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SYNTHESIS AND *IN VITRO* BIOLOGICAL EVALUATION OF NEW TRIPHENYLETHYLENE PLATINUM (II) COMPLEXES

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Abstract: In our search for a chemotherapeutic agent with a better therapeutic index and selectivity for the treatment of breast cancer, we have synthesized cytotoxic triphenylethylene derivatives. The synthesis of this type of compound is straightforward and efficient. The biological activity of these compounds was evaluated *in vitro* on ER+ and ER- human breast tumor cell lines: MCF-7 and MDA-MB-231.

Several platinum coordination complexes such as cis-diamminedichloroplatinum (II) (cisplatin) and carboplatin are currently used in chemotherapy of neoplastic diseases.[1] These complexes of a non-essential heavy metal, exhibit a remarkable antitumor effectiveness and a broad spectrum of activity. It is widely believed that the antitumor activity of platinum drugs is a consequence of their interaction with DNA .[2,3] Cisplatin binds readily to guanine residues of DNA molecules.[3] Cisplatin has proved very successful in the treatment of a variety of human solid tumors such as genitourinary and gynecologic tumors as well as head, neck and lung tumors.[1] Unfortunately, the development of cellular resistance to cisplatin in mammalian cells is common[4-6] and is believed to occurs via four main mechanisms: (a) increased efficiency of repair of platinum-DNA lesions,[7-10] (b) increased inactivation of drug by elevated levels of cellular low-molecular weight thiols, particularly glutathione,[11-13] (c) metallothionein,[14-16] and (d) decreased cellular uptake of drug .[17-21] The clinical utility of the drug is also limited by its toxic effects, particularly kidney toxicity.[2] The search for platinum complexes with a broader spectrum of activity, less toxicity, improved clinical effectiveness against tumors characterized by intrinsic or acquired resistance to cisplatin is ongoing.

In order to improve the low activity of platinum (II) complexes against breast cancer,[22] we investigated the covalent attachment of dichloroplatinum to diamino analogs of triphenylethylene. These derivatives could be considered cytotoxic analogs of tamoxifen, the latter being an efficient drug for the treatment [23] and prevention [24] of breast cancer. Therefore, considering the fact that hormonal therapy will be ultimately followed by chemotherapy or a combination of both, we synthesised a number of non-steroidal cytotoxic estrogens hoping to obtain products with dual activity ie. antiestrogenic and cytotoxic. Moreover, these compounds might also be multidrug resistance (MDR) modulators (chemosensitizers) as per their lipophilic character.[25,26] The present communication describes the synthesis and *in vitro* biological activity of eleven members of this new family of cytotoxic triphenylethylenes.

Synthesis of Triphenylethylene Platinum (II) Complexes 1Aa-c, 1Ba-e, 6Bd-OCH_3 and 1Cd,e.

As shown in Scheme 1, five new platinum (II) complexes **1Ba-e** (**R = R' =OH, R'' = H**) were obtained with a 30% overall yield from commercially available benzyl-4-hydroxyphenyl ketone, after eight steps. The appropriate iodotetrahydropyranyl ethers were prepared in high yield as described previously.[27] Benzyl-4-hydroxyphenyl ketone was initially protected as a methyl ether **2** (**R = OCH_3, R'' = H**) by using dimethylsulfate and sodium hydroxide.[28] The yield for this reaction was around 75% (98% based on recovered starting material).

Alkylation of **2** with the iodotetrahydropyranyl ethers was achieved using sodium hydride in tetrahydrofuran to give compounds **3Ba-e** with an average yield of 75% (98% based on recovered alkyliodide). Addition of an excess of *p*-methoxyphenylmagnesium bromide to the ketones **3Ba-e** and subsequent treatment of the crude tertiary alcohol intermediates with pyridinium-*p*-toluenesulfonate (PPTS) in ethanol at reflux afforded the triphenylethylene alcohols **4Ba-e** as the result of dehydration of the tertiary alcohols and simultaneous deprotection of the tetrahydropyranyl ethers (85% average yield for the two steps).

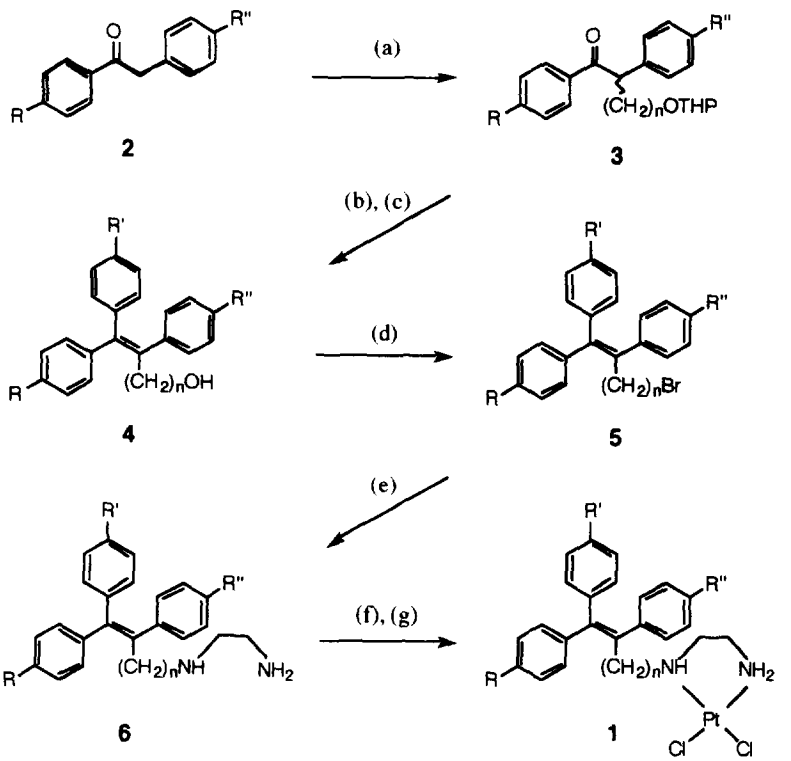
With the desired triphenylethylenes **4Ba-e** in hand, the following sequence of reactions are simple functional group transformations. Initially, alcohols **4Ba-e** were transformed to the bromides **5Ba-e** with carbon tetrabromide and triphenylphosphine in dry dimethylether (85% average yield). The amines **6Ba-e** were obtained with an average yield of 90% by refluxing the bromides **5Ba-e** in the presence of an excess of ethylenediamine in dry methanol. Finally, demethylation with boron tribromide gave the intermediate bis-phenols, which, upon treatment with potassium tetrachloroplatinate (II) in a mixture of dimethylformamide (DMF) and water, led to the desired platinum (II) complexes **1Ba-e** (**R = R' =OH, R'' = H**) (60% average yield for the two steps).[27] The platinum (II) complex **6Bd-OCH_3** was easily obtained by reacting the amine **6Bd** with potassium tetrachloroplatinate (II) in a mixture of DMF and water (80% yield).

The triphenylethylene platinum (II) complexes **1Aa-c** (**R = R' = R'' = H**) were synthesized from commercially available starting material deoxybenzoin **2** (**R = R'' = H**), in a similar sequence of reactions as used early for compounds **1Ba-e**. However, *p*-toluenesulfonic acid (APTS) was used instead of PPTS for the dehydration step. The total yield exceeded 40%. These are the reference derivatives which should not possess any affinity for the estrogen receptor (ER). Also, two new derivatives **1Cd,e** were made from desoxyanisoin as described previously for compounds **1Ca-c** in order to complete the series.[27] All new compounds obtained were characterized by their IR, ^1H NMR, ^{13}C NMR and mass spectrum. The final products **1Aa-c, 1Ba-e, 6Bd-OCH_3 and 1Cd,e** passed element analysis (C, H, N).

***In Vitro* Antitumor Activity**

Two human breast tumor cell lines were chosen based on their estrogen receptor content, to evaluate the antitumor activities of our new platinum (II) complexes.[29] The cytotoxicity of our compounds was tested along with controls (cisplatin and tamoxifen) on both ER^+ (MCF-7) and ER^- (MDA-MD-231) human mammary

carcinomas in order to assess the potential selective anti-neoplastic effect on hormone-dependent breast cancer. The antitumor activity was evaluated with a colorimetric assay that uses the ability of viable cells to reduce a colorless tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), into a thiazolyl blue MTT formazan.[30] A recent report indicates that the MTT assay can be used to replace the [^{3}H] -uridine assay for chemosensitivity screening. The colorimetric assay has the advantages of being safer, less costly and simpler than the radiometric assay.[31]



SCHEME 1

A R = R' = R" = H, **a-c**
B R = R' = OCH_3 or OH, R" = H, **a-e**
C R = R' = R" = OCH_3 or OH, **a-e**

n = 6(a),8(b),10(c),11(d),12(e)

Reagents: (a) NaH, I-$(CH_2)_n$-OTHP, 25 ^{0}C, 17 h, 70% (98%); (b) R'C_6H_4MgBr, Et_2O, 25 ^{0}C, 17 h; (c) crude tertiary alcohol, PPTS or APTS, EtOH, reflux, 5 h, 85% from **3**; (d) CBr_4, Ph_3P, Et_2O, 25 ^{0}C, 24 h, 85%; (e) $H_2NCH_2CH_2NH_2$, CH_3OH, reflux, 24 h, 95%; (f) BBr_3, CH_2Cl_2, -60 ^{0}C to 25 ^{0}C, 15 h to reflux 2 h; (g) K_2PtCl_4, DMF:H_2O, 2 days 60% from **6**.

As shown by the MTT assays on two human breast cancer cell lines, our new compounds demonstrated cytotoxicity on both ER^+ (MCF-7) and ER^- (MDA-MD-231) cells (Table 1). Clearly, the more lipophilic the compound, the better the cytotoxicity. The compounds **1Aa-c** without hydroxy group and **6Bd-OCH3** showed similar cytotoxicity as cisplatin and higher cytotoxicity than tamoxifen, particularly on the MDA-MD-231 (ER^-) cell line. This result can be explained by the following fashion: a more lipophilic compound could theoretically penetrate the lipophilic cell membranes more easily, therefore concentrate sufficiently in the cell to produce its biological activity.[27]

Table 1. Inhibitory concentration of drug on both ER^+ and ER^- breast cancer cell lines.

Drug \ Cell Line	MCF-7 (ER^+) IC_{50} (μM)[a]	MDA-MD-231 (ER^-) IC_{50} (μM)[a]	Number of hydroxy groups
Cisplatin	3.0	3.4	--
Tamoxifen	17	28	--
1Aa	8.0	5	0
1Ab	4.3	1.6	0
1Ac	4.9	1.2	0
1Ba	64	32	2
1Bb	56	29	2
1Bc	52	29	2
1Bd	23	9.3	2
6Bd-OCH3	14	2.3	0
1Be	74	21	2
1Cc[27]	67	18	3
1Cd	77	23	3
1Ce	15	4.3	3

a. Inhibitory concentration as obtained by the MTT assay.

As expected, platinum (II) complexes with a longer side chain are more active than their lower homologues. The compound **1Bd** with eleven carbon atoms side chain was significantly more cytotoxic as compared with compounds **1Ba-c** containing less carbon atoms. Interestingly, derivative **1Be** is less active than its lower homologue **1Bd** indicating that a very subtle change in the chemical structure can drastically alter the biological activity. Moreover, compound **1Ce** is more active than its lower homologues and showed similar activity as derivative **1Bd**. The complexes **1Ba-e** and **1Cc-e** with two and three hydroxy groups respectively showed cytotoxic activities by inhibiting proliferation of the MCF-7 (ER^+) cells, which appears not to be mediated by the ER. This seems to be the case since the proliferation of the MDA-MD-231 (ER^-) cells was inhibited at a lower concentration as it was for the inhibition of MCF-7 (ER^+) cells. However, it is important to indicate that the desired selectivity of the compounds **1Ba-e** and **1Cc-e** might be expressed more clearly (and possibly only) *in vivo* as demonstrated previously for similar types of derivatives.[32,33] The hypothesis of ER mediated selectivity of compounds **1Ba-e** and **1Cc-e** should be and will be further evaluated *in vivo* in the future.

Estrogen Receptor Binding Affinity

The relative binding affinities (RBA) of derivatives **1Aa-c, 1Ba-c and 1Ca-c** for the estrogen receptor were determined by a competitive cytosolic binding assay.[34] The binding affinity for estradiol (E_2) was set to 100%. As expected, compounds **1Aa-c** do not bind to the estrogen receptor (RBA = 0%). Derivatives containing two or three hydroxy groups possess the following RBA values: **1Ba** = 1.1%, **1Bb** = 1.4%, **1Bc** = 0.8%, **1Ca** = 0.2%, **1Cb** = 0.8%, **1Cc** = 1.0% and tamoxifen = 1.3%. A sufficient binding of the drug to the ER is essential for specificity of these cytotoxic agents.[35] Moreover, it is estimated on the basis of the number of receptors per cell (1 000-10 000) and the possible drug concentration that the RBA value should be at least 1% of the E_2 RBA.[35] Consequently, some of the new triphenylethylene platinum (II) complexes should be specific *in vivo*.

In summary, the lipophilicity of the triphenylethylene platinum (II) complexes is an important factor for the *in vitro* cytotoxic activity. Moreover, derivatives with two or three hydroxy groups possess affinity for the ER. The latter compounds might be specific towards ER^+ breast cancer cells in an *in vivo* biological test and will be further studied in our laboratory.

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