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Advances in Breast Cancer Drug Discovery: A Review of Therapeutic Strategies and Studies Involving Photosensitizers, Caged Xanthones and Thiosemicarbazones Derivatives

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Cancer is one of the major causes of death worldwide, and breast cancer is the most prevalent and deadly type among women. Despite the side effects and the phenomena of chemoresistance associated with the drugs involved, chemotherapy remains the main therapeutic strategy to combat and control breast cancer. Therefore, several new classes of compounds against breast cancer have been explored, in an effort to identify new drug candidates with alternative mechanisms of action. The principal results of such exploration, focusing on caged xanthones, thiosemicarbazones and photosensitizers, are presented in this review, along with the main aspects of the drug discovery process against breast cancer. More specifically, the design, structure-activity relationship investigations and anti-breast cancer properties of these three classes are described and discussed in this work.

Keywords: breast cancer, drug discovery, caged xanthones, thiosemicarbazones, photosensitizers

1. Introduction

Cancers are a group of diseases characterized by the uncontrolled growth of mutated cells, resulting in the formation of localized tumors.[1,2] The exact causes for the occurrence of cancers are often individual-dependent and unclear, due to the wide variety of possible risk factors. Indeed, the development of cancers can be linked, among others, to age, diet (excessive processed food and/or alcohol consumption), lifestyle (physical activity and/or tobacco consumption) or working routine (occupational exposure to carcinogen products) and can also be associated to environmental factors (exposure to UV and ionizing radiation) or to mutations naturally occurring in biological processes.[2-4] As a result, cancer is one of the leading causes of premature death and the second highest cause of death in the world, with 9.6 million deaths in 2018.[5]

Breast cancer is leading in terms of incidence, mortality, and prevalence among women worldwide. Indeed, 2.3 million breast cancer cases and 685 000 deaths from breast cancer were reported in 2020 at the global scale,[6] and these values are highly underestimated due to the discrepancy in data availability between the different regions of the world.[5] Current chemotherapeutic treatment for breast cancer involves different classes of drugs that

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act through different mechanisms of action. However, side effects and chemoresistance reduce the therapeutic efficacy of these compounds, reinforcing the need to develop new drugs against breast cancer.[7,8]

In this review, we discuss the main aspects of drug discovery against breast cancer, describing a general overview of the disease, including prognosis, therapeutic strategies, drugs already used in chemotherapy, their mechanism of action and limitations. We also highlight the main findings obtained with caged xanthones and thiosemicarbazones, including metal complexes of the latter, which are two widely explored chemical classes that we selected based on both their relevance in terms of chemical diversity and their recent results regarding cytotoxicity against breast cancer cells. Caged xanthones have an unusual chemical architecture, while thiosemicarbazones tolerate high chemical versatility, allowing different modes of coordination with metal centers. In addition to breast cancer, both classes are studied against a wide range of different cancers, providing a suitable therapeutic window for clinical applications as anticancer agents.[9,10] Additionally, we discuss the treatment of breast cancer using photodynamic therapy (PDT), emphasizing the large diversity of photosensitizers (phenothiazines, porphyrins, chlorins, anthraquinones and organometallics) tested *in vitro* and *in vivo* against breast cancer.

2. Breast Cancer Prognosis and Therapeutic Strategies

In recent decades, scientific and technological progress has allowed the characterization of breast tumors, which have, histologically, been classified as *in situ* or invasive, mainly affecting the epithelial cells of the ducts and/or breast lobules. Diagnoses of breast carcinoma *in situ* have increased, however, invasive ductal carcinoma, now defined as 'no special type' (NST) is the most commonly, accounting for about 70 to 90% of cases worldwide. In addition, about 10% of *in situ* carcinomas can progress to NST.[11-13] However, given their expressive heterogeneity, breast tumors are also molecularly categorized. In this sense, the activation of human epidermal growth factor receptor 2 (HER2, encoded by the oncogene *ERBB2*), the expression of hormone receptors (estrogen receptor-ER and progesterone receptor-PR) and/or penetrating mutations in the suppressor genes *BRCA1* (17q21) and *BRCA2* (13q13) are also considered.[14]

The molecular classification of breast cancer was initially proposed by Perou *et al.*[15] and the five surrogate intrinsic subtypes typically used clinically are: (*i*) Luminal A, (*ii*) Luminal B-like HER2–, (*iii*) Luminal B-like HER2+, (*iv*) HER2-enriched, and (*v*) Triple-negative (TN). Tumors that express ER or PR are considered luminal and differ in the staining pattern and proliferative profile. The Luminal A breast cancer is strongly ER+ and PR+; HER2– with low proliferation rates; the Luminal B-like HER2– expresses high Ki67 index and the Luminal B-like HER2+ overexpresses HER2 with lower levels of ER and/or PR. HER2-enriched tumors are ER–, PR–, HER2+ and the TN does not express none of these receptors.[14] Currently, the staging of the disease considers not only the histological types, but also the molecular aspects of the tumor, being essential in the prognosis of the disease.[16] The traditional TNM system of the American Joint Committee on Cancer (AJCC) evaluates the size of the tumor, represented by the letter "T", the involvement of the lymph nodes or "N" and the occurrence of distant metastases, defined by "M".[17,18] This system was, for a long time, widely used in clinical practice using numbers ranging from 0 to 4 to progressively represent the progression and aggressiveness of the disease.[17,19] The TNM system was update, and the molecular characteristics of the lesions and the histological grade were incorporated. Tumor grade is defined by cell differentiation and the histological distance between normal and transformed cells. As a result, staging has become more complex, and with greater accuracy in patient management[20] which is defined as:

- Stage 0: no evidence of invasion of other tissues (metastasis);
- Stage I (IA and IB): tumors measuring up to 2 cm and the presence of tumor cells in the lymph nodes (0.2-2 mm);
- Stage II (IIA and IIB): tumors measuring between 2 and 5 cm, with or without invasion of up to three lymph nodes;
- Stage III (IIIA, IIIB and IIIC): tumors that measure > 5 cm and/or that have invaded more than four nearby lymph nodes;
- Stage IV: invasive BC that have already compromised other organs in the body, such as the lungs, distant lymph nodes, skin, bones, liver, or brain.[17,20]

However, the expression of ER, PR and/or HER2 alters the classification of tumors, as they respond to hormone treatment or anti-HER2 antibodies. As an example, a tumor between 2 and 5 cm and HER2+, PR+ and ER+ is classified as stage I, not stage II.[17,20] Thus, staging directs the therapeutic decision, along with the hormonal status of the patient, the expression of specific targets and the presence of comorbidities. Locoregional treatment and systemic therapy are the main pillars for breast cancer control.[21] Although operable when initially performed, most women with the disease require systemic treatment. Early

tumors and those classified as luminal and HER2– receive hormone therapy and may not be eligible for chemotherapy. However, proliferative index markers such as Ki67 may influence this therapeutic regimen. HER2+ tumors and TNs are treated with chemotherapy combined or not with targeted therapy.[22] TN breast cancer challenges clinical practice, with a worse prognosis, higher proliferative, invasive and metastatic capacity and higher rates of recurrence.[23] They do not respond to hormone therapy and anti-HER2 antibodies.[24] Despite important advances with the use of poly (ADP-ribose) polymerase (PARP) inhibitors (Anti-PARP) and programmed cell death ligand 1 (PD-L1) inhibitors (Anti-PD-L1),[25] a small number of patients are eligible for these drugs and current chemotherapy drugs remains the main therapeutic strategy, being responsible for reducing the risk of recurrence in about 30% of cases.[26]

3. Main Chemotherapy Agents Used in The Treatment of Breast Cancer

In chemotherapy, compounds with cytotoxicity or with cell proliferation inhibitory activity are used, with systemic action, administered to patients with breast cancer by oral or intravenous route. The tumor microenvironment (TME) favors the greatest influx of these substances, however, as they lack specificity, they also reach non-transformed cells.[27] Chemotherapy, by targeting circulating tumor cells, aims to increase the chance of cure, decrease the risk of recurrence, increase the chances of survival and improve the quality of life of patients.[28] It can be adopted before surgery, in a neoadjuvant system, enabling tumor reduction and a better delimitation of the surgical area, reducing the chances of formation of residual tumors. It has been adopted in TN and HER2+ breast tumors, with significant improvement in the prognosis of patients.[29] When adopted after surgery, chemotherapy is called adjuvant and is considered the gold standard in the control of advanced cases of the disease.[30,31] The absolute benefit of neoadjuvant or adjuvant chemotherapy depends on the risk of recurrence, which is closely related to tumor characteristics.[26]

In this context, the grade and size of the lesion, presence of tumor cells in lymph nodes, age, recurrence, general health status of the patient, molecular classification and genetic profile are considered in determining the chemotherapy regimen.[18,26] Currently, drugs used in the treatment of breast cancer (Figure 1 and Table 1) are classified according to their mechanism of action (Figure 2), with emphasis on alkylating, antimitotic, antimetabolite and anthracyclic agents.[8,61]

Chemotherapeutics compounds that can interact with deoxyribonucleic acid (DNA) and block its replication are known as DNA alkylating agents.[62,63] More specifically, these drugs interact with the double helix, replace alkyl groups with hydrogen atoms, resulting in the formation of cross-links between the two strands and preventing their separation during replication, and therefore resulting in cytotoxic, mutagenic and carcinogenic effects.[64,65] These chemotherapy drugs are subdivided into six classes:

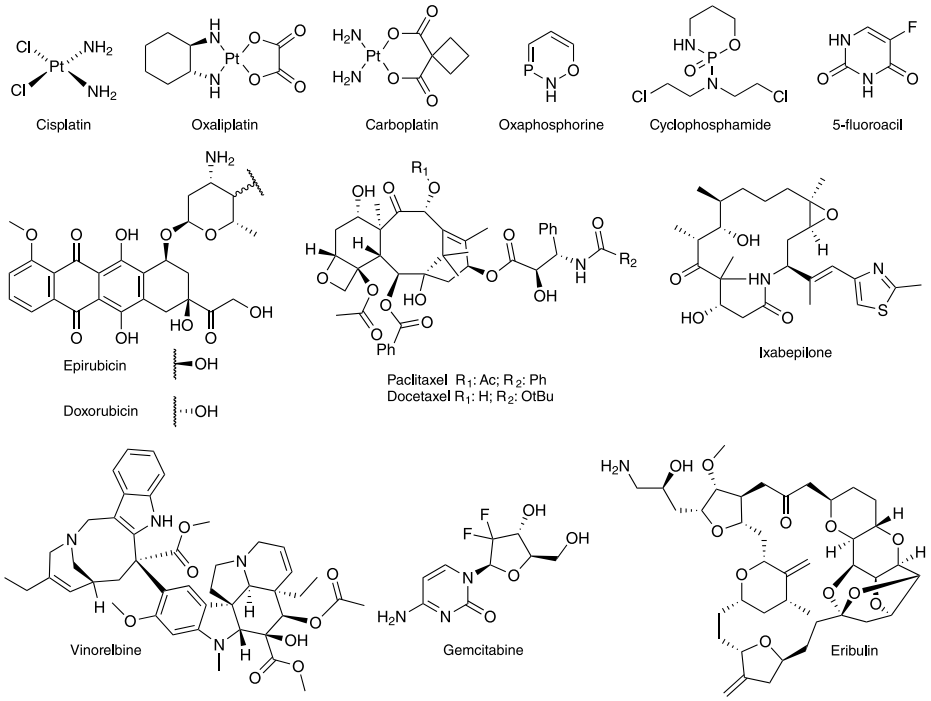


Figure 1. Chemical structure of drugs used in the chemotherapeutic treatment of breast cancer.

Table 1. Details of drugs routinely used in the chemotherapy treatment of breast cancer

Class	Mechanism of action	Drugs	Breast cancer treatment	Side effects
Alkylating agents	bind to DNA, affecting its replication and repair and inducing cell death by apoptosis or necrosis[32-34]	cisplatin, oxaliplatin, and carboplatin[32,34]	neoadjuvant, adjuvant, and metastatic cancer therapy (mostly combined with taxanes or anthracyclines)[34,36]	about 40 side effects and dose limiting; main side effects: nephrotoxicity, myelosuppression, neurotoxicity, cardiotoxicity[37,38]
		oxazaphosphorines-cyclophosphamide[35]		main side effects: nephrotoxicity, neurotoxicity, cardiotoxicity[33,39,40]
Antimitotic agents	stabilize microtubules[41]	ixabepilone, eribulin	therapy of patients with breast cancer resistant to multiple chemotherapeutic agents (anthracycline, taxane, and capecitabine);[43] can be used alone or in combination with capecitabine[44]	may lead to peripheral neuropathy, but reversible[44]
		taxanes-paclitaxel and docetaxel[41]	neoadjuvant, adjuvant and metastatic cancer therapy[45]	dermatological,[47] ophthalmological,[48] and hypersensitivity reactions[49]
		vinorelbine	neoadjuvant, adjuvant and metastatic cancer therapy; can be used in combination with other chemotherapy drugs[46]	neutropenia and leukopenia[50]
Antimetabolites	inhibit the activity of thymidylate synthase[51]	capecitabine and its active 5-fluorouracil	treatment of patients after treatment failure with anthracyclines and taxanes, or in residual cancers[43,52]	hepatotoxicity[55] and dermatological effects[56]
		gemcitabine	therapy of metastatic cancers (usually in combination with anthracyclines or taxanes)[53,54]	neutropenia and myelosuppression[53,54]
Anthracyclines	hydrophobic drugs that attract the DNA molecule; intercalate the DNA molecule, inhibiting topoisomerase II and increasing the production of reactive oxygen species[33,57]	doxorubicin and epirubicin[33,57,58]	neoadjuvant, adjuvant and metastatic cancer therapy[33,57]	cardiotoxicity with cumulative profile[33,57,59]

DNA: deoxyribonucleic acid.

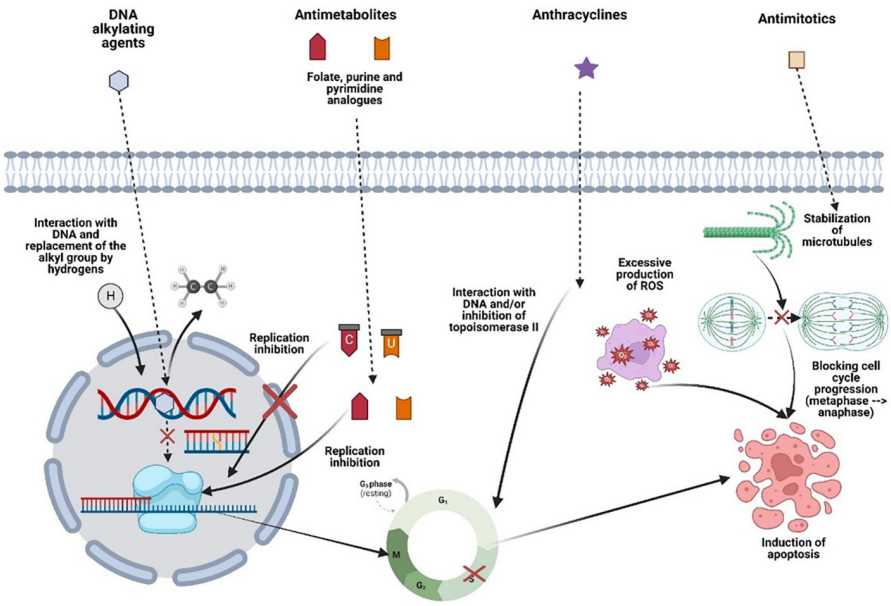


Figure 2. Representation of the mechanism of action of the main chemotherapy drugs used in the treatment of breast cancer. Including DNA alkylating agents, antimetabolites, anthracyclines and antimitotics. Figure created with BioRender.[60]

nitrogen mustards, ethyleneamine and methylenamine derivatives, alkyl sulfonates, nitrosoureas, triazenes and platinum-containing agents.[65] Among the class of alkylating agents, the most used in breast cancer therapy are platinum-based chemotherapy and oxazaphosphorines.[33]

The main representatives of antimitotic are taxanes, ixabepilone, eribulin and vinorelbine. They are compounds with similar functions, stabilizing the mitotic spindle fibers.[66] In terms of chemical structure, taxanes are characterized by the presence of the taxane ring and a bulky ester side.[67] Ixabepilone is a semi-synthetic analogue of epothilone B,[68] eribulin, in turn, is a synthetic macrocyclic ketone, analogue of Halichondrin B (macrolide polyether)[69] and vinorelbine is a representative of vinca alkaloids (*Vinca rosea*).[70] Microtubule stabilization prevents cell cycle progression and consequently induces apoptosis.[71]

Regarding antimetabolites, they are drugs that activate apoptotic mechanisms by disrupting metabolic pathways that are directly or indirectly involved in the synthesis phase (Phase S) of the cell cycle.[72,73] These compounds are analogous substances to folate, purines and pyrimidines.[63,74] In terms of structure, they are similar to cytosine and uracil.[72] For this reason, antimetabolites can significantly affect the activity of enzymes involved in DNA replication. The chemotherapy drugs 5-fluorouracil, capecitabine and gemcitabine are included in this class for breast cancer treatment.[75]

Finally, anthracyclines are glycosidic drugs that have in their chemical structure the amino sugar daunosamine linked to an aglycone hydroxyanthraquinone. These drugs interact with the DNA molecule, induce the excessive production of reactive oxygen species and inhibit the enzyme topoisomerase II.[76,77]

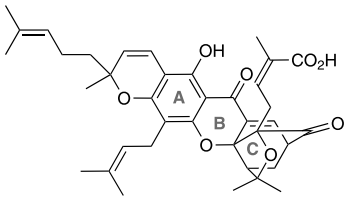
Chemotherapy drugs traditionally used have limitations, which are reflected in the significant number of relapse cases, especially in more advanced cases. In addition to side effects, chemoresistance also stands out, which involves different mechanisms including changes in membrane permeability, activation of drug efflux pathways, and changes in gene expression profile and TME modulation.[78]

Therefore, tumor cells resistant to anthracyclines, taxanes, ozaphosphorines and platinum-based drugs that already commercialized are common.[33] In this context, the search for new drugs, the targeted synthesis of chemotherapy drugs and new therapeutic strategies are urgently needed.[22] In addition, changing the structure, route of administration and conjugating the current chemotherapeutic compounds with other drugs can increase the efficiency of the treatment and avoid chemoresistance pathways.[79,80]

Different synthesis strategies can be adopted, and the targeted one has been shown to be interesting in targeting pathways known to be important for tumor progression.[81] Therefore, as some mechanisms of resistance to chemotherapy have already been described, it is possible to design and synthesize compounds that act on different targets, thus increasing the chance of therapeutic success.[82] However, it has been pointed out that the directed synthesis can make the research more biased, limiting its effects and, over time, promoting again chemoresistance, relapse and death. In this context, libraries of compounds with distinct groups and characteristics are created, seeking to select the most effective ones and, subsequently, describe their mechanisms of action.[83-85]

4. Caged Xanthones against Breast Cancer

Caged xanthones is a class of natural and synthetic compounds that are structurally defined by the presence of a xanthone motif in which its C-ring has been converted into a caged structure. Gambogic acid (GA), a caged *Garcinia* xanthone and the main bioactive compound of this chemical class, is a natural compound readily isolated from various plant species of the *Garcinia* genus, such as *Garcinia hanburyi* or *Garcinia morella*. More specifically, GA can be extracted from the resin of these two species, which is traditionally used in folk medicine in Southeastern Asia. This compound exhibits a highly peculiar structure, composed of the three fused rings (A, B, and C, Figure 3) characterizing its xanthone moiety, which bears an additional caged ring on ring C.[9,86]



GA

IC_{50} (MCF-7) = 0.4 - 2.79 μM
IC_{50} (MDA-MB-231) = 0.24 - 1.50 μM
IC_{50} (T47D) = 0.2 - 0.78 μM
IC_{50} (ZR751) = 0.4 - 1.64 μM

Figure 3. Structure of gambogic acid (GA) and cytotoxicity ranges reported against breast cancer (BC) cell lines. IC_{50}: half-maximal inhibitory concentration.

GA has been identified as a promising agent against breast cancer, after having shown low-micromolar cytotoxicity *in vitro* against diverse breast cancer cell lines such as MCF-7, MDA-MB-231, T47D, and ZR751 (Figure 3).[87-97] GA has been shown to efficiently depolymerize microtubules and increase the phosphorylation levels of both p38 and JNK-1(C-Jun N-Terminal Kinase-1) enzymes in MCF-7 cells, resulting in G2/M cycle arrest and apoptosis of breast cancer cells.[98] The induction of cell cycle arrest and

MCF-7 cell apoptosis by GA could also be attributed to MDM2 (Murine double minute 2) oncogene inhibition and degradation,[99] and to p53 and Bcl-2 (B-cell lymphoma 2) downregulation.[100] Regarding other breast cancer cell lines, the apoptosis-inducing activity of GA in MDA-MB-231 cells could be linked to reactive oxygen species accumulation and activation of the mitochondrial apoptotic pathway,[101] and its autophagy-inducing activity in these cells was proven to be due to mutant p53 (mutp53) inhibition.[102] Furthermore, the apoptosis of MDA-MB-231 cells could be induced by GA in both TfR1(transferrin receptor 1)-dependent and TfR1-independent ways,[103] highlighting the wide range of biochemical pathways available for GA to target breast cancer cells.

In addition to inducing apoptosis in breast cancer cell lines, GA managed to inhibit *in vitro* the adhesion, migration, and cell invasion of MDA-MB-231 and MDA-MB-435 cells via MMP-2 (matrix metalloproteinase 2) and MMP-9 (matrix metalloproteinase 9) inhibition through MAPK (mitogen-activated protein kinase) and kinase C signaling pathways.[104,105] Such results suggested that GA could efficiently reduce the formation of breast cancer metastases, which was confirmed *in vivo*, as GA significantly inhibited (50% inhibition) the formation of lung metastases in MDA-MB-435 xenografted mice.[105] Other *in vivo* studies further confirmed the inhibitory effect of GA against both BC tumor growth and lung metastases (MDA-MB-231 xenograft).[101]

Given the promising results shown by GA *in vitro* and *in vivo*, its pharmacokinetic properties were evaluated in order to assess its viability as a drug candidate. The low water solubility of GA (intrinsic solubility: 13 μg mL^{-1} or 15 μM) was highlighted,[106,107] which could hamper its potential as an oral drug. Even though *in vivo* ADMET (absorption, distribution, metabolism, excretion and toxicity) studies revealed a non-toxic dosage of GA in rats eighteen times higher than the dose used in human clinical trials, they also showed that GA was mainly distributed in the liver and kidneys, which might be harmful at the concentrations necessary for its cytotoxic effect.[108] Finally, this compound displays low metabolic stability *in vivo*, and a few studies already identified diversified metabolites and metabolic pathways of GA in rats or even humans.[109,110]

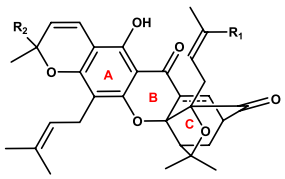
Therefore, despite the interesting anticancer properties of GA, these pharmacokinetic limitations highlighted the need for new GA-based caged xanthones derivatives to treat breast cancer, which could retain its high therapeutic potential while overcoming its pharmacokinetic limitations. As a result, a significant amount of research has been performed in the last two decades, focusing on both the development of synthetic strategies for the construction of the caged C-ring fragment of caged xanthones, and on the structure-activity relationship and optimization of this class against breast cancer. The main strategies towards optimized compounds involved the chemical simplification of GA, mainly by removing the prenylated groups, and the insertion of polar and ionizable groups in the different fragments, which enabled the discovery of pharmacophoric groups, the optimization of the potency against breast cancer, and an overall improvement of the pharmacokinetic properties. In this topic, we will discuss the main strategies for optimizing this chemical class and present the most promising compounds (half-maximal inhibitory concentration (IC_{50}) < 20 μM) against various breast cancer cell lines, highlighting compounds with nanomolar potency (IC_{50} < 1 μM). Quite regrettably, all the relevant studies performed with caged xanthones did not determine the selectivity of those derivatives towards cancer cells *vs.* unmuted cells, despite its crucial importance in anticancer drug discovery.

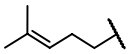
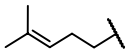
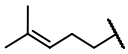
4.1. Structural modifications on the side chains of GA

GA derivatives with modifications on the prenylated side chains of A and C (removal of the carboxyl group) rings have been investigated, to assess their apoptosis-inducing activity (Table 2). These compounds maintained the activity against the MCF-7 cell line, as compounds **1** and **2** displayed potency comparable to GA (IC_{50} = 0.40 μM) with IC_{50} values of 0.90 and 1.10 μM, respectively.[87] However, the removal of the unsaturation in the C ring (compound **3**) resulted in an important decrease in activity against T47D cells, suggesting that the α,β-unsaturated ketone of GA is critical for its apoptosis-inducing activity.[90]

The introduction of diverse esters and amides on the side chain of the dihydropyran ring of GA, while maintaining a methoxy group on ring A and a methyl ester on ring C, delivered derivatives with anti-proliferative activity against MCF-7 cells (measured in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays). Indeed, the IC_{50} obtained for these compounds (**4**-**9**, Table 3) ranged from 0.98 to 5.05 μM, among which the *N*-methylpiperazinamide **8** exhibited a potency three times higher than GA (IC_{50} = 2.79 μM).[92] Other derivatives bearing similar modifications, introducing aliphatic amines, anilines and ethers at the end of the prenylated side chain, were also assayed against the MDA-MB-231 cell line. These compounds (**10**-**25**) exhibited a considerable variation in their cytotoxic activities, with IC_{50} varying from 0.68 to 5.25 μM. Three of them, namely **13** (IC_{50} = 0.99 μM), **15** (IC_{50} = 0.68 μM), and **21** (IC_{50} = 0.80 μM) reached nanomolar levels of potency, even though they slightly lost potency when compared to GA

Table 2. Cytotoxic activity of GA and derivatives **1**-**3** against MCF-7 and T47D cells



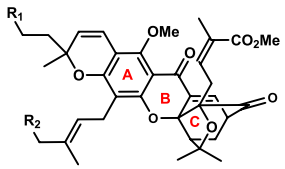
Compound	---	R_1	R_2	IC_{50} / μM		Reference
				MCF-7	T47D	
1	═	H	Me	0.9	ND	87
2	═	Me		1.1	ND	87
3	—	CO_2H		ND	> 20	90
GA	═	CO_2H		0.4	0.7	87,90

IC_{50}: half maximal inhibitory concentration; GA: gambogic acid; ND: not determined.

(IC_{50} = 0.24 μM) (Table 3).[94] Performing similar changes on the other prenylated chain of the A ring delivered compounds with cytotoxic activity against the same cell line comprised between 0.22 and 5.31 μM, with compounds **31** (IC_{50} = 0.89 μM), **34** (IC_{50} = 0.40 μM), **43** (IC_{50} = 0.22 μM) and **44** (IC_{50} = 0.87 μM) displaying nanomolar activity, and only compounds **34** and **43** having an antitumoral activity comparable to GA (Table 3).[89]

As mentioned before, one of the limitations of GA is its poor water solubility, which negatively affects its

Table 3. Cytotoxic activity of GA and derivatives **4**-**44** against MCF-7 and MDA-MB-231 cells



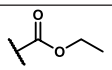
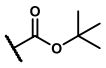
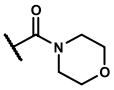
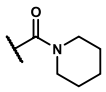
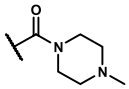
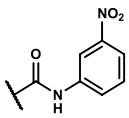
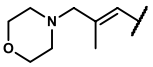
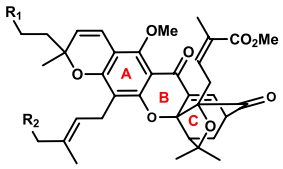
Compound	R_1	R_2	IC_{50} / μM		Reference
			MCF-7	MDA-MB-231	
4		H	5.04	ND	92
5		H	5.05	ND	92
6		H	2.82	ND	92
7		H	1.52	ND	92
8		H	0.98	ND	92
9		H	3.80	ND	92
10		H	ND	4.00	94

Table 3. Cytotoxic activity of GA and derivatives **4**-**44** against MCF-7 and MDA-MB-231 cells (cont.)



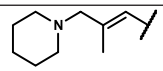
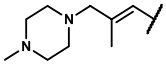
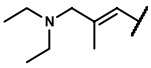
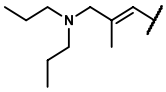
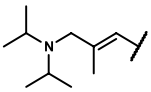
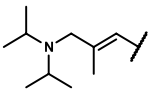
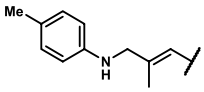
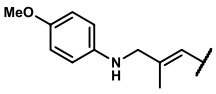
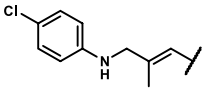
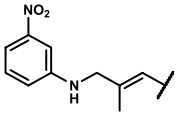
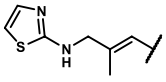
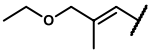
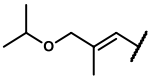
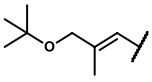
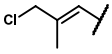
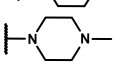
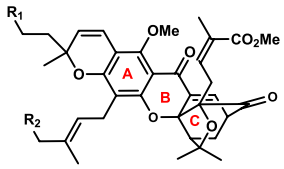
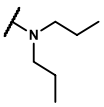
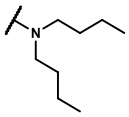
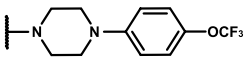
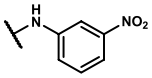
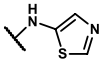
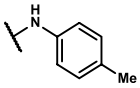
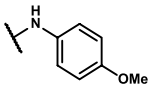
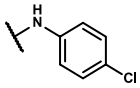
Compound	R_1	R_2	IC_{50} / μM		Reference
			MCF-7	MDA-MB-231	
11		H	ND	4.44	94
12		H	ND	1.62	94
13		H	ND	0.99	94
14		H	ND	1.56	94
15		H	ND	0.68	94
16		H	ND	1.48	94
17		H	ND	5.25	94
18		H	ND	4.91	94
19		H	ND	3.73	94
20		H	ND	4.27	94
21		H	ND	0.80	94
22		H	ND	2.28	94
23		H	ND	1.88	94
24		H	ND	5.13	94
25		H	ND	1.15	94
26			ND	5.31	89
27			ND	2.62	89
28			ND	1.09	89
29			ND	1.20	89

Table 3. Cytotoxic activity of GA and derivatives **4**-**44** against MCF-7 and MDA-MB-231 cells (cont.)



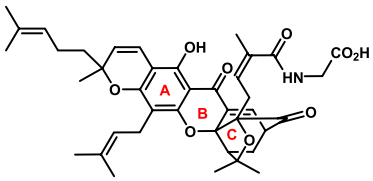
Compound	R_1	R_2	IC_{50} / μM		Reference
			MCF-7	MDA-MB-231	
30			ND	1.56	89
31			ND	0.89	89
32			ND	2.40	89
33			ND	5.25	89
34			ND	0.40	89
35			ND	3.73	89
36			ND	4.25	89
37			ND	1.34	89
38			ND	2.28	89
39			ND	1.88	89
40			ND	4.13	89
41			ND	1.15	89
42			ND	1.02	89
43			ND	0.22	89
44			ND	0.87	88
GA	–	–	2.79	0.24	89,92,94

IC_{50}: half maximal inhibitory concentration; GA: gambogic acid; ND: not determined.

potential to becoming an oral drug. Thus, to improve this parameter, the compound **45** was designed to incorporate a glycine linker in the carboxyl fragment. However, despite encouraging results obtained against MDA-MB-435 (IC_{50} = 1.90 μM) and MDA-MB-468 (IC_{50} = 1.85 μM) cells, SRB (sulforhodamine B) assays performed with **45** revealed a certain disparity in terms of activity against different BC cell lines, with a marked loss of activity against drug-resistant MCF-7/ADR cells (Table 4).[111]

Similarly, ester and amide derivatives of GA bearing either a hydroxyl, a methoxy or an acetyl group on the A ring, were evaluated in HTS (high-throughput screening) caspase activation assay against T47D and ZR751 cells. In a general way, such structural modifications led to

Table 4. Structure and cytotoxic activity of **45** against MCF-7, MCF-7/ADR, MDA-MB-468 and MDA-MB-435 cells

Compound	Structure	IC_{50} / μM				Reference
		MCF-7	MCF-7/ADR	MDA-MB-468	MDA-MB-435	
45		5.55	25.40	1.85	1.90	111

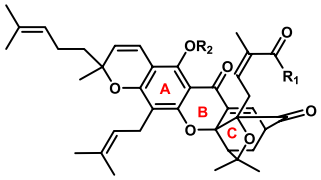
IC_{50}: half maximal inhibitory concentration.

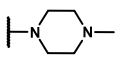
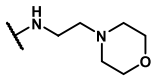
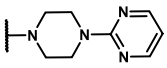
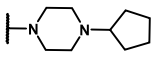
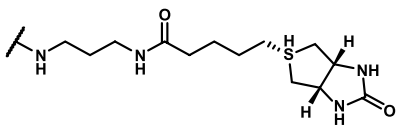
improvements in terms of potency against both BC cell lines in comparison with GA (IC_{50}^{T47D} = 0.78 μM, IC_{50}^{ZR751} = 1.64 μM). Specifically, the piperidinamide **47** and the piperazinylpyrimidinamide **50** exhibited nanomolar activities respectively 4- and 3-fold higher than GA against T47D cells, and both proved to be twice as potent as GA against ZR751 cells (Table 5).[97]

4.2. Structural modifications based on simplified structures of GA

In addition to the extensive research work that delivered derivatives based on the full core of GA, more recent studies suggested that the dihydropyran moiety on the A ring does not exert a substantial effect on the cytotoxicity, and that

Table 5. Cytotoxic activity of GA and derivatives **46**-**55** against T47D and ZR751 cells



Compound	R_1	R_2	IC_{50} / μM		Reference
			T47D	ZR751	
GA	OH	H	0.78	1.64	97
46	OMe	H	0.44	1.29	97
47		H	0.21	0.78	97
48		H	1.39	3.85	97
49		H	0.68	1.76	97
50		H	0.24	0.78	97
51		H	0.38	1.24	97
52		H	0.56	1.14	97
53	OMe	Me	0.80	2.31	97
54		Me	0.42	1.01	97
55	OH	Ac	0.51	1.39	97

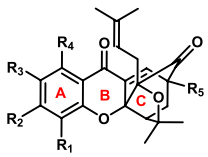
IC_{50}: half maximal inhibitory concentration; GA: gambogic acid.

cluvenone (CLV) represents the minimum pharmacophore of the caged xanthones compounds (Table 6).[91,112] Thus, the cytotoxic activity against human breast carcinoma MCF-7 cells of CLV derivatives with different substitution patterns for the hydroxyl and prenylated groups on ring A was evaluated in MTT assays. However, the activity of these CLV derivatives only remained in the low micromolar range, with IC_{50} values varying between 11.90 and 6.36 μM (compounds **56**-**60**, Table 6).[91] Other A- and C-ring-modified CLV analogs displayed moderate to strong cytotoxicity against the MCF-7 and MDA-MB-231 cell lines, with compound **61** exhibiting an IC_{50} of 0.42 and 2.17 μM against MCF-7 and MDA-MB-231 cells, respectively.[113] The introduction of relatively small substituents on the A ring, such as a hydroxyl or MOM (methoxymethyl) group or a fluorine atom, identified compounds with potency similar to or twice higher than CLV in Spheroids^{MARY-X} model (compounds **63** and **64**, Table 6).[112] The OH group was given slightly more attention, with hydroxycluvenones **65**-**68** being tested against the MCF-7 cell line in MTT assays. Among this small subset of derivatives, the two *meta*-hydroxycluvenones (*meta vs*. B-ring ketone, compounds **66** and **68**, Table 6) only reached half of the potency of CLV, while the potency of the two others (compounds **65** and **67**, Table 6) remained similar, but slightly better, than CLV against MCF-7.[95]

Attempts to improve the drug-like properties of CLV have been carried out, by introducing a carbamate moiety on the A ring (compounds **69**-**78**). Even though no pharmacokinetics evaluation of these derivatives has been published yet, the initial *in vitro* structure-activity relationship (SAR) studies revealed a potent antiproliferative activity against MDA-MB-231 cells. Indeed, all carbamate CLV derivatives but one, namely the pyrrolidinocarbamate **73**, were 1.5 to 10 times more potent than **GA** (Table 7),[96] which constitutes encouraging initial results for this class of compounds.

Natural product analogs of CLV isolated from *Garcinia cantleyana*, bearing fused furan and dihydrofuran rings on the A ring, have also been considered as potential

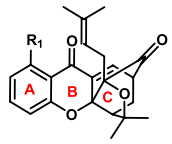
Table 6. Cytotoxic activity of CLV and derivatives **56**-**68** against MCF-7 cells, MDA-MB-231 cells, and in Spheroids^{MARY-X}

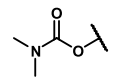
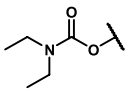
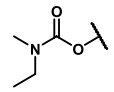
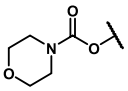
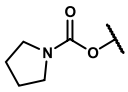
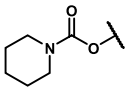
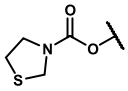
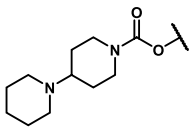
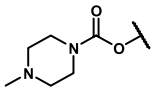
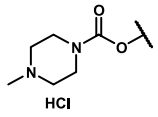


Compound	R_1	R_2	R_3	R_4	R_5	IC_{50} / μM			Reference
						MCF-7	MDA-MB-231	Spheroids^{MARY-X}	
CLV	H	H	H	H	H	13.9[95]	ND	0.63[112]	95,112
56		OH	H	H	H	11.9	ND	ND	91
57	H	H	OH		H	6.97	ND	ND	91
58	H	H		OH	H	9.06	ND	ND	91
59	OH	H	OH		H	6.36	ND	ND	91
60	OH		OH	H	H	8.15	ND	ND	91
61		OH	H	OH	OH	0.42	2.17	ND	113
62		OH	H	OH	OMe	4.40	17.17	ND	113
63	H		H	OH	H	ND	ND	0.38	112
64	H	F	H	F	H	ND	ND	1.12	112
65	H	H	H	OH	H	9.16[95]	0.32[96]	0.51[112]	95,96,112
66	OH	H	H	H	H	19.40	ND	ND	95
67	H	OH	H	H	H	12.40	ND	ND	95
68	H	H	OH	H	H	20.60	ND	ND	95

IC_{50}: half maximal inhibitory concentration; CLV: cluvenone; ND: not determined.

Table 7. Cytotoxic activity of GA and derivatives **69**-**78** against MDA-MB-231 cells

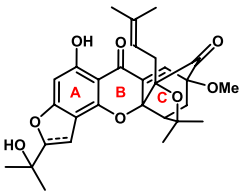


Compound	R_1	IC_{50} / μM	Reference
		MDA-MB-231	
69		0.17	96
70		0.47	96
71		0.25	96
72		0.58	96
73		4.40	96
74		0.87	96
75		1.00	96
76		0.88	96
77		0.76	96
78		0.66	96
GA	–	1.50	96

IC_{50}: half maximal inhibitory concentration; GA: gambogic acid.

hits against breast cancer (BC). The assessment of their cytotoxicity against the MDA-MB-231 and MCF-7 cell lines revealed a huge disparity in terms of potency against these cells, with cytotoxicity 4 to 7 times higher against MCF-7 than against MDA-MB-231 cells. Nonetheless, the activity of compounds **79** and **80** against the MCF-7 line proved of valuable interest, remaining in the low micromolar and nanomolar range (Table 8).[113]

Table 8. Cytotoxic activity of derivatives **79**-**80** against MCF-7 and MDA-MB-231 cells



Compound	---	IC_{50} / μM		Reference
		MCF-7	MDA-MB-231	
79	—	0.83	6.23	113
80	=	1.48	6.70	113

IC_{50}: half maximal inhibitory concentration.

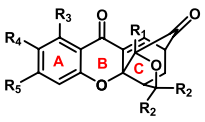
The C ring of CLV has also been explored, bringing structural modifications either on the caged ring itself (methyl removal) or to the prenylated side chain of this ring. From these, it has become clear that the two methyl groups on the caged ring are essential to the cytotoxicity of caged xanthones against T47D cells, as compound **81** exhibited a 7-fold drop in potency when compared to **82** (Table 9).[90] Regarding alterations to the prenylated side chain, replacing the prenyl group by either a diol or an epoxy ring or appending a bromine or hydroxyl to a methylene of the prenyl moiety improved the activity against MCF-7 by up to three orders of magnitude. However, when comparing to GA, only compounds **84** and **86** displayed similar potency against the same MCF-7 cell line (Table 9).[93]

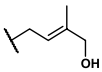
Despite the promising *in vitro* and *in vivo* anti-breast cancer activities of GA, the low aqueous solubility and metabolic stability have limited its progress as an anticancer drug candidate. The main strategies explored to optimize these properties involved the removal of the prenylated groups, and the insertion of polar and ionizable groups in the different fragments, which generated compounds with improved *in vitro* anti-breast cancer and pharmacokinetic properties. Further studies focusing on the *in vivo* evaluation against breast cancer of the most active derivatives reported here, and the elucidation of their mechanism of action, should be expected to be underway, to bring relevant data for comparison with GA.

5. Thiosemicarbazone Derivatives against Breast Cancer

During the last decades thiosemicarbazones have been highlighted in medicinal chemistry due to their chemical and pharmacological properties. Based on that, thousands of compounds have been reported with different chemical structures and biological activities

Table 9. Cytotoxic activity of GA and derivatives **81**-**87** against MCF-7 and T47D cells



Compound	R_1	R_2	R_3	R_4	R_5	IC_{50} / μM		Reference
						T47D	MCF-7	
81		H	H	H	H	> 20	ND	90
82		Me	H	H	H	3.0	ND	90
83		Me	OH	H		ND	4.68	93
84		Me	OH	H		ND	1.78	93
85		Me	OH	H		ND	3.66	93
86		Me	OH	H		ND	1.38	93
87		Me	OH	Br		ND	1.92	93
GA	–	–	–	–	–	0.70[90]	0.90[93]	90,93

IC_{50}: half maximal inhibitory concentration; GA: gambogic acid.

such as antitubercular, antiviral, antibacterial, antifungal and anticancer acticity.[114-118] In this topic, we discuss the potential of free thiosemicarbazone Schiff base and complexes based on thiosemicarbazones as anti-breast cancer drugs candidates involving the recent families of palladium(II), platinum(II) and other metal complexes.

The remarkable anticancer action of thiosemicarbazone derivatives is highlighted by compounds Dp44mT and Triapine (compounds **88** and **89**, respectively, Table 10). Dp44mT is an iron chelator with anticancer activity on human cervical carcinoma-derived (KB3), the small cell lung carcinoma cell line (DMS-53), the colon adenocarcinoma cell line (HCT-15), and the MCF-7 and MDA-MB-231 breast carcinoma.[119] Additionally, Dp44mT is able to induce apoptosis and non-apoptotic lysosomal cell death through the destabilization of the lysosomal membrane by the generation of cytotoxic reactive oxygen species (ROS), leading to intracellular release of lysosomal hydrolases.[120] This compound overcomes the common resistance phenomena expressed by human cancer cells and exhibits outstanding potency and selectivity against the MCF-7 breast cancer cell line with an IC_{50} of 0.38 nM and a selectivity index over 40,000.[119] Triapine is a well-known thiosemicarbazone derivative, which has undergone clinical trials as a chemotherapeutic potential, being studied against numerous cancer cell lines, including cervical cancer, pancreatic cancer, stage IV ovarian epithelial cancer, prostate cancer, among others.[121,122] Triapine has presented also good results against breast cancer cell line with an IC_{50} of 3.00 μM. Its metal chelation proprieties are essential for its cytotoxicity capabilities, as elucidated by Thelander *et al.*[123] in 1983. The constant presence of oxygen and iron is fundamental for the mammalian ribonucleotide reduction (an important step in cellular duplication). Therefore, this thiosemicarbazone induces cellular apoptosis by capturing the iron necessary for this step.[124]

In addition to the broad pharmacological profile of thiosemicarbazones, this class of compounds also presents a high chemical versatility, allowing a plenty of structural modifications. These structures can also act as ligands to metal complexes, being able to coordinate via sulfur and/or nitrogen atoms, depending on the different substituents of the thiosemicarbazone scaffold (R_1, R_2, R_3, R_4, Figure 4), which bring a range of systematic variation possibilities. Such variations can change the physicochemical properties of the ligands, as well as their biological activity and those of their respective complexes. Aldehyde-based thiosemicarbazones, for instance, have a hydrogen atom as one of their substituents (R_1), while R_2 can be either an alkyl, aryl or heterocyclic group. Similarly, the substituents R_3 and R_4 can either be both hydrogen or one hydrogen and the other an alkyl (or aryl)

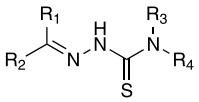


Figure 4. General chemical structure of thiosemicarbazones.

group. The thiosemicarbazones exist as tautomers of thione-thiol and can bind to metallic centers, regardless of if they are found in their neutral or anionic form. The anionic form is generated after the deprotonation of the NH or SH groups. Table 10 highlights thiosemicarbazones with promising results against the MCF-7 cell line (IC_{50} < 20 μM) reported in the last five years. In addition to their potency, one detail worth noting about this class is their overall high selectivity towards this cell line. The detailed discussion of their structure and anticancer activity that follows, is based only in the most active compounds (IC_{50} < 1 μM) and some important compounds that advanced in clinical trials.

Table 10. Selected thiosemicarbazones with IC_{50} < 20.00 μM against MCF-7 cell line

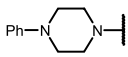
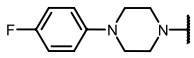
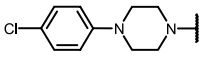
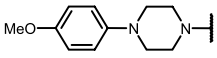
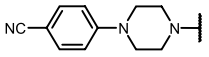
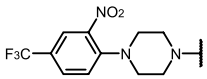
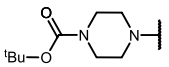
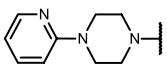
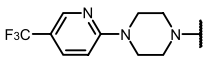
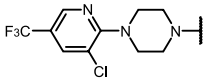
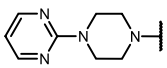
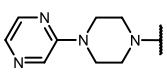
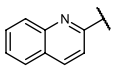
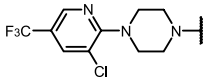
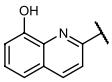
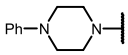
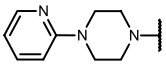
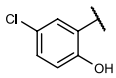
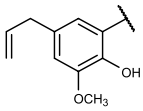
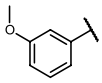
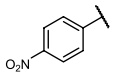
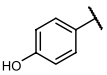
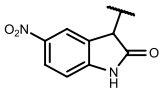
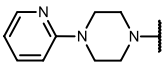
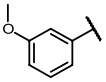
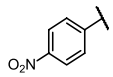
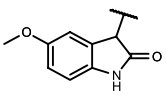
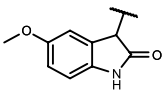
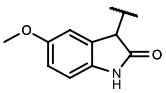
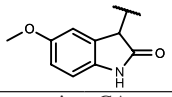
Compound	Thiosemicarbazone				IC_{50} / μM	Reference
	R_1	R_2	R_3	R_4	MCF-7 (SI)	
88 (Dp44mT)			Me	Me	0.00038 (40474)	117
89 (Triapine)	H		H	H	3.00 (> 8)	125
90	H				0.53 (> 47)	126
91	H				0.36 (> 69)	126
92	H				0.20 (> 125)	126
93	H				0.42 (> 60)	126
94	H				3.81 (> 7)	126
95	H				0.47 (> 53)	126
96	H				2.54 (> 10)	126
97	H				1.12 (> 22)	126
98	H				0.20 (> 125)	126
99	H				0.26 (> 96)	126
100	H				1.73 (> 14)	126
101	H				2.29 (> 11)	126
102	H		H	Ph	13.21 (> 6)	127
103	Me		H	Ph	7.53 (> 8)	127
104	Ph		H	Ph	1.33 (41)	127

Table 10. Selected thiosemicarbazones with IC_{50} < 20.00 μM against MCF-7 cell line (cont.)

Compound	Thiosemicarbazone				IC_{50} / μM	Reference
	R_1	R_2	R_3	R_4	MCF-7 (SI)	
105			H	Ph	0.65 (62)	127
106	Me		H	Ph	11.32 (> 5)	127
107	H				0.017 (1004)	117
108	H				0.011 (967)	117
109					0.012 (0.1)	117
110					0.004 (39)	117
111	H		H	^{i}Pr	14.3 (> 35)	128
112	H		H	Ph	15.8 (> 32)	128
113	Me	Me	H	H	2.27 (NA[a])	129
114	H		H	H	3.36 (NA)	129
115	H		H	H	2.74 (NA)	129
116	H		H	H	7.08 (NA)	129
117	H		H	H	3.61 (NA)	129
118					3.28 (NA)	130
119	H		H	H	2.82 (> 7)	131
120	H		H	H	7.10 (> 3)	131
121					2.93 (NA)	132
122					4.41 (NA)	132
123					5.41 (NA)	132
124			Me	Me	7.41 (NA)	132

IC_{50}: half maximal inhibitory concentration; GA: gambogic acid; SI: selectivity index; NA: not available.

Rejmund *et al.*[126] synthesized in 2018 a series of twelve thiosemicarbazones, based on the Triapine skeleton with the inclusion of pyrazine fragments (Figure 4). All the compounds were tested against five human cancer cell lines: HCT116 $p53^{+/+}$, HCT116 $p53^{-/-}$, MCF-7, U-251 and Hs683. The compounds reported in Table 10 as **90**, **91**, **92**, **93**, **95**, **98** and **99** displayed IC_{50} values under 1 μM (0.53, 0.36, 0.20, 0.42, 0.47, 0.20 and 0.26 μM, respectively). The mechanism of action of these compounds is still not fully understood.

A series of five thiosemicarbazone derivatives (**102**, **103**, **104**, **105** and **106**, Table 10) was investigated by Qi *et al.*[127] and demonstrated moderate to high anti-breast cancer activity. Their growth inhibition activities were evaluated against three human cancer cell lines: A549, MCF-7 and T24. The results found against the MCF-7 cell line were 13.21, 7.53, 1.33, 0.65 and 11.32 μM, respectively. Compound **105** was the most active and, interestingly, its chemical structure is very similar to Dp44mT, only replacing the methyl group of Dp44mT by a phenyl. It induces cellular apoptosis through the mitochondrial apoptotic pathway, by controlling the expression of cyclins, cyclin-dependent kinases (Cdk) and Cdk inhibitors, impeding the cell cycle distribution.

5.1. Metal-based compounds containing thiosemicarbazone ligands

The versatility afforded by metal-based complexes in the field of medicinal chemistry and drug development have been proved since the discovery of the arsphenamine, an effective drug for syphilis treatment[133] constituted on an arsenic-based compound. Other examples of metals encountered in the field of inorganic medicinal chemistry include lithium against bipolar disorders,[134] gold(I) for the treatment of rheumatoid arthritis[135] and silver(I), used to provide antibacterial effects.[136] Additionally, cisplatin proved to be an efficient anticancer agent against many kinds of tumors and remains in clinical uses in combination with doxorubicin for breast,[137] bladder,[138] endometrial, ovarian and lung cancer.[139] The success of cisplatin and its platinum(II) analogues as carboplatin and oxaliplatin have incentivized researchers to give special attention to metal complexes, due to their potential anticancer activity.

Thiosemicarbazone derivatives have garnered considerable interest due to their versatile nature as ligands, with high π delocalization, configurational flexibility, and *N*,*S*-donor properties, making them suitable for a plenty of metal ions,[140-144] including palladium(II) and platinum(II). Additionally, thiosemicarbazones can achieve alternative targets and not only DNA as demonstrated by platinum(II)-based drugs. Thus, the complexation of thiosemicarbazones with Pd^{II} and Pt^{II} is an interesting alternative and is a growing area in the field of inorganic medicinal chemistry.[145-150] Metal-based therapeutics provide diverse electronic and structural features, encompassing a wide range of oxidation states, coordination geometries, and types and numbers of ligands.[151,152] These compounds offer novel chemistry, including different types of ligand substitution and metal- and ligand-based redox processes.[151,152] In contrast to organic drugs, they are often "pro-drugs" that undergo activation in the target pathway or site. The importance of studies involving thiosemicarbazone compounds is evident from their basic structure, which provides numerous possibilities for coordination with different metals due to the presence of electron-donating atoms. This characteristic enables the discovery of several new, biologically promising compounds with interesting chemotherapeutic properties, including antitumor effects.[123,134-136]

Therefore, research aimed towards the synthesis and evaluation of thiosemicarbazone compounds as metal-based therapeutics is relevant to the development of new alternative drugs that could be used for cancer treatment or in a combination chemotherapy. Below, we highlight in detail derivatives of Pd^{II}, Pt^{II}, Cu, Ga^{III}, Zn^{II}, Sn^{IV}, Ag^{I} and Mn^{II} complexes containing thiosemicarbazone ligands reported in the last 5 years that obtained promising results against the MCF-7 cell line (Figure 5).

Lin *et al.*[153] performed a study in which three platinum(II) complexes with thiosemicarbazone ligands (complexes **125**, **126** and **127**, Figure 5) were investigated against the MCF-7 cell line. Complexes **125** and **126** exhibit in their chemical structure a chlorine atom coordinated to the metal center, while the complex **127** presents an additional thiosemicarbazone ligand coordinated to the platinum in a monodentate way through its S atom. The authors observed that these platinum(II) complexes exhibited better IC_{50} values (IC_{50} of 6.6, 8.3, and 1.7 μM for **125**, **126**, and **127**, respectively) than cisplatin (IC_{50} = 13.5 μM against MCF-7), used as a positive control. The addition of an extra thiosemicarbazone to the complex **127** significantly improved the *in vitro* anticancer activity.

Six copper(I) complexes containing thiosemicarbazones were prepared and had their *in vitro* cytotoxicity against MCF-7 cells investigated by Mahendiran *et al.*[154] in 2018. Complexes **134** and **135** (Figure 5), with IC_{50} values of 10.9 and 11.2 μM, respectively, exhibited better activity than cisplatin (IC_{50} of 12.1 μM), while the complex **136** (IC_{50} of 13.9 μM) showed the lowest activity of this subset of compounds. The chemical structure of the three complexes reported here differs by the presence of substituents on the benzene ring attached to thiosemicarbazone. The

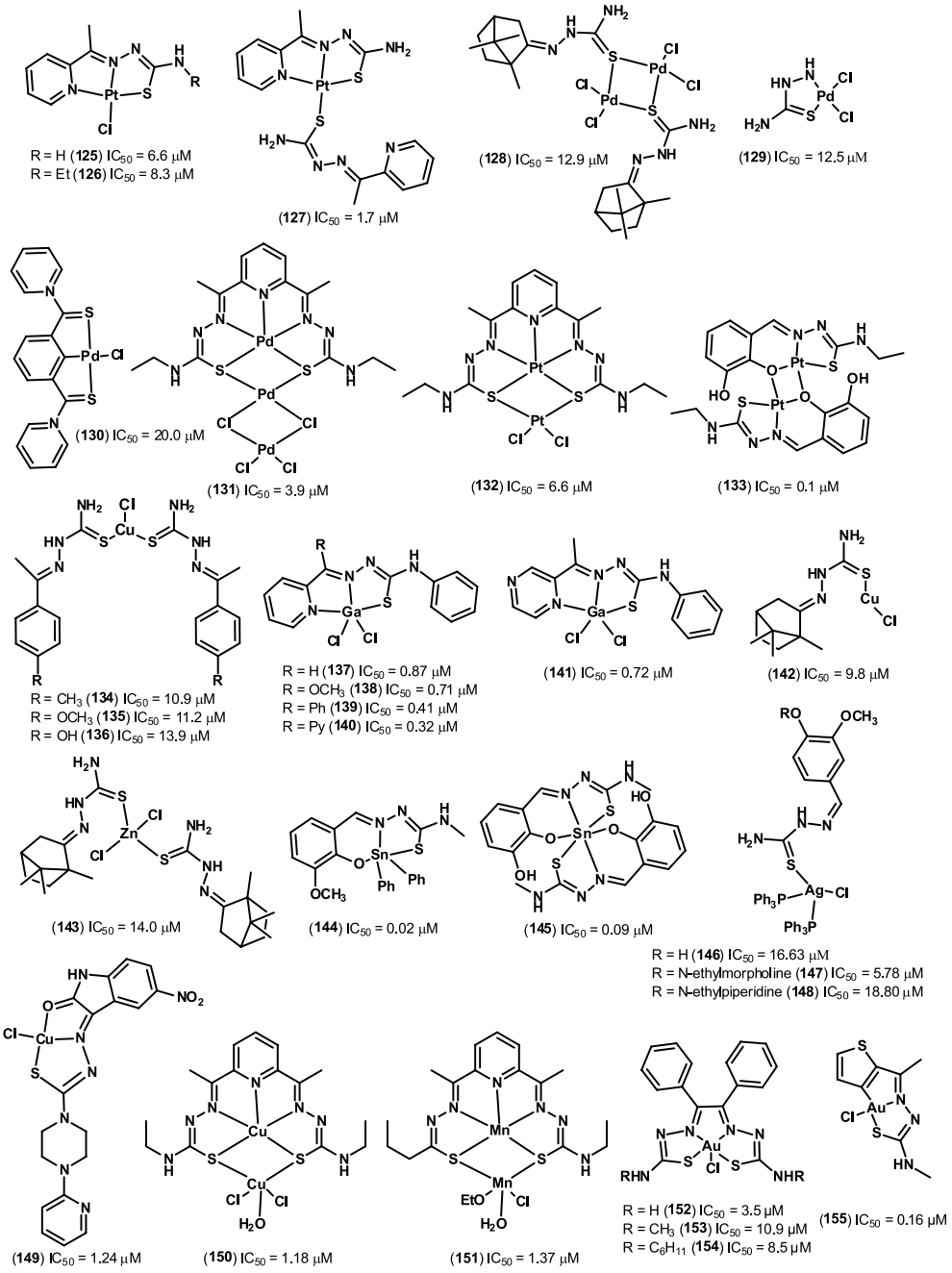


Figure 5. Selected thiosemicarbazone complexes with IC_{50} against MCF-7 cell line under 20 μM.

results suggest that the complexes with electron-donate substituents on the aromatic ring, such as complexes **135** and **136** (R = OMe and OH, respectively), exhibit greater activity due to resonance between the benzene ring and the substituent.

Gallium(III) thiosemicarbazone complexes showed improved anti-proliferative activity against MCF-7 cells when compared to the corresponding free ligands. The IC_{50} values ranged from 0.89 to 15.29 μM for the ligands, while the potency of the corresponding complexes (**137**-**141**, Figure 5) varied from 0.37 to 2.53 μM, indicating that the presence of the metal center can directly influence the anticancer activity.[127] A detailed study of the anticancer mechanism of gallium(III) complexes showed that they act as typical agents in the mitochondrial apoptotic pathway. The gallium(III) complexes were able to inhibit the cell cycle by decreasing the amount of of G2 phase cells by 17% when compared to vehicle-treated controls. Interestingly, the ability of the gallium(III) complexes to inhibit the cell cycle was independent from their concentration, while their apoptosis-promoting ability was concentration-dependent.[127]

Kokina *et al*.[155] synthesized five zinc(II), palladium(II), and copper(I) thiosemicarbazone complexes, and evaluated the effect of ligands and complexes on MCF-7 cell line viability. Their study showed that the ligands had no activity at the tested concentrations (1-50 μM), while the complexes had a significant dose-dependent cytotoxic activity, with IC_{50} values between 9 and 20 μM. The Cu^{I} complex (**142**, Figure 5) exhibited the highest potency among the complexes (IC_{50} = 9.8 μM), comparable to that of cisplatin (IC_{50} = 5.75 μM). However, the palladium(II) complexes (**128** and **129**, Figure 5) and zinc(II) complex (**143**, Figure 5) showed low activity. Thus, the development of metal complexes with natural thiosemicarbazones derived from terpenes has the potential to generate new biologically active compounds.

Twelve tin(IV) complexes with thiosemicarbazone Schiff bases were obtained by Yusof *et al*.[156] in 2020 and tested against ten cancer cell lines, including MCF-7 cells. The diphenyltin(IV) derivatives showed the most promising anticancer potency. The same study also investigated the structure-activity relationship of these complexes, evaluating the effect of different substituents on the thiosemicarbazone backbone, the phenyl ring, and the tin center. Results against MCF-7 showed that almost all complexes were more active than cisplatin (IC_{50} = 6.5 μM), with a special highlight on complexes **144** and **145** which reached nanomolar potency (IC_{50} = 0.02 and 0.09 μM, respectively). It is worth noting that all these twelve tin(IV) complexes were around 300 and 70 times more active than cisplatin, underlining the promising potential of such complexes.

Silva *et al*.[157] presented a breakthrough in the synthesis of tetrahedral silver compounds featuring O-alkylated vanillin thiosemicarbazone ligands. Their complexes exhibited significant antiproliferative activity against lung cancer A549 and breast cancer MDA-MB-231 and MCF-7 cell lines. After 48 h of incubation, the IC_{50} values of the compounds ranged from 5.78 to 18.8 μM, compound **147** being the most active (IC_{50} = 5.78 μM) against MCF-7 cells. All complexes were more potent than the control drug cisplatin. Compound **146** presented the ability to induce cell death by apoptosis and cause mitochondrial membrane depolarization, suggesting that the compounds target mitochondria rather than DNA. Even though the complex **148** was the less potent of this set of compounds, its activity was still almost 3 times higher than cisplatin.

Hosseini-Kharat *et al.*[158] investigated the production of nickel and palladium-based antitumor agents aimed at treating the highly aggressive and drug-resistant triple-negative breast cancer. The compounds were evaluated against MCF-7, MC4L2 and triple-negative breast cancer cell lines with different exposure times (24, 48 and 72 h). The study revealed that the palladium derivatives were better anticancer agents than the nickel-based ones, as complex **130** (Figure 5) showed an activity of 20 μM against the MCF-7 cells, while the nickel complexes showed IC_{50} values between 25 and 200 μM after 48 h of treatment. All the nickel and palladium complexes were less potent than cisplatin, used as a positive control (IC_{50} = 12 μM).

Recently, the copper(II) complex **149** demonstrated a potency of 1.24 μM against the MCF-7 cell line, about twice as high as the control used (IC_{50} = 2.60 μM).[130] This suggests that the high anticancer potency of **149** can be attributed to the copper center, which generates excessive ROS and exhibits high binding affinity towards DNA.

The paper from Jaragh-Alhadad *et al.*[159] highlights the global challenge posed by cancer and the limitations of non-specific treatments causing side effects on healthy tissues. It proposes the use of low-density lipoprotein (LDL) particles to deliver drugs to cancer cells with greater precision and reduce collateral damage. The article details the successful use of LDL particles to encapsulate thiosemicarbazone metal complexes to target tubulin protein in breast, lung, and prostate cancer cells. Using this treatment approach, the authors observed low IC_{50} values for all complexes tested (IC_{50} from 1.18 to 6.61 μM). The copper complex (**150**, Figure 5) with an IC_{50} of 1.18 μM and the manganese complex (**151**) deserve to be highlighted, since they displayed activities three times higher than the palladium(II) complex (**131**) (IC_{50} of 3.89 μM) and six times higher than the platinum(II) complex (**132**, IC_{50} of 6.56 μM). Thus, the approach proved effective in decelerating the growth of cancer cells and mimicked the natural metabolic pathway of LDL. Taken together, these findings suggest that using LDL particles for active drug delivery could be a promising strategy in cancer treatment.

Ali *et al*.[160] in their study, have synthesized a new thiosemicarbazone ligand and used it to coordinate with the following metal ions: Ni^{II}, Cu^{II}, Pd^{II}, and Pt^{II}. The resulting complexes were then tested for their antiproliferative activity against MCF-7 breast cancer cells. The data showed that the complexes had moderate to highly potent antiproliferative activity, reaching nanomolar levels of potency for the most active ones (IC_{50} = 0.1-31.6 μM). Among them, the Pt^{II} complex **133** exhibited the best antiproliferative activity with an IC_{50} of 0.1 μM, while the corresponding Ni^{II} complex remained the less active od all (IC_{50} = 31.6 μM).

Gold(III) compounds have received increasing attention in cancer research. Rodríguez-Fanjul *et al*.,[161] in 2018, presented synthesis of three gold complexes of thiosemicarbazone ligands. The complexes exhibited

significant antiproliferative activity against MCF7, MDA-MB-231 and MCF10A cell lines after 24 h of incubation. Compound **152** was the best for the three strains tested with IC_{50} of 10.9 μM to MCF7, 8.5 μM to MDA-MB-231 and 2.5 μM to MCF10A. However, compounds **153** and **154** are also worth mentioning. In assays against MCF10A, the IC_{50} values of **153** was 3.5 μM and **154** was 17.4 μM. Almeida *et al*.[162] reported the synthesis of a new Au^{III} complex with the ligand 2-acetylthiophene-*N*(3)-methylthiosemicarbazone. The biological activity of free thiosemicarbazone and its complexes were evaluated against the human cancer cell line MCF-7. After 24 h of treatment, the results against MCF-7 showed that complex **155** (IC_{50} = 0.16 μM) was 600 times more active than the ligand (IC_{50} > 100 μM), showing that the inclusion of the metal potentiates the biological activity of the compound.

In most studies reported, the coordination of thiosemicarbazones to a metal ion has been found to increase the anticancer activity against breast cancer cells.[153-160] It is notable that inorganic compounds can offer alternative mechanisms of action and reach different molecular targets when compared to organic molecules.[127] It should be noticed that there are still few studies on the mechanism of action of inorganic compounds against cancer cells. This can be justified by the high versatility of these structures, which allows a huge diversity of reactions with metal complexes. For instance, they can undergo ligand exchange and redox reactions, changes in their oxidation state, and their geometry can be altered in a biological environment.[151,152] The molecular self-aggregation exhibited by thiosemicarbazones cause the poorly water-soluble character of this class. In most part of the studies the drug candidate is primarily solubilized in dimethyl sulfoxide (DMSO) and the maximum achievable concentration in solution is often limited by the DMSO cytotoxicity. Furthermore, derivatives of thiosemicarbazones precipitate very quickly during *in vitro* assays, leading in some cases to unreliable concentration (IC_{50}) data.[163-165] Thus, the extremely low aqueous solubility content of thiosemicarbazones remains a key obstacle to their biological evaluation.

6. The Potential of Photodynamic Therapy (PDT) in Breast Cancer

Light has been used as a therapeutic agent for thousands of years.[166] The combination of light and certain chemicals compounds, called photosensitizers (PS), can oxidize biomolecules (proteins, lipids and DNA) and promote the death of cancer cells.[167,168] This is the basic principle of PDT, a therapeutic modality that uses light and a PS in presence of oxygen to treat some types of cancers, including breast cancer.[169-171] The advantages of PDT, in comparison to conventional treatments such as chemotherapy, radiotherapy and surgery, are its lower invasiveness than surgery, the possibility of repeating PDT without facing the risks of cumulative toxic effects encountered in radiotherapy, and the absence of the side effects frequently occurring in chemotherapy since PS does not exhibit toxicity without light.[172]

Differently from main chemotherapy agents used in the treatment of breast cancer, the mechanism of action of PDT does not involve DNA alkylating agents, antimetabolites, anthracyclines and antimitotics (Table 1 and Figure 2). Actually, the cytotoxic effects induced by PDT on cancer cells are the consequence of a generation of ROS by the PS. The mechanism of action starts with the absorption of a photon from a light of suitable wavelength by the PS, which promotes an electron from the ground state to an orbital of higher energy, known as the excited state. The PS in this singlet excited state is very unstable and can undergo diverse photophysical processes, including intersystem crossing, to form a more stable triplet excited state. This triplet excited state lasts long enough to allow the transfer of its energy to molecular oxygen (O_2), producing singlet oxygen ($^1O_2$), or to allow electron transfer reactions forming other ROS such as hydroxyl radicals ($OH^•$), superoxide ions ($O_2^{-•}$) and hydrogen peroxide (H_2O_2).[167,168] As a result, intracellular biomolecules can be oxidized by these ROS, which may trigger cell death depending on the extent of cellular damage.[167]

Most of the approved protocols of PDT and involving PS are for treating accessible and/or non-deep tumors such as non-melanoma skin cancers,[173] head, neck, lung, gastrointestinal and prostate cancers.[174] The recent advances in PDT technology resulted in the broadening of the applications of PDT to breast cancer treatment.[171] Generally, an ideal PS should exhibit low toxicity in the dark; high molecular absorption (≥ 20000-30000 M^{-1} cm^{-1}) in the phototherapeutic window (ca. 600-900 nm), high quantum yield of ROS, and good solubility in water and chemical stability.[174] One of the difficulties to the clinical use of PS is the depth of penetration of light into biological tissues. During rational drug design it is important to consider chemical structures of PS that absorbs photons on "optical transparency windows" of biological tissues allowing deeper penetration and less attenuation during the irradiation process. Another limitation is the balance of lipophilic/hydrophilic nature of PS, a more lipophilic PS usually aggregate losing its photophysical properties and water solubility, while a more hydrophilic PS may

not permeate through cellular membranes and have poor interaction with target biomolecules.[175] Although significant advances through PS design, up to nowadays, there are no PS presenting a "magic bullet" containing all ideal characteristics required for a powerful PS. Therefore, the search for new, tissue-specific PS is constantly ongoing, in order to identify photosensitizers capable of meeting these requirements. As a result, several *in vitro* studies have underlined the potential of different PS for applications in PDT against human breast cancer cell lines (Figure 6).

Methylene blue (MB, compound **156**, Figure 6) a phenotiazinium dye with a variety of applications, was assayed using 2 or 20 μM of MB followed by light irradiation (4.5 J cm^{-2}) against MCF-7, MDA-MB-231 and MCF-10A cell lines.[176] PDT with MB was found to induce larger percentages of cell death in two human breast cancer cell lines (MCF-7 and MDA-MB-231) than in non-tumorigenic human MCF-10A cells, reaching a difference of up to 80% of MB-PDT-induced cell death between malignant and normal-like cells.[176] The MDA-MB-231 line showed the highest rate of cell death after 24 h PDT

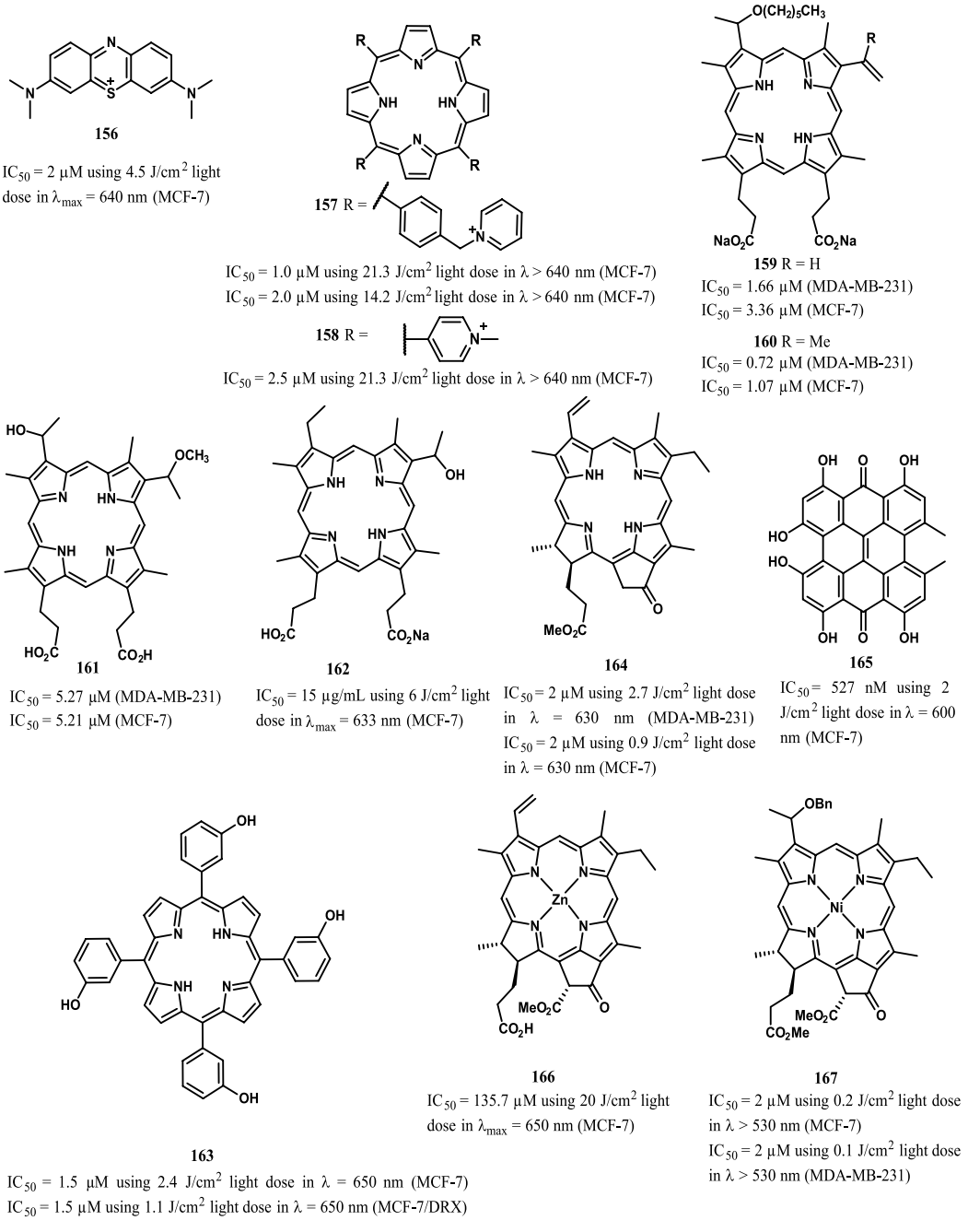


Figure 6. Chemical structures of common PS (phenothiazines, porphyrins, chlorins and hypericin) tested *in vitro* for PDT to breast cancer.

(97.3%), followed by the MCF-7 cell line (78.3%) and finally by the normal-like MCF-10A cells (18.0%) using 2 μM of MB and 4.5 J cm^{-2} light dose (640 nm). This shows that MB-PDT selectively induces cell death in breast cancer cells.[176]

Porphyrin derivatives were also tested *in vitro* against breast cancer cell lines. Gamelas *et al*.[177] evaluated the photosensitizing properties of 5,10,15,20-tetrakis [4-(pyridinium-1-yl-methyl)phenyl]porphyrin (compound **157**, Figure 6) and compared them with the ones of the cationic 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin (TMPyP, compound **158**, Figure 6), a promising PS for PDT. In MCF-7 cells, **157** demonstrated a significant photodynamic efficiency (50% of MCF-7 cells survival) at 1 μM after an irradiation period of 15 min, and the maximum efficiency was reached within 10 min using a concentration of 2.5 μM of **157** and red-light irradiation ($\lambda > 640$ nm, 23.7 mW cm^{-2}). For **158**, its photodynamic effect induces 50% of cell mortality at a concentration of 2.5 μM, using a similar red-light irradiation ($\lambda > 640$ nm, 21.3 J cm^{-2}).[177]

Other porphyrin based structures were studied by Feng *et al*.[178] who synthesized three porphyrin derivatives from protoporphyrin dimethyl ester (compounds **159** and **160**, Figure 6) and hematoporphyrin monomethyl ether (HMME, compound **161**, Figure 6), The activity of these compounds was evaluated against the MCF-7 and MDA-MB-231 cell lines, and both dimethyl ester derivatives **159** and **160** exhibited higher phototoxicity under low light doses (0.5, 1 and 2 J cm^{-2}) when compared to their homologous compound **161**. Porfimer sodium (compound **162**, Figure 6) managed to reach a cytotoxic activity of **15** 2.48 μM (PS concentration) against MCF-7 cells under 633 nm light irradiation (6 J cm^{-2} for 4 h).[179]

In addition to the free porphyrins widely used in PDT, porphyrins with some metals coordinated to the nitrogen atoms of the heterocycles can be used to improve the efficiency of PDT. For example, the survival rate of breast cancer cells decreased from 65.3 to 17.8% when a Zn-coordinated porphyrin was used instead of its corresponding free porphyrin, suggesting a higher photodynamic activity of zinc(II) porphyrins comparing to free porphyrins.[180]

Teiten *et al*.[181] tested a PS from chlorin class, *meta*-tetra(hydroxyphenyl)chlorin (*m*THPC, compound **163**, Figure 6) against both the MCF-7 cell line and its doxorubicin-resistant subline MCF-7/DXR (doxorubicin-resistant) overexpressing the P-glycoprotein efflux-pump. Due to the larger surface area of the resistant cell line, the uptake of **163** was greater, leading to an enhanced cytotoxicity against MCF-7/DXR. Indeed, a concentration of 1.5 μM of **163** caused 50% inactivation of MCF-7 and MCF-7/DXR cells when exposed to red light irradiation (650 nm) at light doses of 2.4 and 1.1 J cm^{-2}, respectively.[181] The photodynamic activity of **163**, measured 24 h after irradiation, was significantly greater in MCF-7/DXR than in MCF-7 cells.[181] Another PS from the chlorin class, methyl pyropheophenylchlorin (MPPa, compound **164**, Figure 6), was studied by Zhu *et al*.[182] against the MDA-MB-231 cell line. This compound reached an IC_{50} of 2 μM when irradiated at 630 nm with a light dose of 2.7 J cm^{-2}, and the efficient phototoxicity of MPPa was attributed to the concurrent induction of autophagy and ER stress. Other works also reported similar IC_{50} values (IC_{50} = 2 μM, 630 nm, light dose = 0.9 J cm^{-2}) for **164** in MCF-7 cells and demonstrated that MPPa-based photodynamic therapy inhibits the metastasis of MCF-7 cells, probably through the protein kinase B (Akt)/NF-κB-dependent matrix metalloproteinases (MMP-9) signaling pathway, which plays a crucial role in the degradation of the extracellular matrix and the subsequent invasion and metastasis of tumor cells.[183]

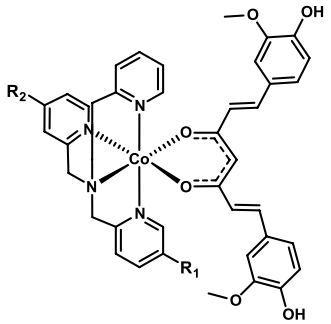
In a similar way to metalloporphyrins, compounds from the chlorin class may also coordinate metals at the center of their chromophore, resulting in altered photophysical properties and, consequently, photodynamic efficiency. Zinc pheophorbide *a* (compound **166**, Figure 6) was found to display an IC_{50} of 527 nM against MCF-7 cells when applying a light dose of 2 J cm^{-2} with a light emitting diode (LED) illuminator equipped with a 600 nm cut-off filter.[184] This nanomolar potency is rather promising, suggesting that zinc pheophorbide *a* might progress to *in vivo* studies. On the other hand, the combination of some metals and PS may not enhance the efficiency of PDT. For example, a pheophorbide *a* nickel complex (compound **167**, Figure 6) exhibited a low activity (IC_{50} = 135.7 μM) against the MCF-7 cell line, even when exposed to a 650 nm red light at a dose of 20 J cm^{-2}.[185]

Hypericin (compound **165**, Figure 6), a polycyclic quinone isolated from *Hypericum perforatum*, is a photosensitizing agent used in PDT. Its photoactivity has been demonstrated against various cancer cells *in vitro*.[186] Theodossiou *et al.*[187] evaluated the photodynamic activity of **165** against MCF-7 and MDA-MB-231 cells, which turned to be 70% higher for MDA-MB-231 than for MCF-7 cells.[187]

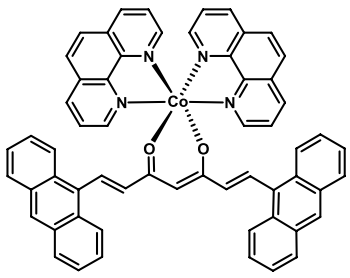
In addition to the different classes of photosensitizers that have been previously presented, and constitute the majority of PS used in PDT, various metal complexes (Figure 7) have also been investigated *in vitro* as promising PS in PDT for human breast cancer. Some tertiary cobalt(III) complexes (compounds **168**-**170**), for example, are low cytotoxic in the dark, but their accumulation in the cell membrane in significant concentrations favors reactions of photooxidation,

conferring them a certain phototoxicity against the MCF-7 and MDA-MB-231 human breast cancer cell lines.[188,189] Co^{II} complex (compound **171**, Figure 7) using 10 J cm^{-2} light dose at visible light (400-700 nm) showed IC_{50} of 12.2 and 14.1 μM for MCF-7 and MDA-MB-231 cells, respectively. Similar results were obtained for cobalt(II) complex (compound **172**, Figure 7) with IC_{50} values 6.1 and 9.3 μM in MCF-7 and MDA-MB-231 cells, respectively.[190] Given that these complexes exhibited some toxicity in the dark, their phototoxic index (PI) was also determined. The PI is one of the various descriptors of the potential of a PS , and is defined as the quotient of the IC_{50} of a PS in the dark divided by its IC_{50} upon light irradiation.[191] This value quantifies the increase in potency of a PS when irradiated, with compounds of PI > 5 being considered as phototoxic.[191,192] Then, the PI values > 6.8 for these cobalt complexes, indicate high phototoxicity of the cobalt complexes containing anthracene-based curcuminoid ligand, which makes them suitable candidates for PDT in breast cancer.[190-192]

Other examples of metal-based PS are ruthenium complexes, which favorable photophysical properties earned them a rising interest from researchers involved in the field of PDT. Among them, Ru^{II} polypyridyl complexes are highly promising photosensitizers that can act as anticancer agents, even though their relative absence of selectivity may lead to cytotoxic effects against normal cells in addition to their toxicity towards cancer cells. Thus, aiming to overcome this limitation of such complexes, a Ru^{II} complex containing a tamoxifen moiety acting as an estrogen receptor specific targeting group (compound **173**, Figure 7) was synthesized and assessed for PDT against breast cancer. To provide an accurate assessment of the impact of the tamoxifen moiety, **173** was compared with the corresponding compound lacking the tamoxifen submit (compound **174**, Figure 7). The inclusion of the tamoxifen fragment in **173** proved to be highly beneficial regarding the cytotoxicity against MCF-7 cells, which remained about 4 times higher

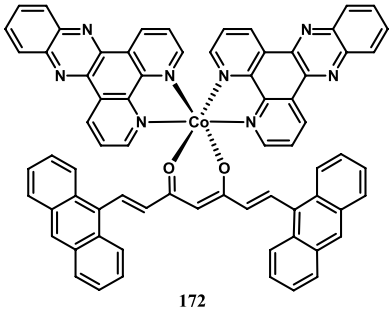


168: $R_1 = R_2 = H$; $IC_{50} = 6$ μM using 15.1 J/cm^2 in $\lambda_{max} = 470$ nm, PI = 6.8 (MCF-7)
169: $R_1 = H$ and $R_2 = CO_2^-$; $IC_{50} = 5$ μM using 15.1 J/cm^2 in $\lambda_{max} = 470$ nm, PI = 17.0 (MCF-7)
170: $R_1 = R_2 = CO_2^-$; $IC_{50} = 11$ μM using 15.1 J/cm^2 in $\lambda_{max} = 470$ nm, PI = 14.1 (MCF-7)



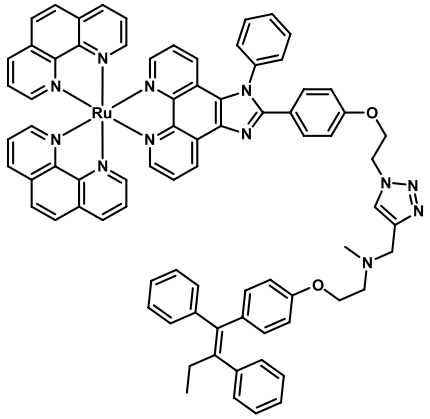
171

$IC_{50} = 12.2$ μM using 10 J/cm² light dose in 400-700 nm (MCF-7)
$IC_{50} = 14.1$ μM using 10 J/cm² light dose in 400-700 nm (MDA-MB-231)



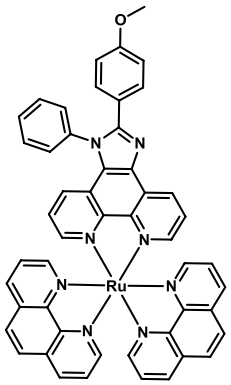
172

$IC_{50} = 6.1$ μM using 10 J/cm² light dose in 400-700 nm (MCF-7), PI = 10.1
$IC_{50} = 9.3$ μM using 10 J/cm² light dose in 400-700 nm (MDA-MB-231), PI = 8.5



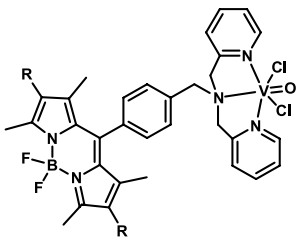
173

$IC_{50} \sim 4$ μM using 12 J/cm^2 in $\lambda_{max} = 450$ nm (MCF-7)



174

$IC_{50} \sim 16$ μM using 12 J/cm^2 in $\lambda_{max} = 450$ nm (MCF-7)



175: R = I; $IC_{50} \sim 0.2$ μM using 10 J/cm² between 400-700 nm (MCF-7)
176: R = H; $IC_{50} = 3.4$ μM using 10 J/cm² between 400-700 nm (MCF-7)

Figure 7. Structure of PS based on metal complexes and tested *in vitro* for human breast cancer.

(IC_{50} ca. 4 μM) than the cytotoxic activity of **174** in the same conditions (450 nm irradiation at 12 J cm^{-2}).[193] The higher photoactivity of **173** was attributed to its tamoxifen moiety, which enhances both the cellular uptake of **173** by breast cancer cells and the production of ROS in lysosomes organelles.[193]

Vanadium-based complexes, especially oxovanadium complexes, have also been considered for a possible use as PS in PDT applied to breast cancer. Boron-dipyrromethene (BODIPY)-containing oxovanadium complexes (compounds **175** and **176**, Figure 7) were synthesized and tested against MCF-7 cells.[194] Both oxovanadium complexes showed low toxicity in the dark (IC_{50} > 100 μM). The diiodo-BODIPY derivative **175** exhibited nanomolar potency against the MCF-7 line (IC_{50} = 0.2 μM) under light irradiation (400-700 nm, 10 J cm^{-2}), while the removal of the two iodine atoms on the BODIPY moiety (compound **176**, Figure 7) led to a 17-fold loss of potency under the same conditions of irradiation (IC_{50} = 3.4 μM).[194] This enhanced photoactivity was attributed to the greater generation of singlet oxygen by **175** (φ_Δ (quantum yield of singlet oxygen) ca. 0.6) than by **176** (φ_Δ ca. 0.14).[194]

All the PS we have described until now have been tested *in vitro*, and were included in this review after a careful selection according to the following criteria: (*i*) we only included PS tested in human breast cancer cell lines (MCF-7 and MDA-MB-231) , i.e., PS only tested in mouse/mice breast cancer cell lines were not considered here; (*ii*) PS conjugated to nanoparticles or incorporated into formulations such as liposomal or polymeric micelles were excluded from our analysis, since our goal was evaluate the impact on photoactivity of the structure of the PS alone; (*iii*) studies on synergistic effects between PS and another drug or conventional therapies were also excluded from our discussion due to the same reason; (*iv*) only PS exhibiting satisfying *in vitro* photoactivity (IC_{50} < 10 μM) were selected.

In addition to their *in vitro* assessment, some clinical studies involving PS are also worth mentioning (Table 11). The first clinical studies described employing PDT for the treatment of breast cancer used a hematoporphyrin derivative (HpD, Photofrin I) and dihematoporphyrin esters (DHE, Photofrin II), commonly known as first-generation photosensitizers. Even though these PS induced a clinical side-effect (prolonged skin phototoxicity for about 4 weeks), the trials revealed a complete response to recurrent breast cancer in 7-20% of patients, using DHE or HpD activated by light doses ranging from 20-359 J cm^{-2} (red light at 630 nm).[195,196]

HpD (Photofrin I) and DHE (Photofrin II) are mixtures of porphyrins in different states of aggregation,[201] that were approved by regulatory agencies for the treatment a broad range of cancers, such as bladder cancer (1993, Canada), early stage of lung cancer (1994, Japan), esophageal cancer (1995, USA, FDA approval) and early non-small cell lung cancer (1998, USA, FDA approval).[202] This drug was also investigated for the treatment of chest wall recurrences of breast cancer.[202] Porfimer sodium was assayed against breast cancer secondary tumors (chest wall) at a dose of 3 mg kg^{-1} with an irradiation of 60-120 J cm^{-2} (laser light). It achieved an interesting 75% of complete recovery, but also induced undesirable side effects (damaged local tissue).[197] Similar recovery rates were observed in studies using a lower dose of porfimer sodium (around 0.75 mg kg^{-1}) and higher light doses (140-182 J cm^{-2}), which allowed to limit the damage to surrounding tissues.[203,204] A light dose of only 100 J cm^{-2} even managed a total response rate of 91% and a complete response in 73%, using low doses of PS, since normal tissues retain lower drug concentrations than tumors, in which drugs tend to accumulate. This allows the use of higher light doses and deeper tissue penetration to destroy the tumor while sparing normal tissues, thanks to photobleaching.[198,205] Indeed, a treatment using 0.8 mg kg^{-1} porfimer sodium and a light dose of 150 J cm^{-2} resulted

Table 11. Photosensitizers (PS) tested *in vivo* for PDT in breast cancer

PS	Concentration / (mg kg^{-1})	Light dose / (J cm^{-2})	λ / nm	Completed recovery / %	Model	Reference
Verteporfin	0.4	30-50	690	–	human[a]	170
Temoporfin (*m*-THPC)	0.10-0.15	5-10	652	–	human[b]	195
	2.5-5.0	20-359	630	7-20	human[b]	196
	3.0	60-120	630	75	human[b]	197
Porfimer sodium	1-2	100	630	91	human[b]	198
	0.8	135-170	630	89	human[b]	199
	0.8	150-200	630	64	human[b]	200

[a]Primary breast cancer; [b]breast carcinoma chest wall recurrence.

in a complete recovery in 89% of the tumors treated.[199] Similar conditions guaranteed a complete recovery of 64% in another study, which also described the immune-modulating effects of PDT.[200] PDT-generated tumor-sensitized lymphocytes were collected after the treatment, and lesions that were not within the area of treatment regressed within 4 to 6 weeks after the beginning of the treatment, due to a possible immune mechanism at these disease sites.

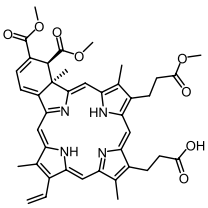
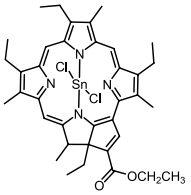
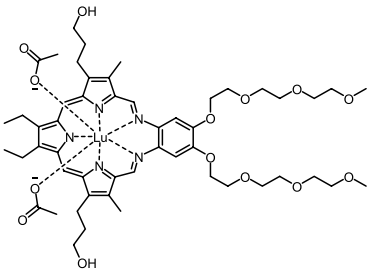
Temoporfin (*m*-THPC, compound **163**), another PS approved for the PDT treatment of squamous cell carcinoma of the head and neck by the European Medicines Agency (EMA) in 2001,[206] was also evaluated in clinical studies to treat breast cancer.[195] A temoporfin dose of 0.1 mg kg^{-1} bodyweight followed by irradiation 48 h after drug administration (light dose: 5-10 J cm^{-2}) resulted in the full recovery of all the 89 patients treated in the study. The superiority of this class of photosensitizing compounds in comparison to the first generation of photosensitizers like porfimer sodium can be attributed to the higher purity of temoporfin (single-compound formulation, 98% purity), its higher phototoxicity in a specific light dose (5-10 J cm^{-2} required for temoporfin *vs.* 20-359 J cm^{-2} for porfimer sodium) the lower drug doses required (0.1-0.15 mg kg^{-1} *vs.* 1.0-5.0 mg kg^{-1}), and it maximum absorbance of 652 nm, allowing in a deeper light penetration in body tissues when compared to the absorbance of 630 nm of porfimer sodium.[181,195]

Verteporfin, a PS approved by FDA to treat age-related macular degeneration using PDT in 2000,[207] was administered at a dose of 0.4 mg kg^{-1} to primary breast cancer patients and exposed to laser irradiation (690 nm, 30-50 J cm^{-2}).[170] Such treatment managed to increase the extent of tumor necrosis in a light dose-dependent manner. Unfortunately, due to the lack of candidates for this study, no statistical analysis of the clinical response could be performed.

Although many preclinical studies have been reported in order to identify a PS suitable for approval for breast cancer treatment, to date, only three compounds managed to reach the stage of clinical trials, which are currently ongoing (Table 12).

To summarize this section, PDT is a type of treatment approved for skin cancers, head, neck, lung, gastrointestinal and prostate cancers. Recently research advances have shown the applicability of this promising method for breast cancer treatment. The PDT mechanism of action are based on ROS generation that oxidize biomolecules

Table 12. Clinical trials of PS to PDT in breast cancer

PS chemical structure	Commercial name	Stage	Status	Reference
 Benzoporphyrin derivate monoacid ring A (BPDMA)	Verteporfin	phase I/IIA	completed	208
		phase II	unknown	209
 Tin ethyl etiopurpurin (SnET2)	Rostaporfin	phase II/III	completed	206,210
 Motexafin lutetium	Lutrin, Antrin	phase II	completed	211

and may trigger cells to death. ROS are generated by PS, thus chemical structure of PS is crucial to PDT efficiency. We showed different PS compounds tested *in vitro* against breast cancer cell line, which porphyrin and chlorin derivatives were the most common. From all PS tested, three derivatives are ongoing in clinical trials against breast cancer.

7. Conclusions

Chemotherapy is the main therapeutic strategy to treat breast cancer, despite the chemoresistance and side effects presented by the currently used drugs. Therefore, the search for new drugs candidates is urgently needed, and the *in vitro* screening of different classes of chemical compounds against breast cancer have been widely explored. From the extensive *in vitro* results of caged xanthones, thiosemicarbazones and photosensitizers reported in this review, compounds based on these scaffolds appear as promising against breast cancer, highlighting the potential of these classes of compounds for providing new anticancer agents.

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C.O.R.J., T.M.T., T.G.A., C.G.O. were responsible for conceptualization, data curation, resources, validation, visualization, writing original draft, writing-review and editing; D.D.S.O., T.J.W.D.L., F.C.S., I.V.C., R.A.C.S., P.A.M., G.M.A. for investigation, writing original draft, writing-review and editing.

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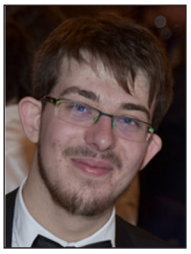

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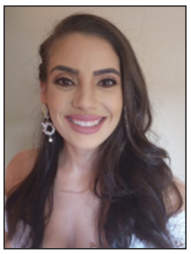

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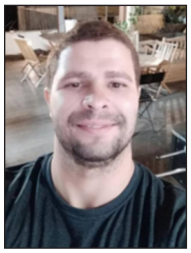

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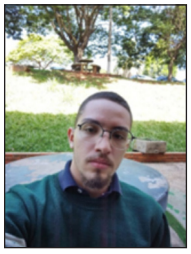

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