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The Role of Metallodrugs in Cellular Senescence

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Dedicated to Prof. M. C. Gimeno on the occasion of her receipt the Rafael Usón Medal.

Delivering alternative strategies to deal with cancer is a huge milestone in research. Cancer cells can give a senescence response as consequence of cellular stress or external stimuli such as the use of chemotherapies, which could end up eventually in cancer relapse. Controlling cellular senescence will surely open new cancer treatment approaches. Cancer senescence induction can be used as an added timeframe to look for alternative treatments and senolytic drugs to avoid cancer relapse destroying the senescent cells (SnCs). Within cancer senescence research, metal complexes are underdeveloped in comparison with that of organic molecules or nanoparticles. Herein, we highlight the scarce investigation performed with metal complexes in the field of senescence and how a great input on them could be a huge step towards the search of alternative cancer treatments.

1. Introduction

In 1961, Hayflick and Moorhead demonstrated that normal cultured cells displayed a finite number of cellular divisions before entering in a permanent cell cycle arrest in which they remained viable.[1] This phenomenon, termed senescence, is characterized by a high lysosomal activity, elevated secretion of chemokines, cytokines and growth factors,[2] as well as, upregulation of antiapoptotic mechanisms, changes in cellular morphology, in the heterochromatin,[3] and finally, an increment in β -galactosidase activity.[4] Cellular senescence is a highly heterogeneous state, which is induced as a consequence of different factors as telomere shortening,[5] DNA damage, oncogene activation or cellular stress.[6] Diseases associated to aging and cancer metastasis or progression are highly related with the cells entering this stage of cell cycle arrest.[7,8] Additionally, senescent cells (SnCs) could be artificially generated after treatment with certain types of chemotherapies or irradiation sources.[9] For instance, Wang and coworkers reported that the use of cisplatin, a treatment for a nasopharyngeal carcinoma, induced the cells to enter in a stage of senescence.[10] Later studies corroborated that other drugs used in chemotherapy like hydroxyurea,[11] doxorubicin,[12] or camptothecin[13] also promote similar behavior (Figure 1).

Senescence plays a dual role in the regulation of cancer, giving an additional timeframe to the patients and allowing the use of novel therapies for recovery.[14] Specifically, senescence acts like a barrier inhibiting the division of those cells exhibiting active-oncogenes, the cancerous cell promotors. Activation of tumor suppression genes such as p53/p21^{CIP1} and p16^{INK4a}/RB[15] is crucial for promoting a cell cycle arrest and senescence. As a result of that, the immune system is also activated, promoting the elimination of SnCs, Figure 1.[16] Despite the good prospects of inducing senescence in cancer therapy, accumulation of SnCs can increase the probability of cancer relapse and metastasis. SnCs, acquire the senescence-associated secretory phenotype, known as SASP, secreting high levels of different proteins and contributing to generate chronic inflammation in the cellular microenvironment. As consequence, the probability of suffering age-related diseases and tumor progression increases.[17]

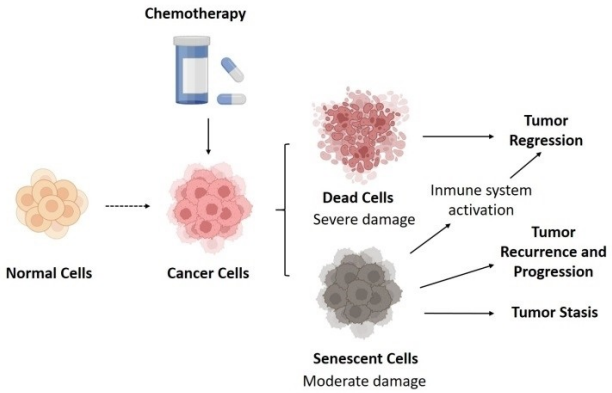

Figure 1. Schematic illustration of chemotherapy's effects in cancer cells.

Taking into account all these facts, it seems clear the necessity of finding novel strategies to target SnCs, identifying their origin and molecular pathways to eventually eliminate them. This new modality in medicine is known as senotherapy[18] and it can be divided into two treatment strategies. Senolytics, drugs that selectively eliminate SnCs and senomorphics, drugs that prevent side effects generated by

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SnCs and suppress SASP. In addition to them, pro-senescence therapy arouses as a great alternative to deal with cancer cells. It is based on the induction of senescence in the tumoral area, blocking temporally cancer to spread along the body and promoting physiological response by the recruitment of immune cells to the tumor. In this way, pro-senescence therapy[19] can be used as an effective starting point for immunotherapies against cancer. In this perspective article, we compile the reported examples of metallic complexes that have shown any activity within SnCs. We will focus on metallic complexes able to induce senescence and those that exhibit senolytic properties, as both of them can open new pathways and hopes in the treatment of cancer. The scope of this perspective is restricted to metal complexes over organic compounds as they could be an interesting alternative to traditional organic drugs. Their diverse mechanism of action and biological targets make these compounds very appealing for delivering multi-targeted therapy. Moreover, the active optical properties presented by some of them, could be used to promote photodynamic therapy[20] as a senolytic treatment and/or to develop theragnostic agents within senolytic/pro-senescence drugs.[21] Noteworthy, nanoparticles have been also used to induce senescence or as senolytics in the recent years.[22,23] Among them, gold nanoparticles[24–26] and silver nanoparticles[27,28] have been widely used. However, we will not consider them for this perspective article as we intended to focus on small metallic molecules. Our objective is to stress the great prospect of using small metallic molecules in the senescence field as new drugs. Finally, we will highlight the weakness and strengths of senescence-targeting strategies and the necessity of finding global markers to target SnCs.[29]

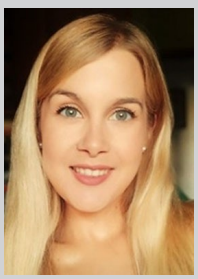
2. Senotherapies

In the last fifty years, senescence has been considered as a double-edge sword to control and eliminate cancer cells. Firstly, it can prevent the malignant transformation of the cells through a permanent cell cycle arrest and secondly, it can promote the activation of the immune system. However, as previously mentioned, the accumulation of these cells is also undesirable because eventually, it can promote tumor recurrence if the cell cycle is re-activated.[30] Having said that, it seems clear that there is a fine line between the advantages and disadvantages of senescence that need to be tackled. Therefore, a growing interest is set on the development of new drugs that can selectively induce (pro-senescence drugs, or senescence inducers) or destroy SnCs (senolytics), being most of them organic compounds (Figure 2).

Among pro-senescence drugs it can be found doxorubicin,[31] palbociclib[32] or camptothecin,[13] all of them typical drugs for the treatments of different cancer diseases. Instead, drugs such as navitoclax (antidiabetic),[33] metformin (anticancer)[34] and fisetin (antioxidant)[35] have demonstrated their ability as senolytic destroying SnCs. Therefore, after seeing the variety of drugs delivering the completely opposite effect, there is still a long way to go in order to identify the specific features for a compound to go from a pro-senescence drug to a senolytic. Metal-based drugs such as cisplatin or carboplatin are also being used in senotherapy as pro-senescence drugs.[36,37] However, this area is still in its infancy, as the gross of investigations are focused in organic compound. Despite that, investigation in the field points towards the good prospects of incorporating metal-based drugs in the race for delivering efficient treatments to tackle senescence, and with that, assisting to find solutions in cancer and aging diseases.

3. Hallmarks of Cellular Senescence

As it was already mentioned, cellular senescence is a highly heterogeneous process that is commonly characterized by different hallmarks such as, permanent DNA damage, permanent cell cycle arrest, secretion of inflammatory factors (SASP), endoplasmic reticulum stress, overexpression of some proteins as p53, p16 or p21 and changes in cellular morphology (increment of size) as well as, accumulation of dysfunctional mitochondria, increased of lysosomal content, apoptotic resistance and changes in the nucleus (heterochromatin) or high β-galactosidase activity.[38] However, not all of them need to take place simultaneously for a cell entering in a senescent stage, which hinders the assessment of cellular senescence. The lack of a global maker is the main limitation to target SnCs. To confirm senescence induction, it is mandatory the identification of at least three of the hallmarks previously described. The most



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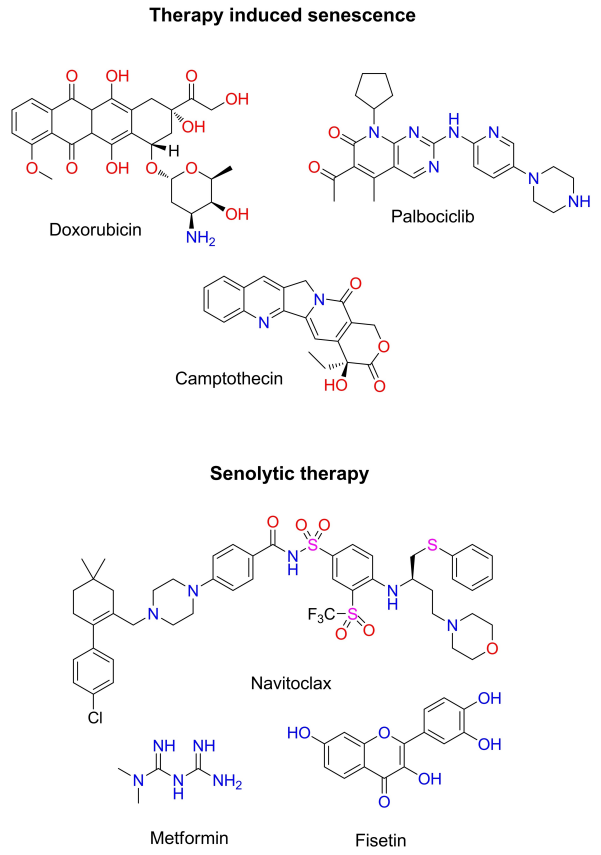

Figure 2. Examples of organic drugs with application in senescence.

commonly used for assessing an ongoing senescence stage are described below.

A) The permanent cell cycle arrest could be easily detected by flow cytometry or even in an easier manner by optical microscopy with the corroboration of a constant percentage of cellular confluency over time.

B) Optical microscopy could be also used to probe changes in the cellular morphology. Generally, SnCs display flat morphology and their size increases at least twice, including the nuclear area. Moreover, SnCs containing polynucleus are also often detected with this technique.

C) Determination of an increment of a β-galactosidase activity (SA- β -gal) by a staining assay. In fact, the simplicity of this assay led many research groups dealing with SnCs to incorporate it in their daily work.[39] It is known that lysosomal-β-galactosidase (GLB1) hydrolyzes β-galactose from glycoconjugates and it is the source of senescence-associated β-gal activity (SA-β-gal).[40] However, it not possible to confirm yet whether this is a distinct enzyme active at pH 6, and differentially expressed in senescence, or just an indicator of an increase in the typical acid lysosomal β-galactosidase.[41] In any case, SA-β-gal activity has been widely used as a biomarker for senescence.

D) The overexpression of some proteins such as p16,[42] p21[43] or p53 could be determined by western blot analysis or flow cytometry. These proteins are involved in the cell cycle progression, and their overexpression could lead to a cell cycle arrest and thus, to cellular senescence.

E) The activation of some factors for avoiding an apoptotic cell death mechanism (apoptotic resistance) is a frequent pathway that SnCs follow. Therefore, performing assays to identify the expression of BCL2 proteins such as p21 or PI3K are also a promising strategy to identify senescence.[44]

F) Detection of SASP. SnCs secret numerous proteins such as cytokines, chemokines and proteinases that, overall, generate inflammation in the cellular microenvironment. It has been determined that SASP is involved in some process that could cause mitochondrial dysfunction[45] and endoplasmic reticulum stress[46] that activate the generation of reactive oxygen species (ROS).[47] Therefore, detection of SASP could be used as an additional strategy to suggest a senescence process taking place.

There are additional assays for the identification of further hallmarks associated with SnCs. However, as the main objective of this perspective lies in addressing the role of metallodrugs in senotherapy, only the most commonly used assays were described in this section.

4. Metals in Senescence

The use of metal complexes in senotherapies is still underdeveloped. Only few examples have recently appeared in the literature, being those of platinum-based complexes the majority. Among the reported metal-based complexes in the field of senescence, there are only examples exhibiting pro-senescence activity, leaving aside metal-based species as senolytics. It is worth remembering that both strategies could be considered as an alternative pathway to traditional chemotherapies, opening the door for the development of new antitumor treatments.

4.1. Platinum Complexes in Senescence

Platinum-based drugs have been by far the most explored complexes in the field of senescence. Among them, traditional anticancer drugs such as cisplatin and carboplatin stand out. Cisplatin is the most widely metal-based drug used in cancer chemotherapy and it can be administered either alone or combined with other drugs as a treatment of solid tumors.[48] However, several reports suggest that the use of this drug increases the possibility of cancer relapse or recurrence, which is a phenomena commonly known as platinum-drugs resistance.[49] Studies made in the last fifteen years indicate that cisplatin, as well as its analogue carboplatin, are able to induce senescence in several cancerous cell lines.[50–52] Interestingly, SnCs generated as a consequence of this chemotherapeutic drug can escape from the arrested condition and regenerate the original tumor, cancer relapse.[53] At the moment, cisplatin is one of the daily based SnCs inducer used in many research laboratories working in the field of senescence.[54] Therefore, there are well stablished protocols to promote the trans-

formation of cancerous or normal cells to SnCs using this drug.[18]

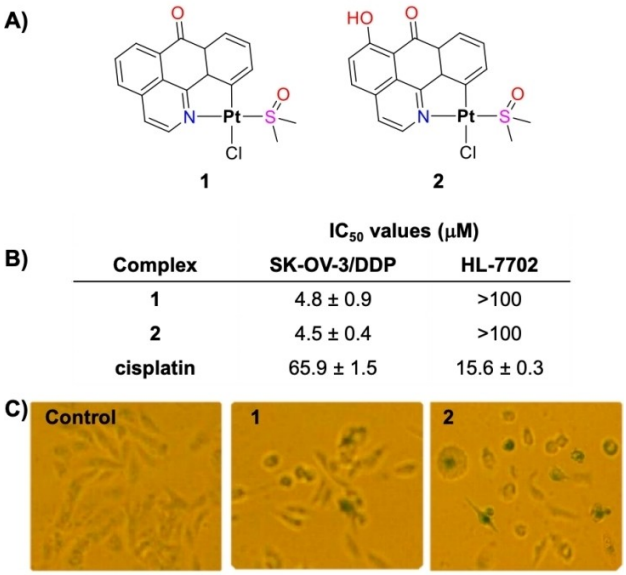

Figure 3. A) Structures of platinum-drugs that can induce senescence in SK-OV-3/DDP at 0.5 μM. B) IC_{50} values of complexes **1**, **2** and cisplatin in SK-OV-3/DDP and HL-7702 cell lines, incubated 48 h. C) Microscopy images of SA-β-galactosidase expression in SK-OV-3/DDP cells treated **1**, **2** (0.5 μM) or 0.1 % DMSO (control) for 7 days. Figure 3(B–C) were adapted with permission from Ref. [55]; Copyright (2019), American Chemical Society.

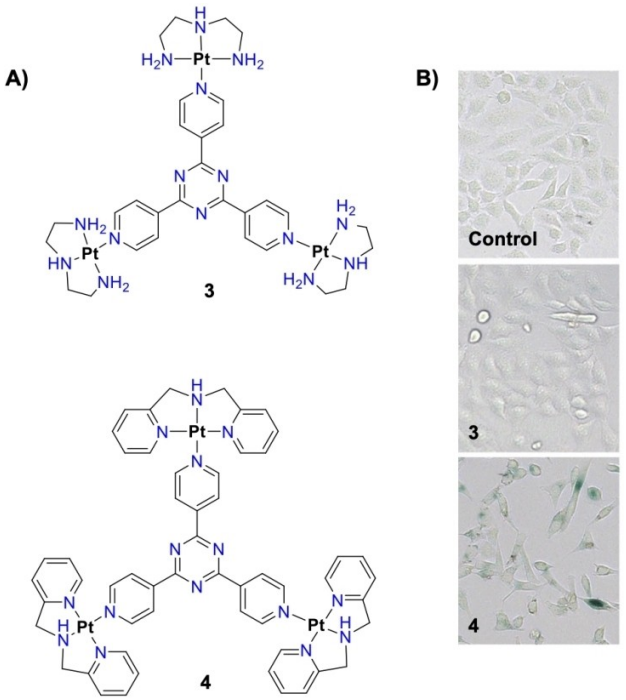

Figure 4. A) Chemical structures of complexes **3** and **4** ; B) SA-β-gal activity test in HeLa cells after 20 days of treatment (6.0 μM). Images acquired using x10 objective. Counter ions are NO_3^- anions. Figure 9B was adapted from Ref. [56] with permission from the Royal Society of Chemistry.

In the recent years, additional platinum-based complexes have shown bioactivity within senescence. In 2015, Liang and coworkers reported a family of square planar Pt^{II} complexes containing an azabenzathrone derivate, complexes **1** and **2**, see Figure 3A. Both of them are able to bind preferentially to G-quadruplex DNA in SK-OV-3/DDP cell line (ovarian carcinoma cells resistant to cisplatin), in comparison with that in normal cells (HL-7702), rendering a higher cytotoxicity in the former case, Figure 3B. Therefore, both complexes displayed preferential anticancer activity in ovarian carcinoma that cannot be treated with cisplatin. Regarding their prospects in senotherapy, authors claimed that **1** and **2** could induce senescence in SK-OV-3/DDP cell line after their treatment for 7 days using lower concentrations (0.5 μM) than that of their IC_{50} values. Their assessment was based on the effect that **1** and **2** exerted over the activity of the enzyme SA- β -gal using a SA- β -gal assay. Figure 3C showed in both cases the overactivity of the SA- β -gal which was stained in blue by a β-Gal staining solution (5-bromo-4-chloro-3-indolyl-β-D-galactoside) in comparison with that of control cells.

Such duality anticancer and pro-senescence activity gives the possibility of selecting the therapeutic use of these complexes, either as a chemotherapeutic drug or a pro-senescence inducer.[55]

Thereafter, two trigerminal star-like Pt^{II} complexes **3** and **4** were developed by Mao et al. in 2016, Figure 4A. Even though both complexes presented less cytotoxic activity than cisplatin in breast cancer HeLa cells (43.5 ± 1.3 μM (**3**), 23.5 ± 0.8 μM (**4**) and 16.8 ± 1.1 μM (cisplatin)), they can induce a strong telomeric DNA damage response, generating cellular senescence and telomere dysfunction.[56] This behaviour is especially remarkable for complex **4**, showing in addition a considerable increment of the SA-β-gal activity, one of the hallmarks for senescence, after incubation at 6 μM for 20 days. Figure 4B clearly revealed the higher activity of SA-β-gal stained in blue in comparison with that seen for the cells incubated with complex **3** and the control cells.

Altogether, these examples may suggest that the use of square-planar Pt^{II} complexes as anticancer treatment could be divided into two therapeutic approaches. In chemotherapy, when the complexes are used at concentration matching that of their IC_{50} for short incubation times, and in senotherapy, when they are incubated at concentrations lower than their IC_{50} values for longer times. This last approach can be useful in the cases of failure to tackle cancer with a chemotherapeutic treatment, rendering a new timeframe to find alternative treatments and/or stimulate an immune response.

4.2. Non Platinum-based Complexes in Senotherapy

Apart from platinum complexes, there is only a handful of other metallic species that have revealed their potential activity as pro-senescence probes. Among them, ruthenium, osmium, iron, and some iridium complexes can be found. Chronological revision will be made to emphasise the novelty of this area and promising prospects awaiting.

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In 2015, Legong, Top and co-workers, developed analogues of hydroxytamoxifen, a drug that has been used to treat breast cancer. The mechanism of action of this drug is based on the strong ability to compete with hormones for binding to the estrogen receptor (ER), proteins overexpressed in many breast cancer cells. Authors intended to modulate the activity of hydroxytamoxifen, with the incorporation of a metal center in a metallocene fashion. Thus, the ferrocenyl (**5** and **8**), ruthenocenyl (**6**) and osmocenyl (**7**) derivates were synthesised,[57] Figure 5A. All synthetized complexes exhibited moderate cytotoxicity in two breast cancer cell lines, MDA-MB-231 and MCF-7, see Figure 5B. Their senescence induction capacity in MDA-MB-231 was reported for the tamoxifen-like complexes of the three metals, species **5**–**7**, and the ferrocenyl diphenol derivative, complex **8**. The assessment was performed based on a SA-β-gal assay where overactivity the enzyme was analysed using a range of concentrations going from 0.5 to 4 μM. Figure 5C specifically showed the overactivity of the enzyme when the cells were incubated at 2 μM with complex **6**. This senescence induction was observed also for incubation at concentration of 0.5 and 3 μM in the case of complexes **5**–**7**, whereas for complex **8** senescence induction was displayed at even higher concentration (4 μM), Figure 5B. Thus, it can be said that incubation conditions seemed to be key for tamoxifen-like complex to induce senescence, being more effective in all cases when low concentrations were used (0.5 μM vs. 4 μM).

The same year, three additional Ru^{II} complexes containing a chiral oxoaporphine derivative (**9** (R/S-(±)), **10** (R-(+)) and **11** (S-(−)) showed to be promising pro-senescence probes, see Figure 6A.[58] The three of them exhibited moderate in vitro anticancer activities in a wide number of cell lines (BEL-7404, A549, MGC80, HeLa, Hep-G2, among others). The induction of senescence was studied on BEL-7404 cells using a subcytotoxic concentration (2.0 μM) to avoid acute cytotoxicity. After 7 days of treatment with complexes **9**–**11**, BEL-7404 cells presented some of the characteristic phenotype of SnCs, as big size and flat morphology. To corroborate the senescence induction, authors performed a β -gal assay, giving a positive response for all the probes. Moreover, it was also suggested that complex **11** was able to induce senescence due to the shortening of telomere length, whereas **9** and **10** could dysfunction telomeres, initiating in this way cellular senescence. On top of that, authors examined the cell cycle progression of BEL-7404 cells by flow cytometry. In view of the results shown in Figure 6B, authors claim an evident perturbation of the cell cycle with an arrest in S phase, which in concordance with a senescence induction process.

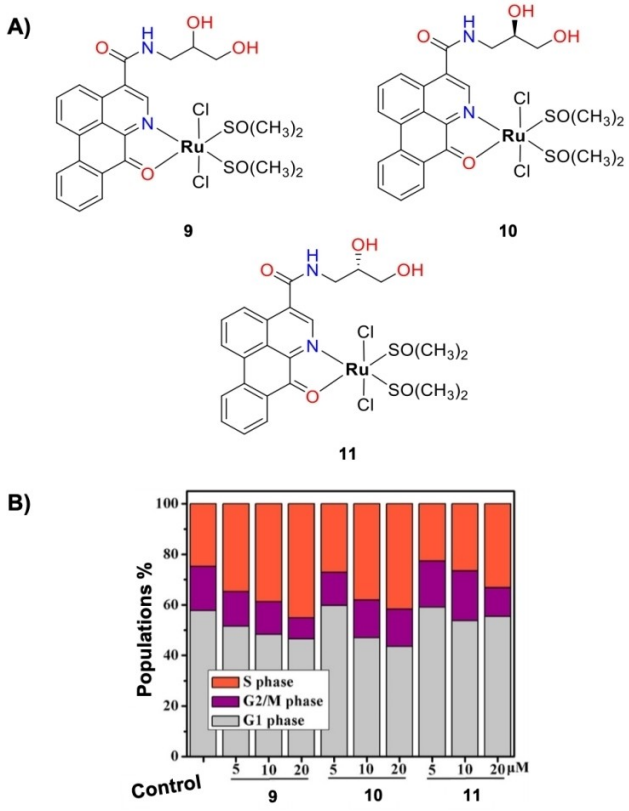
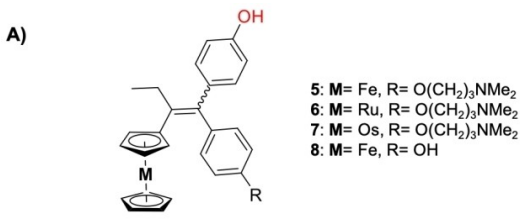


Figure 6. A) Depiction of complexes **9**–**11**. B) Population for the different phases of the cell cycle on BEL-7404 after being treated with **9**–**11** for 48 h using concentrations of 5, 10 and 20 μM by flow cytometry. Figure 6B was adapted with permission from Ref. [58]; Copyright (2019), American Chemical Society.



B)

Compound	IC_{50} values (μM) MDA-MB-231	MCF-7	Senescence Induction 0.5 μM	4 μM
5	0.5	0.8	+	No
6	1.9	2.4	+	No
7	2.7	2.9	+	No
8	0.6	0.9	+	+

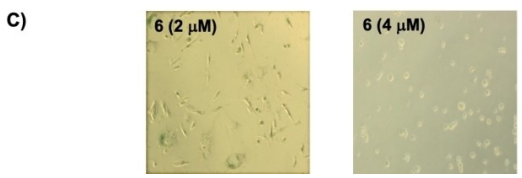


Figure 5. A) Reported metallocifen complexes (**5**–**8**). B) IC_{50} data of **5**–**8** incubated for 3 days in different tumor cell lines. C) SA-β-galactosidase assay to confirm senescence induction on MDA-MB-231 cells after 4 days of treatment using complex **6** (2 μM). Figure 5(B–C) were adapted with permission from Ref. [57]; Copyright (2015), WILEY-VCH.

More recently, in 2019 it was reported the first example of a "piano-stool" Ru^{II} complex which was able to induce senescence.[59] This Ru^{II} complex **12** followed a similar chemical structure to that of a RAPTA–C, but containing a pyridyl-based benzo[g]chromene-3-carbonitrile derivative instead of the PTA ligand, see Figure 7A. This complex exhibited a moderate inhibitory character in several cancer cell lines, with IC_{50} values between 13.5 μM on 518 A2 (melanoma) and 62.4 μM on HDFa

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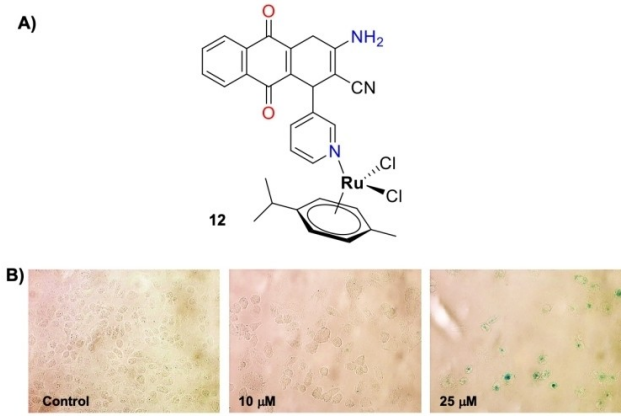

Figure 7. A) Structure of complex **12**. B) Microscopy images of senescence-associated β -galactosidase staining of 518A2 cells treated with **12** at 10 and 25 μM for 72 h. Figure 7B was adapted with permission from Ref. [59] Copyright (2019), Society for Biological Inorganic Chemistry.

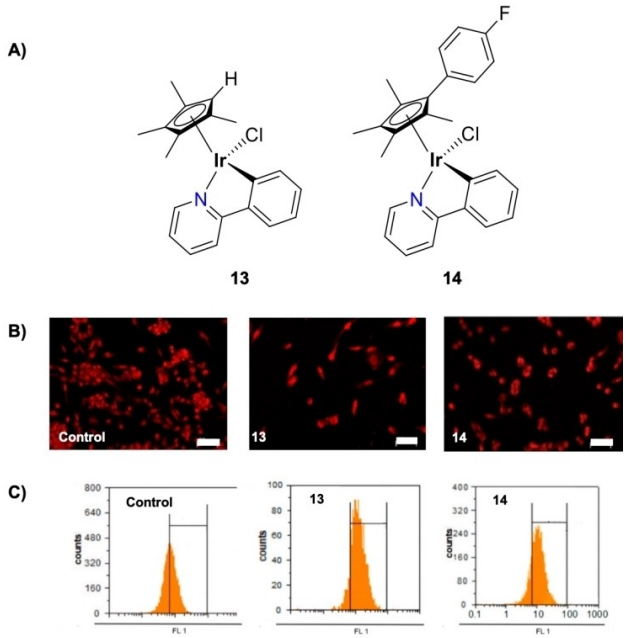

Figure 8. A) Structures of complexes **13** and **14**. B) Study of cell morphology changes using a fluorescence microscope, after the treatment of A2780 cells with Dako (a fluorescent mounting medium) and complexes **13** and **14** for 48 h. Scale bar: 20 μm. C) FDG assay for A2780 cells treated with **13** and **14** during 48 h, using channel FL1 (green emission). Figure 8 (B–C) were adapted with permission from Ref. [60] Copyright (2020), MDPI.

(human dermal fibroblasts) cells. Regarding its senescence induction character, the authors claimed that complex **12** was able to generate 518A2 SnCs in just 72 h using concentrations going from 10 to 25 μM in a SA-β-gal assay and staining in blue the increment of the enzyme activity. Figure 7B shows the blue staining of the SA-β-gal test, when complex **12** is incubated on 518A2 cells for 72 h using concentration of 25 μM. In this case, incubation at lower concentration (10 μM) did not deliver senescence induction. Additional mechanistic studies entailing nuclear uptake and DNA interaction revealed the DNA as the possible final target.

The last example that has been published of a metal complex able to generated senescence was reported by Maksimovic-Ivanic and Marchetti in 2021.[60] These authors described two samples of Ir^{III} complexes, **13** and **14**, which have cytotoxicity in six different human and rodent cell lines within a wide range of concentrations (IC_{50} values between 37–1.2 μM), Figure 8A. Studies made on A2780 cells (ovarian cancer cell line), showed that both complexes follow an apoptotic cell death mechanism by activation of caspases. Moreover, they disrupted the mitochondrial potential and were able to generate morphological changes. These alterations were attributed to the generation of senescence after 48 h of exposition using their IC_{50} concentration. Figure 8B showed those morphological changes in terms of cellular abnormal shape and giant nuclei indicating a senescence induction. Additionally, FDG (fluorescein di(β-D-galactopyranoside)) assay was performed using flow cytometry for detecting the increased activity of β-galactosidase. Population shifts observed at the flow cytometry graphs, demonstrate the higher β-galactosidase activity for the complexes **13** and **14**, being of 38% and 31% higher respectively in comparison with that of the control cells, Figure 8C. However, as the authors already claimed, additional assays need to be performed to corroborate whether the cells have entered a senescence stage.

After analyzing the few examples reported in the literature dealing with metal complexes in senescence, it seems evident that detecting the activity of β-galactosidase seems to be a standard hallmark in all of them. In some cases this assay was used as a single probe to suggest senescence induction (**1** and **2**). In this sense, it is important to remark the necessity of addressing senescence from at least three different hallmarks, some of them described in the previous section. Moreover, the incorporation of a parallel positive control it would provide additional evidence for senescence induction.

5. Complexes as Potential Pro-senescence Drugs

As it was noticeable in the previous section, research on complexes in the field of senescence is scarce. To the best of our knowledge, only six examples have been reported in the literature so far, describing their plausible senescence-inducer character. Despite that, a closer look to other examples on metal-based anticancer agents published, let us to think that this limited number of promising pro-senescence drugs could be higher. Many of these complexes can promote changes in cells that could fit with the induction of senescence. In this section a selection of those will be described.

In 2016, Gasser, Ferrari and coworkers,[61] published a Ru^{II} polypyridyl complex, **15**, which exhibits a nuclear localization in several cellular lines, including healthy and cancerous cell lines, Figure 9A. Authors probed that the mechanism of action entailed DNA intercalation interaction, via guanidine oxidation,

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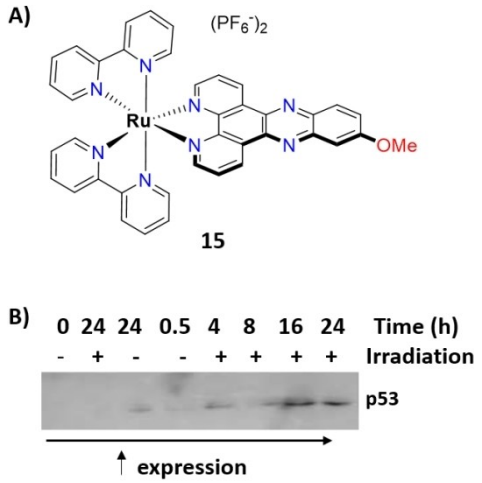


Figure 9. A) Structure of complex **15**. B) Western blot analysis of p53 expression in U2OS cells upon incubation with **15** at different times under irradiation conditions. Figure 9B was adapted from Ref. [61] with permission from the Royal Society of Chemistry.

upon UV irradiation. In addition to that, they showed that **15** exhibited two key features within the induction of senescence, being able to arrest the cell cycle under irradiation conditions at G2/M phase of U2OS cells (bone osteosarcoma) and to activate the protein p53, an important hallmark in senescence (Figure 9B). As consequence, complex **15** could be thought to be a plausible candidate for a pro-senescence drug.

In 2020, Liu and coworkers reported a Au^{I} complex containing as ligand an oleanolic acid derivative, Figure 10.[62] Authors claimed that this gold complex **16**, was not only selective for ovarian cancer A2780 cells, showing IC_{50} values of 10.24 ± 0.21 μM, but it also presented a particular mechanism of action. Complex **16** was able to inhibit the mitochondrial enzyme thioredoxine reductase (TrxR) and to generate dysfunction of mitochondria as well as endoplasmic reticulum stress (hallmark for senescence). Moreover, incubation with complex **16** for 72 h, blocked the cell cycle on A2780 cells. Most of the cell population accumulated at S phase, which is the responsible of the duplication of the genetic material. Such accumulation in S phase is likely to generate variations in the heterochromatin, another hallmark of senescence. Even though authors do not comment on these features in terms of senescence induction, the fact that **16** was able to generate a permanent cell cycle arrest together with the endoplasmic reticulum stress observed, indicate that this complex might be promoting senescence in A2780.

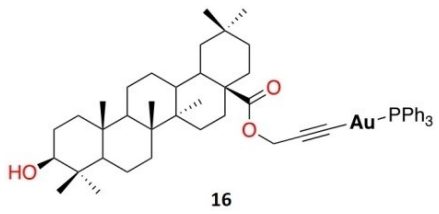
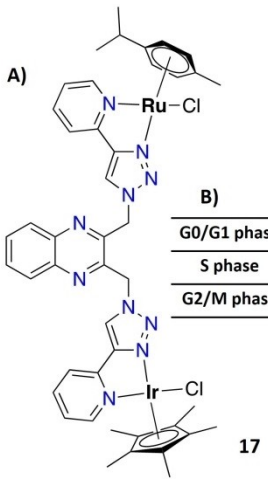


Figure 10. Chemical structure of complex **16**.

The same year, a Ru^{II}-Ir^{III} heterobimetallic complex **17**, was reported by Bose, Paira and collaborators,[63] Figure 11A. This luminescent binuclear complex was studied in several cancer cell lines, as HeLa (ovarian), Caco-2 (colon), HT-29 (colon) and HEK293 (kidney), exhibiting in all of them higher cytotoxicity than cisplatin. This good effect could be achievable thanks to the synergic effect between two different metallic centers, and a thoughtful ligand design that provided solubility, high lipophilicity and stability in the cellular microenvironment. The authors suggested mitochondria as final targets after performing both, co-localization and mitochondrial dysfunction assays. Once again, it is possible to see a permanent cell cycle arrest in G2/M phase in HT-29 cells, that goes from 15.2 to 32.0% of inhibition, upon increasing the complex concentration, Figure 11B. The permanent cell cycle arrest in G2/M phase and the dysfunctional mitochondria detected are in line with the initiation of a senescence process.

In 2021, other examples of plausible pro-senescence drugs have emerged. Wang and coworkers published two Pt^{II} complexes, **18** and **19**, containing a triphenylphosphonium group to promote mitochondrial targeting, Figure 12A.[64] These platinum complexes exhibited high cytotoxicity against human renal carcinoma 786-O and murine prostatic cancer cells RM-1. Both of them were probed to inhibit the activity of DNA topoisomerases, generating an irreparable DNA damage, which was evidenced by the overexpression of proteins p53, a senescence hallmark. On top of that, complexes **18** and **19** also prompted a cell cycle arrest process at G2/M phase, suggesting their ability to induce senescence under the appropriate incubation conditions, Figure 12B.

The same year, Chanda and coworkers,[65] described a Ir^{III} complex containing an indazole derivative, **20**, Figure 13A. Complex **20** presented antitumor effects for triple negative breast cancer cell lines (MDA-MB-231 and MDA-MB-468) and



B)

	Control	17 (10 μM)	17 (15 μM)
G0/G1 phase	46.5%	51.1%	41.6%
S phase	38.3%	34.0%	26.4%
G2/M phase	15.2%	14.9%	32.0%

Figure 11. A) Chemical structure of complex **17**. B) Table of the different phases of the cell cycle obtained by flow cytometry. Figure 11B was adapted with permission from Ref. [63]; Copyright (2020), American Chemical Society.

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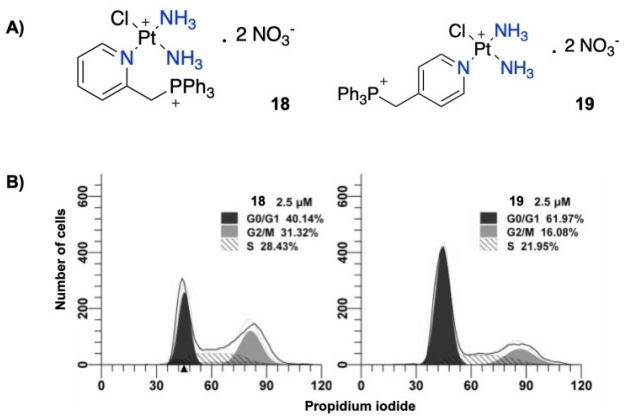

Figure 12. A) Structure of complexes **18** and **19**. B) Study by flow cytometry of their effects on the cell cycle of 786-O cells after an incubation period of 24 h. Figure 12B was adapted from Ref. [64] with permission from the Royal Society of Chemistry.

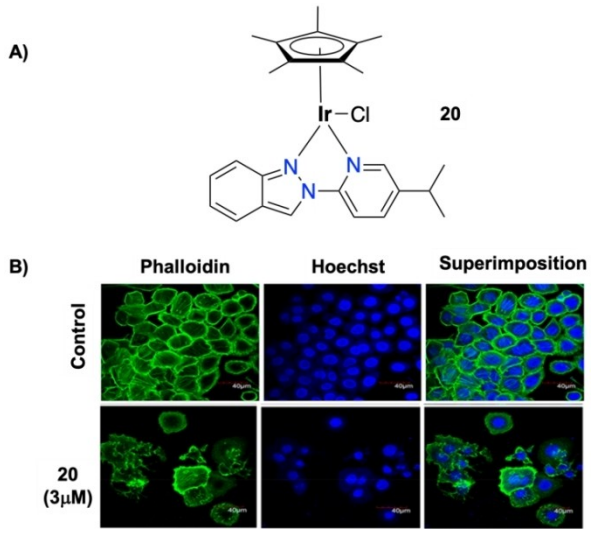

Figure 13. A) Structure of complex **20**. B) Confocal microscopy images of MDA-MB-468 cells incubated with **20**. Commercial trackers Phalloidin and Hoechst were used to for labelling F-actin and nucleus respectively. Scale bar: 40 µm. Figure 13B was adapted with permission from Ref. [65]; Copyright (2021), American Chemical Society.

also for colon cancer line HCT-116. Specifically, this complex was able to generate mitochondrial damage, to block the cellular cycle in G2/S phase and to inhibit cell migration. On top of that, a microscopy fluorescence assay where the cells were treated with complex **20** (3 µM) for 24 h and two internal standards revealed morphological changes in comparison with that of the control. Figure 13B showed an increment of the cell size and irregular cell morphology promoted by **20**. This fact, together with the mentioned cell cycle arrest, let us to think that induction of a senescent state might be taking place.

These examples just described in this section, represents only a small part of those complexes that, having been published in the last years as promising anticancer drug candidates, might also have potential as pro-senescence inducing drugs when they are incubated in the appropriate conditions. Therefore, a new horizon is opened within the field of senotherapy with the addition of metalo-based candidates in the search of novel pro-senescence drugs.

6. Conclusions

The prospects arising from the use of senolytic and pro-senescence drugs to deal with cancer are multiple and variable. From eliminating the cancerous SnCs and avoiding the possibility of cancer relapse, to induction of cancer cells to become senescent opening a new timeframe where alternative drugs can be used and/or the immune system could be activated. Although much work has already been done in senotherapy with the development of organic-based and nano-particle-based senolytic and pro-senescent drugs, only few examples have been reported for metal-based analogous, being all of them exclusively pro-senescent metallodrugs. The scarce amount of examples described herein emphasized the novelty of this field and the big challenge still ahead of us. On top of that, none of the described examples were conceived as specific complexes to be used in senotherapy, instead they were initially designed in their majority as anticancer agents. Therefore, the studies reported herein on the senescence field are, somehow, vague and they do not probe senescence generation thoroughly by identification of at least three of the hallmarks for SnCs. Sometimes only a single assay on the SA-β-gal activity or just a morphological cellular change was enough evidence for the authors to suggest cells entering in a senescent stage. Moreover, none of them have considered the possibility of performing a positive control experiment to corroborate the senescence induction, which weakens the final assessment. Despite that, these examples can be considered as a proof of concept to underline that metallodrugs must be included within senescence research as a new therapeutic approach. Metal-based probes could be good allies or worthy alternatives to conventional drugs due to the diverse bioactivity and/or photophysical properties, mostly diving by the presence of the metal center. The variety and different nature of hallmarks in senescence makes very difficult to list properties expecting from an ideal compound to target SnCs or to induce senescence. Therefore, finding a global marker or hallmark to target senescence is surely becoming a great asset among the research community not only for dealing with cancer, but also for addressing aging and related diseases.[61] Overactivity of SA-β-gal or overexpression of p53, p21 or p16 are some of the hallmarks that have been found, however, the lack of sensitivity or specificity urges to keep searching for better candidates. Identification and elimination of SnCs could highly contribute to tissue regeneration or avoid cancer relapse. Despite that, the elimination of these cells not always is necessarily good. In some cases, their generation could drive the activation of the immune response, prevent cancer cell proliferation when the cancer treatment is not working and allow taking advantage of novel therapies such as senotherapy to deal with cancer.[62,66] Deepen in the role of metallodrugs to promote senescence

and/or eliminate SnCs will surely open new and exciting perspectives in cellular senescence.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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