Synthesis and preliminary in vitro biological activity of non-steroidal cytotoxic estrogens designed for the treatment of breast cancer

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The development of resistance to endocrine therapy as well as chemotherapy is presently a major problem in the treatment of breast cancer. To minimize this obstacle, new, more selective and potent, chemotherapeutic agents should be designed. One way to improve selectivity is to link a cytotoxic moiety to a molecule possessing an affinity to the estrogen receptor (ER). The latter would be used to direct the cytotoxic portion of the molecule towards the target cells. Our initial approach led us to the synthesis of new triphenylethylene–platinum(II) complexes **1***a–c*. The commercially available desoxyanisoin (**10**) was efficiently transformed in seven steps into the platinum(II) complexes **1***a–c* with an overall yield exceeding 30%. The biological activity of compounds **1***a–c* was evaluated in vitro on ER+ and ER− human breast tumor cell lines: MCF-7 and MDA-MD-231.

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Le dévelopement de la résistance aux thérapies hormonales aussi bien qu'à la chimiothérapie est présentement un problème majeur dans le traitement du cancer du sein. De manière à minimiser cet obstacle, de nouveaux agents chemothérapeutiques plus selectifs et puissants devraient être conçus. Afin d'améliorer la sélectivité, il est possible de lier une portion cytotoxique à une molécule possédant une affinité pour le récepteur de l'estrogène (ER). Cette dernière serait utilisée pour diriger la portion cytotoxique de la molécule aux cellules cibles. Notre approche initiale nous a mené à la synthèse de complexes de triphényléthylène–platine(II) **1***a–c*. La désoxyanisoïne (**10**) commerciale fut transformée, en sept étapes, en complexes de platine(II) **1***a–c* avec un rendement global excédent 30%. L'activité biologique des composés **1***a–c* fut évaluée in vitro sur des cellules cancéreuses du sein : ER+ (MCF-7) et ER− (MDA-MD-231).

Introduction

Breast cancer is the most common form of cancer among women in North America. In 1993, it is estimated that more than 5000 women will die of this disease in Canada. Much progress has been accomplished for the treatment and the detection of this malady. Unfortunately, resistance to endocrine therapy as well as cytotoxic chemotherapy is still considered one of the major obstacles to successful treatment of breast cancer (1). Thus, many researchers are developing new drugs that are designed to overcome the problem of drug resistance. An attractive solution to this impediment is drug targeting. As we know, the aim of drug targeting is to deliver drugs only to those sites needing treatment; when this objective is met, not only the efficacy of the treatment will be improved, but toxic side effects will also be minimized (2). Therefore, we believe that new, more selective and potent, chemotherapeutic agents should be designed to rapidly eradicate the disease, preferably before any sign of resistance occurs.

The concept of linking an anti-cancer agent to a ligand with affinity for a tumor cell has some encouraging experimental precedent. As early as the 1960s, researchers investigated the possibility of linking nitrogen mustard groups to the steroidal skeleton in order to induce cytotoxic effects on hormone-dependent tumor cells (3). Since that time, several compounds (steroids and others) have been linked to various alkylating agents, including nitrogen mustards (4), nitrosoureas (5), and platinum complexes (6). However, in the early attempts, the cytotoxic moiety was often inappropriately linked to the estrogenic portion at the binding sites of the molecules, i.e., carbon 3 or 17 (steroid numbering, **3**, Scheme 1). This resulted in an inefficient interaction with the ER, and consequently a low biological activity. More recently, compounds **5** and **6**, synthesized in Germany (7, 8), showed very promising biological activities. The platinum complex **5** was found to be more potent than the parent drug cisplatin (**2**) and was selective on hormone-dependent breast cancer in vivo (in vitro, no selectivity was shown (7)). On the other hand, compound **6** showed selectivity on hormone-dependent breast cancer in both situations, i.e., in vitro and in vivo (8). In both cases, the exact mechanism(s) of action remains to be elucidated.

The new derivatives **1***a–c* were synthesized on the structural basis of the steroidal antiestrogens ICI 164 384 (**3**) and ICI 182 780 (**4**) recently described in the literature (9). Cytotoxic triphenylethylene with a long alkyl chain in the middle part of the molecule will, therefore, mimic the pure antiestrogens ICI 164 384 and ICI 182 780 bearing a 7-α side chain (see Scheme 1). This structural analogy should confer upon the new platinum(II) complexes both antiestrogenic and cytotoxic activity. We wish to report the details of our initial effort towards the synthesis of such derivatives (10) as well as their in vitro biological activity on MCF-7 (ER+) and MDA-MD-231 (ER−) human breast cancer cell lines.

Results and discussion

Synthesis of platinum(II) complexes

As shown in Scheme 3, three new (1,2-diaminoethane)

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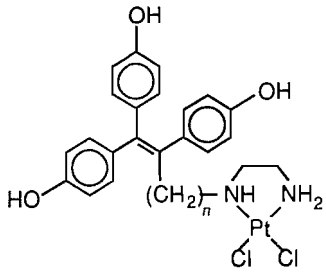
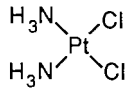


1 *a-c*



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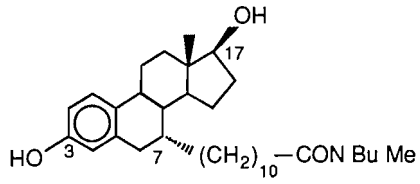

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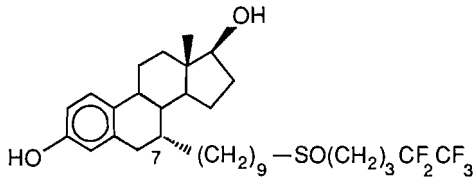

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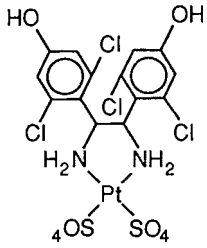

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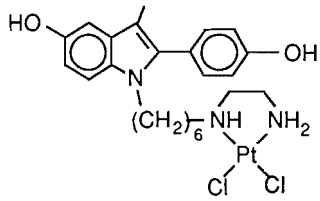
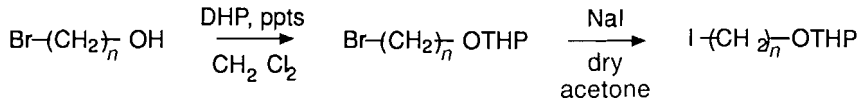


6

n = 6(*a*), 8(*b*), 10(*c*)

SCHEME 1



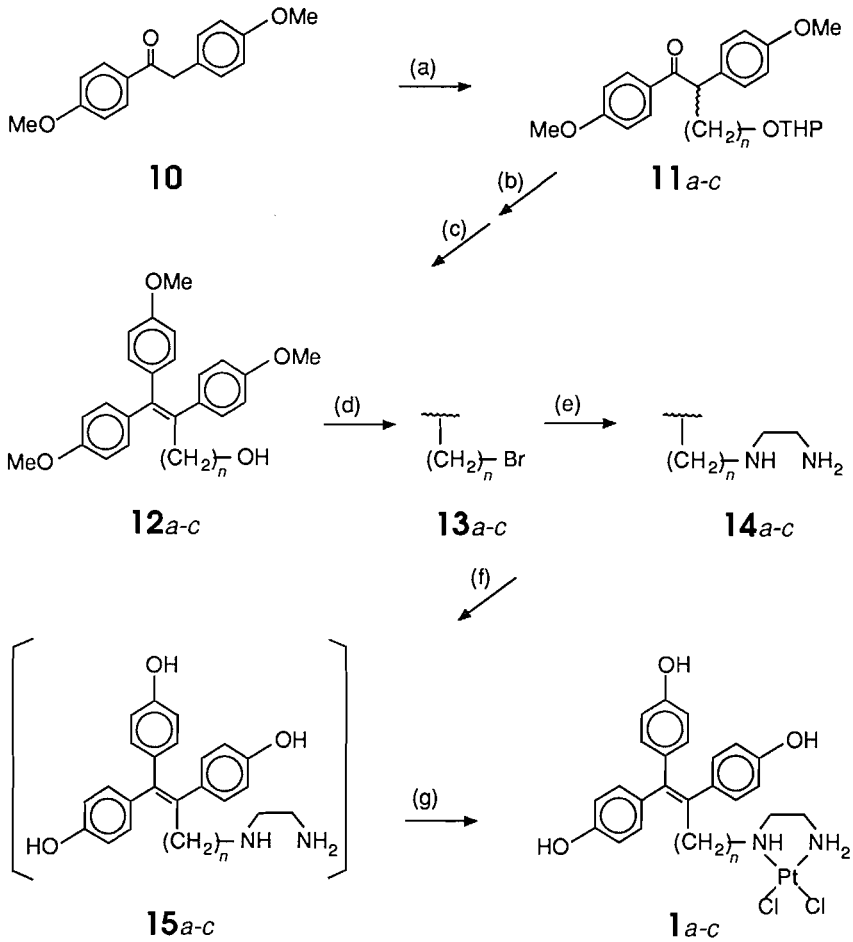
7 *a-c* **8** *a-c* **9** *a-c*

n = 6(*a*), 8(*b*), 10(*c*)

SCHEME 2

dichloroplatinum(II) complexes **1***a–c* were synthesized from commercially available desoxyanisoin (**10**). Initially the appropriate iodotetrahydropyranyl ethers were prepared. The choice of the length of the starting bromoalcohol **7** with 6, 8, and 10 carbon atoms is important since it has been observed that at least 6 carbon atoms are needed to give platinum derivatives such as compound **6** good estrogen receptor binding activity (11). The high affinity of compound **6** was possibly due to the interaction with an additional binding site removed from the receptor (11). As illustrated in Scheme 2, the alcohols **7***a–c* were protected as a tetrahydropyranyl ether (12) to give compounds **8***a–c*, which, upon treatment with sodium iodide in dry acetone, gave the iodotetrahydropyranyl ethers **9***a–c* (95% average yield for the two steps).

Alkylation of desoxyanisoin (**10**) with the iodotetrahydropyranyl ethers **9***a–c* was achieved using sodium hydride in tetrahydrofuran to give compounds **11***a–c* with an average yield of 65% (98% taking in consideration the alkyl iodide recovered, Scheme 3). Addition of an excess of *p*-methoxyphenylmagnesium bromide to ketones **11***a–c* and subsequent treatment of the crude tertiary alcohols with pyridinium-*p*-toluenesulfonate in ethanol at reflux afforded directly the triphenylethylene alcohols **12***a–c* with an average yield of 90% as the result of dehydration of the tertiary alcohol intermediates and simultaneous deprotection of the tetrahydropyranyl ethers (12). With the desired triphenylethylenes in hand the following sequence of reactions are simple functional group transformations. Initially, alcohols **12***a–c* were transformed to the bromides **13***a–c* (85% average yield) with carbon tetrabromide and triphenylphos-



Reagents: (a) NaH, I-$(CH_2)_n$-OTHP, THF, 25°C, 17 h, 65% (98%); (b) $MeOC_6H_4MgBr$, ether, 25°C, 17 h; (c) crude tertiary alochol, ethanol, PPTS, reflux, 5 h, 90% from **11**; (d) CBr_4, Ph_3P, ether, 25°C, 24 h, 85%; (e) $H_2NCH_2CH_2NH_2$, methanol, reflux, 24 h, 95% crude; (f) BBr_3, CH_2Cl_2, -60°C to 25°C (15 h) to reflux (2 h); (g) K_2PtCl_4, DMF: H_2O, 2 days, 60% from **14**

SCHEME 3

phine in dry ether (13). The amines **14***a–c* were obtained with an average yield of 95% by refluxing bromides **13***a–c* in the presence of an excess of ethylenediamine in dry methanol (11). Finally, demethylation with boron tribromide gave the intermediate tris-phenols **15***a–c*, which, upon treatment with potassium tetrachloroplatinate(II) in a mixture of dimethylformamide and water, led to the desired platinum(II) complexes **1***a–c* (60% average yield for the two steps (11, 14, 15)).

In vitro antitumor activity

Two human breast tumor cell lines were chosen, based on their estrogen receptor content, to evaluate the antitumor activity of our new platinum(II) complexes (16). The "cytotoxic antiestrogens" (**1***a* = Pt-6, **1***b* = Pt-8, **1***c* = Pt-10) were tested along with controls (vehicle, cisplatin = Cis-Pt, tamoxifen = TAM) on both ER+, i.e., MCF-7, and ER−, i.e., 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 soluble tetrazolium salt, 3(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide (MTT), into an insoluble formazan precipitate (17). A recent report (18) 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.

As shown by the MTT assays on human breast cancer cell lines, the new compounds demonstrated similar activity on either MCF-7 (ER+) or MDA-MD-231 (ER−) cells (see Table 1). Interestingly, the longer the side chain, the better the activity. This could be explained in the following fashion: a more lipophilic compound (Pt-10) could theoretically penetrate more easily the lipophilic cell membranes, therefore killing the cells more readily and (or), assuming an identical rate of infiltration in the cells, a compound with a longer chain could possibly alkylate DNA more efficiently due to fewer steric interactions between the triphenylethylene moiety and DNA. These interesting biological results also indicate that our new products are not selective to-

TABLE 1. Inhibitory concentration on breast tumor cells[a]

	MCF-7 (ER+) IC_{50} (μM)	MDA-MD-231 (ER−) IC_{50} (μM)
Cis-Pt	31	14
TAM	14	22
Pt-6	225	188
Pt-8	100	94
Pt-10	48	47

[a]IC_{50} as obtained by the MTT assay (see Experimental).

wards ER+ human breast cancer cells. It is important to indicate that the desired selectivity might be expressed more clearly (and possibly only) in vivo as was demonstrated previously for compound **5**. The fact that these compounds exhibit activity against both ER+ and ER− tumor cells may have advantages in that selective accumulation in the ER+ tumor could quench any ER− tumor cells that are known to spring up from the initial ER+ cells or destroy any ER− cells already present in the original, and predominantly ER+, tumor. This hypothesis will be further evaluated both in vitro and in vivo in the future.

Three new platinum(II) complexes have been synthesized and tested for biological activity. The synthesis of this type of compound is straightforward and efficient. The strategy described could be used to synthesize other types of DNA alkylating agents such as nitrogen mustards and nitrosoureas. Work is in progress in our laboratory to synthesize similar derivatives with improved biological activity.

Experimental

Synthesis of Pt(II) complexes

General procedures

Anhydrous reactions were performed under an inert atmosphere, the setup assembled and cooled under dry nitrogen. Unless otherwise noted, starting material, reactant, and solvents were obtained commercially and were used as such or purified and (or) dried by standard means (19). Organic solutions were dried over magnesium sulphate ($MgSO_4$), and evaporated on a rotatory evaporator and under reduced pressure. All reactions were monitored by thin-layer chromatography (TLC). The plates were visualized by UV fluorescence, or by staining with iodine or spraying with an aqueous solution of phosphomolybdic acid followed by heating the plate at ~135°C. Commercial TLC plates were Sigma T 6145 (polyester silica gel 60 Å (0.25 mm)). Preparative TLC was performed on 1 mm silica gel 60 Å, 20 × 20 plates (Whatman, 4861 840). Flash chromatography was performed according to the method of Still et al. (20) on Merck grade 60 silica gel, 230–400 mesh. All solvents used in chromatography had been distilled. Melting points (mp) were recorded on an Electrothermal 9100 apparatus and are uncorrected. The infrared spectra (IR) were taken on a Nicolet model 205 FT-IR spectrophotometer. Mass spectral assays (MS, *m/e*) were obtained using a VG Micromass 7070 HS instrument using an ionization energy of 70 eV. Elemental analyses were conducted by Microanalysis Laboratories Limited, Markham, Ontario. Nuclear magnetic resonance (NMR) spectra were obtained in $CDCl_3$ solution, unless otherwise noted, on a General Electric GE 300-NB (300 MHz) instrument: chemical shifts were measured relative to internal standards: tetramethylsilane (TMS, δ 0.0 ppm) for ^1H and $CDCl_3$ (δ 77.0 ppm) for ^{13}C NMR. Multiplicities are described by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet), tq (triple quartet), mm (multiple multiplets), and so on. The NMR assignments were assisted by ^{13}C–^1H correlation (HETCORR) 2-D spectra.

General procedure for conversion of bromoalcohols to iodotetrahydropyranyl ethers

*1-Tetrahydropyranyloxy-6-bromohexane (**8**a).* A solution of 6-bromo-1-hexanol **7***a* (5.0 g, 27.6 mmol), dihydropyran (2.58 g (2.8 mL), 30.6 mmol), and pyridinium *p*-toluenesulfonate (PPTS, 10 mg, 3.97×10^{-5} mol) in dichloromethane (CH_2Cl_2, 50 mL) was stirred for 5 h under nitrogen. Afterwards, sodium bicarbonate ($NaHCO_3$, 500 mg) and $MgSO_4$ (5.0 g) were added to the reaction mixture and stirred 15 min before being filtered on a short pad of Celite/silica gel (1 cm/4 cm) using CH_2Cl_2 as eluent. The filtrate was evaporated to a viscous oil **8***a* (7.16 g, 98%), which was used without further purification in the next step. IR (neat): 1170–1000 (C—O), cm^{-1}. ^1H NMR (δ ppm): 4.57 (1H, t apparent, J = 3.2 Hz, -OCHO-), 3.87, 3.74, 3.50, and 3.38 (4H, 4 × m, -CH_2OCHOCH_2-), 3.41 (2H, t, J = 6.7 Hz, -CH_2Br), 1.3–2.0 (14H, m, BrCH$_2$-(CH_2)$_4$-CH$_2$O-THP). MS (*m/e*): 265 (M^+ +1), 163 (M^+ −OTHP).

*1-Tetrahydropyranyloxy-6-iodohexane (**9**a).* To a solution of crude bromide **8***a* (7.16 g, 27 mmol) in dry acetone was added sodium iodide (6.07 g, 40.5 mmol). The reaction mixture was stirred at 25°C for 5 h. Then, most of the solvent was evaporated and the residue was transferred to an extraction flask with ether (100 mL) and water (100 mL). The organic phase was washed with water (6 × 50 mL), dried, filtered, and concentrated to a viscous liquid. The crude iodide **9***a* (8.26 g, 98%) was used as such at the alkylation step. IR (neat): 1170–1000 (C—O) cm^{-1}. ^1H NMR (δ ppm): 4.57 (1H, t apparent, J = 3.2 Hz, -OCHO-), 3.87, 3.74, 3.50, and 3.38 (4H, 4 × m, -CH_2OCHOCH_2-), 3.19 (2H, t, J = 7.0 Hz, -CH_2I), 1.3–2.0 (14H, m, ICH$_2$-(CH_2)$_4$-CH$_2$O-THP). MS (*m/e*): 311 (M^+ −H), 211 (M^+ −OTHP).

*1-Tetrahydropyranyloxy-8-bromooctane (**8**b):* 98% yield. IR (neat): 1170–1000 (C—O) cm^{-1}. ^1H NMR (δ ppm): 4.58 (1H, t apparent, J = 3.5 Hz, -OCHO-), 3.87, 3.73, 3.50, and 3.38 (4H, 4 × m, -CH_2OCHOCH_2-), 3.41 (2H, t, J = 6.8 Hz, -CH_2Br), 1.2–2.0 (18H, m, BrCH$_2$-(CH_2)$_6$-CH$_2$O-THP). MS (*m/e*): 293 (M^+ +1).

*1-Tetrahydropyranyloxy-8-iodooctane (**9**b):* 98% yield. IR (neat): 1170–1000 (C—O) cm^{-1}. ^1H NMR (δ ppm): 4.58 (1H, t apparent, J = 3.5 Hz,-OCHO-), 3.87, 3.73, 3.50, and 3.38 (4H, 4 × m, -CH_2OCHOCH_2-), 3.19 (2H, t, J = 7.0 Hz, -CH_2I), 1.2–2.0 (18H, m, ICH$_2$-(CH_2)$_6$-CH$_2$O-THP). MS (*m/e*): 339 (M^+ −H), 239 (M^+ −OTHP).

*1-Tetrahydropyranyloxy-10-bromodecane (**8**c):* 99% yield. IR (neat): 1170–1000 (C—O) cm^{-1}. ^1H NMR (δ ppm): 4.58 (1H, t apparent, J = 3.5 Hz, -OCHO-), 3.87, 3.73, 3.50, and 3.38 (4H, 4 × m, -CH_2OCHOCH_2-), 3.41 (2H, t, J = 6.8 Hz, -CH_2Br), 1.2–2.0 (22H, m, BrCH$_2$-(CH_2)$_8$-CH$_2$O-THP). MS (*m/e*): 321 (M^+ +1).

*1-Tetrahydropyranyloxy-10-iododecane (**9**c):* 98% yield. IR (neat): 1170–1000 (C—O) cm^{-1}. ^1H NMR (δ ppm): 4.58 (1H, t apparent, J = 3.5 Hz, -OCHO-), 3.87, 3.73, 3.50, and 3.38 (4H, 4 × m, -CH_2OCHOCH_2-), 3.19 (2H, t, J = 7.0 Hz, -CH_2I), 1.2–2.0 (22H, m, ICH$_2$-(CH_2)$_8$-CH$_2$O-THP). MS (*m/e*): 367 (M^+ −H).

General procedure for conversion of desoxyanisoin to triphenylethylene platinum(II) complexes

*A. Synthesis of 1,2-bis(4-methoxyphenyl)-X-tetrahydropyranyloxy-alkylones **11**a–c.* To a stirred suspension of sodium hydride (732 mg, 18.3 mmol, 60% dispersion in mineral oil) in dry tetrahydrofuran (THF, 150 mL) was added rapidly desoxyanisoin (**10**) (4.47 g, 17.4 mmol). The reaction mixture was heated (50°C) with a water bath for 1 h under a nitrogen atmosphere. After cooling, the iodotetrahydropyranyl ether (18.3 mmol) was added dropwise and the resulting mixture stirred overnight (17 h). Most of the solvent was then evaporated and the residue was diluted with ether (200 mL) and treated with water (50 mL). The ethereal phase was washed thoroughly with water (6 × 50 mL), dried, and evaporated to give an oil that was purified by flash chromatography (hexane:acetone, 85:15). A viscous oil was obtained. The aver-

age yield was 65% (98% taking into account the alkyl iodide recovered).

*1,2-Bis(4-methoxyphenyl)-8-tetrahydropyranyloxy-octanone (**11**a):* IR (neat): 1674 (C=O), 1599 (C=C), 1254 (C=O) cm^{-1}. ^1H NMR (δ ppm): 7.95, 6.84 and 7.21, 6.80 (8H, 2 × two d, J = 8.88 and 8.66 Hz, 2 × *para*-substituted anisyl group), 4.55 (1H, t, J = 3.45 Hz, -OCHO-), 4.44 (1H, t, J = 7.23 Hz, -CH-), 3.82 and 3.47 (2H, two m, -CH_2OTHP), 3.70 and 3.34 (2H, two m, -CHOCH_2-), 3.77 and 3.71 (6H, two s, 2 × -OCH_3), 2.20–1.10 (16H, br singlets, 8 × -CH_2-). ^{13}C NMR (δ ppm): 198.57, 162.97, 158.23, 132.03, 130.69(2), 129.69, 128.91(2), 113.98(2), 113.44(2), 98.62, 67.37, 62.13, 55.17, 54.93, 52.08, 33.83, 30.60, 29.50, 29.30, 27.47, 25.92, 25.32, 19.53. MS (*m/e*): 440 (M^+), 356 (M^+ −DHP).

*1,2-Bis(4-methoxyphenyl)-10-tetrahydropyranyloxy-decanone (**11**b):* IR (neat): 1674 (C=O), 1599 (C=C), 1255 (C—O) cm^{-1}. ^1H NMR (δ ppm): 7.95, 6.86 and 7.21, 6.81 (8H, 2 × two d, J = 8.87 and 8.62 Hz, 2 × *para*-substituted anisyl group), 4.56 (1H, t, J = 3.45 Hz, -OCHO-), 4.44 (1H, t, J = 7.23 Hz, -CH-), 3.82 and 3.47 (2H, two m, -CH_2OTHP), 3.70 and 3.35 (2H, two m, -CHOCH_2-), 3.81 and 3.74 (6H, two s, 2 × -OCH_3), 2.20–1.10 (20H, br singlets, 10 × -CH_2-). ^{13}C NMR (δ ppm): 198.77, 163.07, 158.32, 132.20, 130.82(2), 129.84, 129.03(2), 114.09(2), 113.55(2), 98.75, 67.58, 62.26, 55.32, 55.10, 52.24, 33.98, 30.72, 29.65, 29.53, 29.33(2), 27.64, 26.14, 25.44, 19.64. MS (*m/e*): no molecular ion, 384 (M^+ −DHP).

*1,2-Bis(4-methoxyphenyl)-12-tetrahydropyranyloxy-dodecanone (**11**c):* IR (neat): 1674 (C=O), 1599 (C=C), 1254 (C—O) cm^{-1}. ^1H NMR (δ ppm): 7.95, 6.87 and 7.21, 6.81 (8H, 2 × two d, J = 8.88 and 8.67 Hz, 2 × *para*-substituted anisyl group), 4.57 (1H, t, J = 3.45 Hz, -OCHO-), 4.40 (1H, t, J = 7.23 Hz, -CH-), 3.82 and 3.47 (2H, two m, -CH_2OTHP), 3.70 and 3.34 (2H, two m, -CHOCH_2-), 3.80 and 3.73 (6H, two s, 2 × -OCH_3), 2.20–1.10 (24H, br singlets, 12 × -CH_2-). ^{13}C NMR (δ ppm): 198.74, 163.04, 158.31, 132.18, 130.78(2), 129.82, 129.00(2), 114.05(2), 113.52(2), 98.73, 67.58, 62.24, 55.28, 55.04, 52.20, 33.97, 30.69, 29.65, 29.55, 29.44(2), 29.39(2), 27.61, 26.14, 25.42, 19.63. MS (*m/e*): no molecular ion, 412 (M^+ −DHP).

*B. Synthesis of X,Y,Y-tris(4-methoxyphenyl)-X-alken-1-ols **12**a–c.* A Grignard reagent was prepared from 460 mg (18.9 mmol) of magnesium and 2.98 g (2 mL, 15.9 mmol) of 4-bromoanisole in the presence of a crystal of iodine in 100 mL of dry ether. The Grignard reagent was usually ready after stirring at room temperature overnight (17 h), but sometimes required heating at reflux to initiate the reaction. A solution of ketone **11** (3.0 mmol) in dry ether was treated with the excess of Grignard reagent for 3 h under nitrogen at 25°C and was then hydrolysed with 50 mL of 10% aqueous ammonium chloride. The ethereal phase was washed with water (5 × 50 mL), dried, and evaporated to give the crude tertiary alcohol intermediate. The oily residue was dehydrated and deprotected in 100 mL of 95% ethanol in the presence of PPTS (100 mg, 0.34 mmol) at reflux for 2.5 h. After evaporation of the solvent, the residue was taken with ether and extracted with water (5 × 50 mL), dried, and evaporated to an oil. Flash chromatography (hexanes:acetone, 85:15) gave **12** in 90% average yield as a viscous oil.

*7,8,8-Tris(4-methoxyphenyl)-7-octen-1-ol (**12**a):* IR (neat): 3640–3150 (OH), 1605 (C=C), 1242 (C—O), cm^{-1}. ^1H NMR (δ ppm): 7.13, 6.85 and 7.01, 6.69 and 6.78, 6.54 (12H, 3 × two d, J = 8.70, 8.70, and 8.78 Hz, 3 × *para*-substituted anisyl group), 3.78, 3.71, and 3.64 (9H, 3 × s, 3 × -OCH_3), 3.51 (2H, t, J = 6.65 Hz, -CH_2OH), 2.40 (2H, m, -C=CCH_2-), 1.90 (1H, br s, -OH), 1.50–1.10 (8H, m, -CH_2-(CH_2)$_4$-CH_2-). ^{13}C NMR (δ ppm): 157.93, 157.48, 157.09, 139.06, 137.40, 136.34, 135.88, 134.88, 131.76(2), 130.46(4), 113.25(2), 113.11(2), 112.58(2), 62.61, 55.02, 54.88, 54.82, 35.69, 32.43, 29.35, 28.81, 25.25. MS (*m/e*): 446 (M^+), 359 (M^+ −$C_5H_{10}OH$).

*9,10,10-Tris(4-methoxyphenyl)-9-decen-1-ol (**12**b):* IR (neat): 3690–3150 (OH), 1605 (C=C), 1242 (C—O) cm^{-1}. ^1H NMR (δ ppm): 7.13, 6.87 and 7.02, 6.72 and 6.78, 6.55 (12H, 3 × two d, J = 8.70, 8.73, and 8.81 Hz, 3 × *para*-substituted anisyl group), 3.81, 3.75, and 3.68 (9H, 3 × s, 3 × -OCH_3), 3.59 (2H, t, J = 6.65 Hz, -CH_2OH), 2.40 (2H, m, -C=CCH_2-), 1.90 (1H, br s, -OH), 1.60–1.10 (12H, m, -CH_2-(CH_2)$_6$-CH_2-). ^{13}C NMR (δ ppm): 158.02, 157.55, 157.22, 139.29, 137.42, 136.50, 136.03, 135.04, 131.87(2), 130.56(4), 113.34(2), 113.19(2), 112.67(2), 62.99, 55.16, 54.99(2), 35.82, 32.71, 29.59(2), 29.19, 28.91, 25.62. MS (*m/e*): 474 (M^+), 359 (M^+ −$C_7H_{14}OH$).

*11,12,12-Tris(4-methoxyphenyl)-11-dodecen-1-ol (**12**c):* IR (neat): 3640–3150 (OH), 1605 (C=C), 1242 (C—O), cm^{-1}. ^1H NMR (δ ppm): 7.13, 6.87 and 7.02, 6.70 and 6.78, 6.55 (12H, 3 × two d, J = 8.70, 8.73, and 8.80 Hz, 3 × *para*-substituted anisyl group), 3.81, 3.74, and 3.67 (9H, 3 × s, 3 × -OCH_3), 3.62 (2H, t, J = 6.65 Hz, -CH_2OH), 2.40 (2H, m, -C=CCH_2-), 1.80 (1H, br s, -OH), 1.60–1.10 (16H, m, -CH_2-(CH_2)$_8$-CH_2-). ^{13}C NMR (δ ppm): 157.92, 157.47, 157.12, 139.29, 137.32, 136.46, 136.02, 135.02, 131.84(2), 130.54(4), 113.28(2), 113.14(2), 112.63(2), 62.92, 55.10, 54.98, 54.92, 35.80, 32.62, 29.62, 29.45, 29.32(2), 29.19, 28.90, 25.63. MS (*m/e*): 502 (M^+), 359 (M^+ −$C_9H_{18}OH$).

*C. Synthesis of 1-bromo-X,Y,Y-tris(4-methoxyphenyl)-X-alkenes **13**a–c.* A solution of alcohol **12** (5.83 mmol), carbon tetrabromide (7.73 g, 23.3 mmol), and triphenylphosphine (6.11 g, 23.3 mmol) in dry ether (250 mL) was stirred at room temperature (25°C) for 20 h under a nitrogen atmosphere. The triphenylphosphine oxide precipitate was filtered and the resulting solution was washed thoroughly with water (5 × 50 mL), dried, and evaporated to an oil. The crude material was purified by flash chromatography (hexanes:acetone, 95:5) to give the bromide **13** in 85% average yield as a viscous oil.

*1-Bromo-7,8,8-tris(4-methoxyphenyl)-7-octene (**13**a):* IR (neat): 1605 (C=C), 1242 (C—O), cm^{-1}. ^1H NMR (δ ppm): 7.13, 6.86 and 7.02, 6.69 and 6.78, 6.54 (12H, 3 × two d, J = 8.67, 8.69, and 8.74 Hz, 3 × *para*-substituted anisyl group), 3.79, 3.72, and 3.64 (9H, 3 × s, 3 × -OCH_3), 3.30 (2H, t, J = 6.84 Hz, -CH_2Br), 2.41 (2H, m, -C=CCH_2-), 1.73 (2H, p, J = 7.20 Hz, -CH_2CH_2Br), 1.26 (6H, m, -CH_2-(CH_2)$_3$-CH_2CH_2Br). ^{13}C NMR (δ ppm): 158.02, 157.55, 157.19, 138.91, 137.55, 136.31, 135.83, 134.80, 131.77(2), 130.46(4), 113.29(2), 113.17(2), 112.61(2), 55.06, 54.91, 54.84, 35.62, 33.87, 32.53, 28.66(2), 27.72. MS (*m/e*): 508 (M^+), 510 (M^+ +2), 359 (M^+ −$C_5H_{10}Br$).

*1-Bromo-9,10,10-tris(4-methoxyphenyl)-9-decene (**13**b):* IR (neat): 1605 (C=C), 1242 (C—O), cm^{-1}. ^1H NMR (δ ppm): 7.13, 6.87 and 7.02, 6.71 and 6.77, 6.55 (12H, 3 × two d, J = 8.73, 8.79, and 8.79 Hz, 3 × *para*-substituted anisyl group), 3.82, 3.76, and 3.69 (9H, 3 × s, 3 × -OCH_3), 3.37 (2H, t, J = 6.87 Hz, -CH_2Br), 2.42 (2H, m, -C=CCH_2-), 1.80 (2H, p, J = 7.35 Hz, -CH_2CH_2Br), 1.40–1.10 (10H, m, -CH_2-(CH_2)$_5$-CH_2CH_2Br). ^{13}C NMR (δ ppm): 158.05, 157.57, 157.21, 139.22, 137.46, 136.46, 136.01, 134.00, 131.87(2), 130.57(4), 113.33(2), 113.19(2), 112.67(2), 55.18, 55.04, 54.98, 35.80, 34.08, 32.74, 29.53, 29.02, 28.87, 28.56, 28.05. MS (*m/e*): 536 (M^+), 538 (M^+ +2), 359 (M^+ −$C_7H_{14}Br$).

*1-Bromo-11,12,12-tris(4-methoxyphenyl)-11-dodecene (**13**c):* IR (neat): 1605 (C=C), 1242 (C—O) cm^{-1}. ^1H NMR (δ ppm): 7.13, 6.85 and 7.02, 6.69 and 6.78, 6.54 (12H, 3 × two d, J = 8.70, 8.77, and 8.84 Hz, 3 × *para*-substituted anisyl group), 3.78, 3.71, and 3.64 (9H, 3 × s, 3 × -OCH_3), 3.36 (2H, t, J = 6.86 Hz, -CH_2Br), 2.40 (2H, m, -C=CCH_2-), 1.81 (2H, p, J = 7.35 Hz, -CH_2CH_2Br), 1.40–1.10 (14H, m, -CH_2-(CH_2)$_7$-CH_2CH_2Br). ^{13}C NMR (δ ppm): 157.98, 157.51, 157.14, 139.20, 137.36, 136.39, 135.95, 134.94, 131.80(2), 130.50(4), 113.24(2), 113.11(2), 112.60(2), 55.04, 54.89, 54.83, 35.78, 33.95, 32.68, 29.60, 29.28(2), 29.14, 28.88, 28.62, 28.05. MS (*m/e*): 564 (M^+), 566 (M^+ +2), 359 (M^+ −$C_9H_{18}Br$).

*D. Synthesis of 1-[(2-aminoethyl)amino]-X,Y,Y-tris(4-methoxyphenyl)-X-alkenes **14**a–c.* Under a nitrogen atmosphere, ethylenediamine (592 mg (658 μL), 9.84 mmol) was added to a solution of triphenylethylene bromide **13** (0.984 mmol) in 50 mL of dry methanol. After boiling for 2 days under reflux (sometimes a longer

reaction period is required), the solvent was evaporated. The resulting residue was dissolved in ether (100 mL) and washed with a solution of $NaHCO_3$ (30 mL, 5% aqueous) and with water (5 × 50 mL). The ethereal phase was dried and evaporated to a viscous oil. The average yield was 75%.

*1-[(2-Aminoethyl)amino]-7,8,8-tris(4-methoxyphenyl)-7-octene (**14**a):* IR (neat): 3565–3130 (N—H), 1605 (C=C), 1242 (C—O) cm^{-1}. ^1H NMR (δ ppm): 7.13, 6.85 and 7.01, 6.67 and 6.78, 6.52 (12H, 3 × two d, J = 8.28, 8.33, and 8.21 Hz, 3 × *para*-substituted anisyl group), 3.74, 3.64, and 3.59 (9H, 3 × s, 3 × $-OCH_3$), 2.75 (2H, br s, $-CH_2NH_2$), 2.61 (2H, br s, $-CH_2CH_2NH_2$), 2.51 (2H, t, J = 7.0 Hz, $-CH_2NH$-), 2.42 (2H, t, J = 7.0 Hz, $-C{=}CCH_2$-), 2.29 (3H, br s, -NH and $-NH_2$), 1.50–1.10 (8H, m, $-CH_2-(CH_2)_4-CH_2$-). ^{13}C NMR (δ ppm): 157.68, 157.21, 156.87, 138.76, 137.11, 136.00, 135.55, 134.53, 131.47(2), 130.16(4), 112.96(2), 112.82(2), 112.28(2), 54.66, 54.50, 54.44, 51.69, 49.32, 41.02, 35.44, 29.49, 29.22, 28.56, 26.62. MS (*m/e*): 488 (M^+), 458 ($M^+ - CH_2NH_2$), 445 ($M^+ - CH_2CH_2NH$).

*1-[(2-Aminoethyl)amino]-9,10,10-tris(4-methoxyphenyl)-9-decene (**14**b):* IR (neat): 3660–3130 (N—H), 1605 (C=C), 1242 (C—O) cm^{-1}. ^1H NMR (δ ppm): 7.13, 6.86 and 7.02, 6.68 and 6.78, 6.53 (12H, 3 × two d, J = 8.32, 8.35, and 8.52 Hz, 3 × *para*-substituted anisyl group), 3.95 (3H, br s, -NH and $-NH_2$), 3.77, 3.70, and 3.63 (9H, 3 × s, 3 × $-OCH_3$), 2.93 (2H, m, $-CH_2NH_2$), 2.82 (2H, m, $-CH_2CH_2NH_2$), 2.66 (2H, m, $-CH_2NH$-), 2.40 (2H, m, $-C{=}CCH_2$-), 1.60–1.10 (12H, m, $-CH_2-(CH_2)_6-CH_2$-). ^{13}C NMR (δ ppm): 157.77, 157.30, 156.94, 138.95, 137.17, 136.15, 135.73, 134.70, 131.60(2), 130.29(4), 113.06(2), 112.95(2), 112.40(2), 54.85, 54.69, 54.64, 50.24, 48.85, 39.60, 35.58, 29.38, 28.95(2), 28.69, 28.37, 26.84. MS (*m/e*): 516 (M^+), 486 ($M^+ - CH_2NH_2$), 473 ($M^+ - CH_2CH_2NH$).

*1-[(2-Aminoethyl)amino]-11,12,12-tris(4-methoxyphenyl)-11-dodecene (**14**c):* IR (neat): 3660–3140 (N—H), 1605 (C=C), 1242 (C—O) cm^{-1}, ^1H NMR (δ ppm): 7.13, 6.86 and 7.02, 6.70 and 6.78, 6.55 (12H, 3 × two d, J = 8.66, 8.70, and 8.80 Hz, 3 × *para*-substituted anisyl group), 3.81, 3.74, and 3.67 (9H, 3 × s, 3 × $-OCH_3$), 2.80 (2H, t, J = 6.0 Hz, $-CH_2NH_2$), 2.66 (2H, t, J = 6.0 Hz, $-CH_2CH_2NH_2$), 2.59 (2H, t, J = 7.2 Hz, $-CH_2NH$-), 2.40 (2H, m, $-C{=}CCH_2$-), 2.04 (3H, br s, -NH and $-NH_2$), 1.50–1.10 (16H, m, $-CH_2-(CH_2)_8-CH_2$-). ^{13}C NMR (δ ppm): 157.97, 157.49, 157.14, 139.26, 137.31, 136.42, 136.00, 134.99, 131.81(2), 130.51(4), 113.25(2), 113.12(2), 112.61(2), 55.08, 54.91(2), 52.32, 49.81, 41.55, 35.80, 30.02, 29.66, 29.46(3), 29.23, 28.92, 27.28. MS (*m/e*): 544 (M^+), 514 ($M^+ - CH_2NH_2$), 501 ($M^+ - CH_2CH_2NH$).

*E. Synthesis of 1-[(2-aminoethyl)amino]-X,Y,Y-tris(4-hydroxyphenyl)-X-alkenes **15**a–c.* A solution of trimethyl ether **14** (0.665 mmol) in dry CH_2Cl_2 (30 mL) was treated with a solution of boron tribromide (2.33 mL (1 M), 2.33 mmol) at −60°C, under a nitrogen atmosphere. After the addition, the reaction mixture was allowed to warm to room temperature (25°C) and was stirred for 17 h. The mixture was refluxed during 2 h. The reaction was cooled with an ice bath before adding 15 mL of methanol. The resulting solution was concentrated to 2–3 mL, treated with saturated $NaHCO_3$ solution (30 mL), and extracted with ethyl acetate (5 × 30 mL). The crude yields range from 50 to 85%. Compounds **15***a–c* were used without further purification in the next step.

*F. Synthesis of 1-[*cis*-[(2-aminoethyl)amino]dichloroplatinum-(II)]-X,Y,Y-tris(4-hydroxyphenyl)-X-alkenes **1**a–c.* A solution of potassium tetrachloroplatinate(II) (219 mg, 0.527 mmol) in 7.5 mL of a mixture of dimethylformamide (DMF) and water (2:1) was added to a warm (35°C) solution of diamine **15** (0.527 mmol) in 5 mL of DMF. The resulting mixture (pH 9–10) was stirred in the dark for 2–3 days until the pH value reached 4–5. Then, 1 drop of dimethyl sulfoxide was added and the stirring was continued for 2 h. The solvent was evaporated and the residue was suspended in saturated potassium chloride solution (30 mL; **note**: a vigorous stirring was essential to pulverized the lumps of platinum(II) complex). The resulting suspension was filtered, washed with water (100–250 mL), and dried in a desiccator. The product can be further purified either by flash chromatography or by preparative TLC (CH_2Cl_2:methanol, 9:1). The crude yields range from 70 to 95%.

*1-[*cis*-[(2-Aminoethyl)amino]dichloroplatinum(II)]-7,8,8-tris(4-hydroxyphenyl)-7-octene (**1**a):* mp >148°C (dec.). IR (KBr): 3640–3050 (N—H, O—H), 1605 (C=C), 1223 (C—O) cm^{-1}. ^1H NMR (acetone-d_6, δ ppm): 8.6–8.2 (3H, br s, 3 × -OH), 7.02, 6.83 and 6.94, 6.64 and 6.69, 6.48 (12H, 3 × two d, J = 8.57, 8.59, and 8.59 Hz, 3 × *para*-substituted phenol), 5.64, 5.08, and 4.97 (3H, 3 × br s, $-NHCH_2CH_2NH_2$), 3.15 (6H, br s, $-CH_2NHCH_2CH_2NH_2$), 3.03 (2H, br s, $-CH_2CH_2NH$-), 2.80–2.55 (2H, br s, CH_2CH_2-CH_2NH-), 2.39 (2H, m, $-C{=}CCH_2$-), 1.80–1.10 (4H, m, $-C{=}CCH_2-(CH_2)_2$-). ^{13}C NMR (acetone-d_6, δ ppm): 156.70, 156.20, 155.88, 139.39, 138.78, 136.16, 135.93, 134.64, 132.55(2), 131.43(2), 131.26(2), 115.61(2), 115.44(2), 114.84(2), 56.11, 53.43, 47.67, 36.21, 27.58, 26.81. (N.B. 2 carbons are hidden by acetone.) Anal. calcd. for $C_{28}H_{34}Cl_2N_2O_3Pt$: C 47.26, H 4.78, N 3.94; found: C 47.52, H 4.50, N 3.81.

*1-[*cis*-[(2-Aminoethyl)amino]dichloroplatinum(II)]-9,10,10-tris(4-hydroxyphenyl)-9-decene (**1**b):* mp >150°C (dec.). IR (KBr): 3700–3000 (N—H, O—H), 1605 (C=C), 1223 (C—O) cm^{-1}. ^1H NMR (acetone-d_6, δ ppm): 8.53, 8.39, and 8.32 (3H, 3 × br s, 3 × -OH), 7.02, 6.83 and 6.94, 6.64 and 6.69, 6.48 (12H, 3 × two d, J = 8.50, 8.60, and 8.64 Hz, 3 × *para*-substituted phenol), 5.75, 5.19, and 5.05 (3H, 3 × br s, $-NHCH_2CH_2NH_2$), 3.24 (6H, br s, $-CH_2NHCH_2CH_2NH_2$), 3.08 (2H, br s, $-CH_2CH_2NH$-), 2.73 (2H, br s, $-CH_2CH_2CH_2NH$-), 2.39 (2H, m, $-C{=}CCH_2$-), 1.80–1.10 (8H, m, $-C{=}CCH_2-(CH_2)_4$-). ^{13}C NMR (acetone-d_6, δ ppm): 156.01, 155.62, 155.20, 138.87, 138.10, 135.54, 135.25, 134.03, 131.88(2), 130.71(2), 130.58(2), 114.91(2), 114.76(2), 114.19(2), 55.43, 52.90, 46.99, 35.69, 27.12, 26.54. (N.B. 4 carbons are hidden by acetone.) Anal. calcd. for $C_{30}H_{38}Cl_2N_2O_3Pt$: C 48.71, H 5.14, N 3.79; found: C 48.82, H 5.14, N 3.68.

*1-[*cis*-[(2-Aminoethyl)amino]dichloroplatinum(II)]-11,12,12-tris(4-hydroxyphenyl)-11-dodecene (**1**c):* mp >150°C (dec.). IR (KBr): 3775–3050 (N—H, O—H), 1605 (C=C), 1223 (C—O) cm^{-1}. ^1H NMR (δ ppm): 8.53, 8.39, and 8.32 (3H, 3 × s, 3 × -OH), 7.02, 6.81 and 6.93, 6.63 and 6.69, 6.48 (12H, 3 × two d, J = 8.51, 8.62, and 8.65 Hz, 3 × *para*-substituted phenol), 5.72, 5.11, and 4.98 (3H, 3 × br s, $-NHCH_2CH_2NH_2$), 3.14 (6H, br s, $-CH_2NHCH_2CH_2NH_2$), 3.05 (2H, br s, $-CH_2CH_2NH$-), 2.72 (2H, br s, $-CH_2CH_2CH_2NH$-), 2.39 (2H, m, $-C{=}CCH_2$-), 1.80–1.10 (12H, m, $-C{=}CCH_2-(CH_2)_6$-). ^{13}C NMR (acetone-d_6, δ ppm): 156.72, 156.23, 155.88, 139.59, 138.70, 136.23, 135.63, 134.70, 132.58(2), 131.39(2), 131.27(2), 115.52(2), 115.40(2), 114.89, 114.84, 56.13, 53.51, 47.82, 36.43, 27.82, 27.26. (N.B. 6 carbons are hidden by acetone.) Anal. calcd. for $C_{32}H_{42}Cl_2N_2O_3Pt$: C 50.20, H 5.49, N 3.66; found: C 50.53, H 5.62, N 3.70.

In vitro antitumor activity

Microcytostasis assay

MTT was dissolved in phosphate-buffered saline (PBS) at a concentration of 5 mg/mL and stored in a dark environment at 4°C for a maximum of 3 weeks. Cell lines were grown until they were subconfluent, then trypsinized, washed in PBS, and resuspended in fresh media. A total of 100 μL medium containing 10^4 viable cells was plated per well into 96-well microtitre plates. Cells were allowed to attach to the plates for 24 h at 37°C in a 5% CO_2 atmosphere. The new drugs were dissolved in 0.25 mL of 95% ethanol and 4.75 mL of fresh media to a concentration of 1000 μ*m*. Drug dilutions were prepared in culture medium (range 1–1000 μ*m*). Medium was removed from the cells and test dilutions were added in 100 μ*m* fresh medium. Tests were carried out with 10 control wells (cells, no drug treatment) and 3 wells for each test dilution. Cells were exposed to the drug for 24 h at 37°C in 5% CO_2. Medium was removed and the cells were washed three times with sterile PBS. Next, 100 μL fresh medium was added to each well, followed by a 24 h recovery period. Subsequently, the medium was aspirated from each well and 100 μL 1:10 dilution of

MTT stock (5 mg/mL) was added to each well, followed by a 4 h incubation period at 37°C. MTT/medium blank controls with no cells also received 100 μM MTT/well. After incubation, medium was aspirated from each well, 100 μL DMSO was added per well, and the plates were agitated on a plate shaker for 20 min at room temperature (RT). The plates were read spectrophotometrically using a dual wavelength filter (570 and 630 nm) with a BIO-Tek EL310 E1A reader interfaced with a Samsung Deskmaster 386 microcomputer. Absorbance at 630 nm was used as the reference wavelength for detecting artefacts in the plastic plates and was subtracted from the 570 nm values. Wells with medium only (no cells) were processed in exactly the same manner as the rest of the plate, and the mean absorbance for these wells was subtracted from the absorbance values in the other wells.

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