) have been recently shown to greatly increase their cytotoxicity (from >100 μM to 3 μM IC_{50}) upon 590–700 nm light irradiation.[46] However, the light activity of these compounds originated entirely from the porphyrin moiety as the Re complex present in the conjugate does not produce any singlet oxygen upon irradiation at 590–700 nm. The Re conjugation, however, was found to improve the uptake of these porphyrin-Re conjugates. Hot ^{99m}Tc analogues of **49** and **50** have also been prepared and shown to be stable in cell culture medium for at least 24 h by HPLC studies.[89]

Re organometallic compounds can also function as photosensitizers in the visible light range as recently demonstrated with Re(I) tricarbonyl indolato complexes (**51**).[43] These compounds' UV/vis absorption extends up to 600 nm so that visible light irradiation (>505 nm) results into up to a 1000 fold increase in anti-cancer activity on HeLa cells and the most active of them

significantly reduced the size of melanoma spheroids. Singlet oxygen formation was detected for all complexes of the study by indirect *para*-nitrosodimethylaniline/imidazole assay[90] The most cytotoxic complex **51c** (0.1 μM HeLa) was studied in more detail. Curiously, it underwent pyridine ligand substitution after irradiation in presence of 4-dimethyaminopyridine. Considerable ROS production was also detected *in vitro* after irradiation. Fluorescence and phase-contrast microscopy revealed that this complex was localizing in the cellular membrane and induced severe morphological changes in HeLa cells upon irradiation. In fact, addition of a membrane-localized anti-oxidant protected the cells from the anti-proliferative effect of the Re complex.

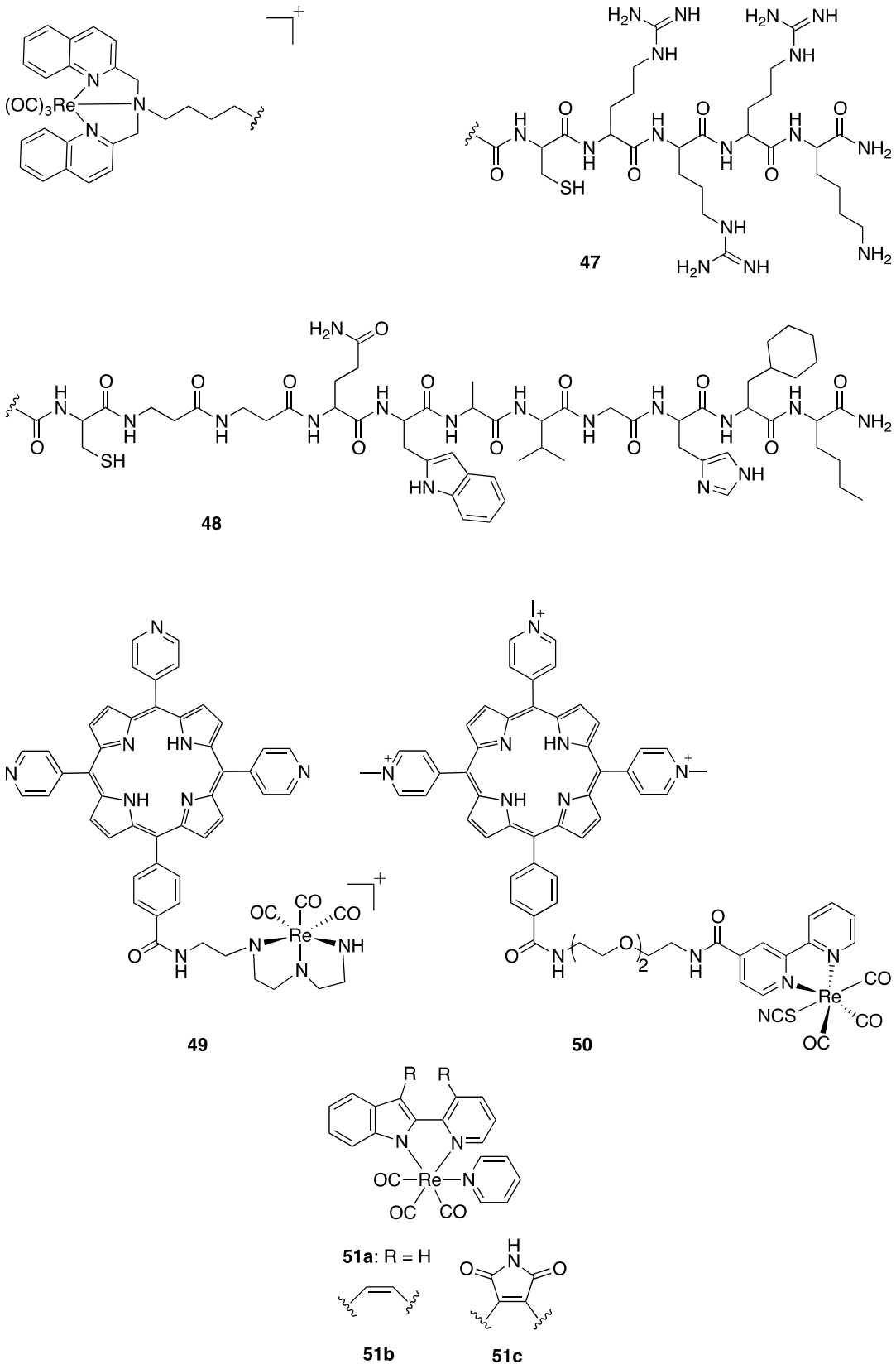
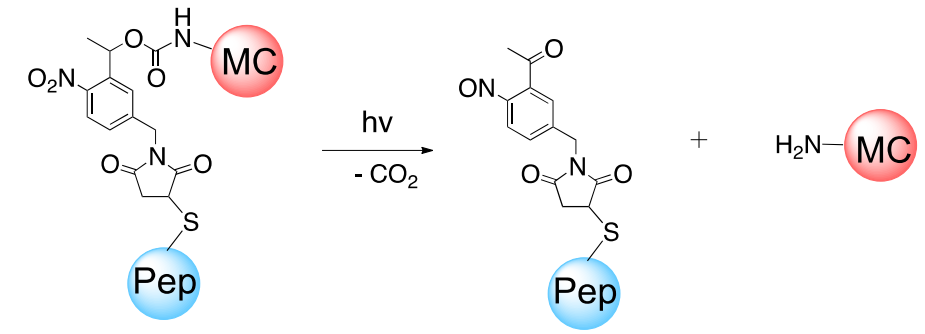
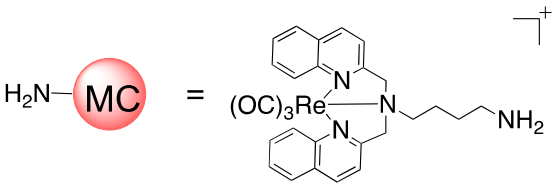
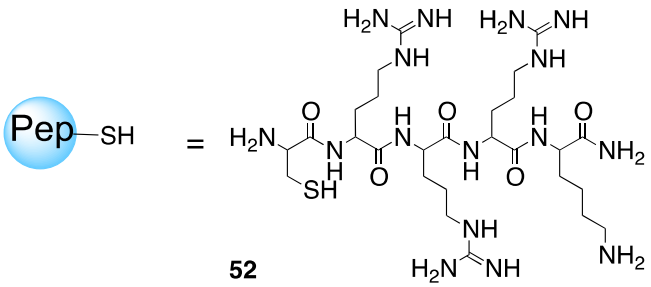


Figure 8. Photo-toxic Re organometallic compounds.

Recently, our group has incorporated a UVA (355 nm) photo-labile protecting group (PLPG) into the Re(I) *N,N*-bisquinoline bioconjugates **47** and **48** described above.[47] Upon irradiation at a very low light dose (1.2 J/cm^2), the resulting photo-caged bioconjugates **52** and **53** (Scheme 1) liberated the Re complex **17** in addition to singlet oxygen generation. IC_{50} values of c.a. 9 μM were thus reached. Biological studies demonstrated that the most cytotoxic compound **52** accumulated considerably in nucleoli where it could affect both DNA and RNA triggered cell death *via* both apoptosis and necrosis.







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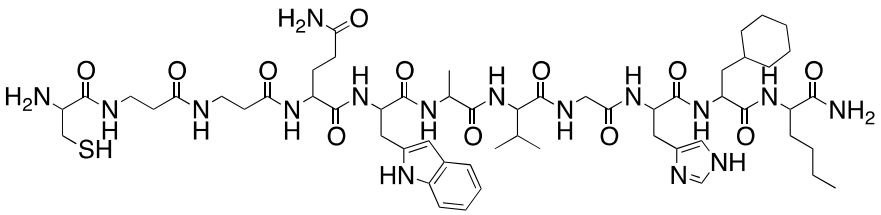

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Scheme 1. Photo-caged Re organometallic compounds.

CONCLUSIONS AND PERSPECTIVES

In this review, we have summarized the currently known Re organometallic compounds with anti-cancer activity, as well as highlighted their modes of cytotoxic action when possible. The majority of anti-proliferative Re organometallic complexes act as "traditional" chemotherapeutic agents, although a few photo-active Re complexes have also been recently reported. Potentially, an even larger number of Re organometallic compounds with PDT activity could be identified by screening the singlet oxygen production of known Re photocatalysts.

Most of the compounds presented in this review are potent anti-cancer agents with IC_{50} values reaching or exceeding the activity of cisplatin. The underlying mechanisms of toxicity are, however, not always fully elucidated and, to the best of our knowledge, only one *in vivo* study has been conducted on cold Re organometallic complexes.[41] This study as well as a couple of successful *in vivo* evaluations of coordinative Re compounds[91, 92] have demonstrated a synergistic action of Re compounds with cisplatin. Undoubtedly, more thorough biological investigations both *in vitro* and *in vivo* would give us a better understanding of the cytotoxicity of Re organometallic complexes. In this context, preparation of "hot" analogues of promising Re compounds to visualize their *in vivo* behavior is an indispensable step and the most helpful tool to allow for the development of powerful and selective anti-cancer drugs.

ACKNOWLEDGEMENTS

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