Chemistry & Biology, Vol. 11, 557–564, April, 2004, ©2004 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.chembiol.2004.03.024

Synthesis, Characterization, and Cytotoxicity of a Series of Estrogen-Tethered Platinum(IV) Complexes

Katie R. Barnes, Alexander Kutikov, and Stephen J. Lippard*
Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Summary

Several estrogen-tethered platinum(IV) complexes were prepared and characterized by ESI-MS and ^{1}H NMR spectroscopy. Their design was inspired by the observation that estrogen receptor-positive cells exposed to the hormone are sensitized to cisplatin. Intracellular reduction of *bis*-estrogen-*cis*-diamminedichloroplatinum(IV), BEP_n (where n = 1–5 methylene groups between Pt and estrogen), occurs to afford cisplatin and two equivalents of the linker-modified estrogen. The ability of BEP_n to induce overexpression of HMGB1 was established by immunofluorescence microscopy. The cytotoxicity of the compounds was evaluated in ER(+) MCF-7 and ER(−) HCC-1937 human breast cancer cell lines. BEP3 selectively induces overexpression of HMGB1 in MCF-7 cells, compared to HCC-1937 cells, and enhances their sensitivity (IC_{50} = 2.1 ± 0.4 μM versus 3.7 ± 0.9 μM, respectively) to the compound. The difference in compound activities and the potential of compounds of this class for treating breast and ovarian cancer are discussed.

Introduction

The anticancer activity of cisplatin was discovered serendipitously more than three decades ago [1]. Today, cisplatin is used to treat testicular cancer, with success rates of greater than 90% [2, 3], as well as breast, ovarian, bladder, lung, and head and neck carcinomas, either alone or in combination therapy [4]. Because cisplatin has adverse side effects, is limited to a narrow range of cancers, and can be rendered inactive due to acquired resistance [2], research has focused on elucidating its mechanism of action to guide the rational synthesis of improved platinum analogs.

Cisplatin can bind to RNA, proteins, and other sulfur-containing biomolecules, but DNA is its primary biological target [5–7]. Various adducts form when cisplatin binds to DNA, with 1,2-intrastrand d(GpG) and d(ApG) crosslinks accounting for approximately 90% of such interactions [6, 8]; a smaller number of 1,3-intrastrand and interstrand crosslinks also form. These adducts block transcription, inhibit replication, and ultimately induce apoptosis [6]. The DNA duplex is significantly distorted upon formation of cisplatin 1,2-intrastrand crosslinks [9]. The resulting structure serves as a recognition motif for a variety of cellular proteins, including DNA repair components, histones, and high-mobility group (HMG) domain proteins, such as HMGB1 [6, 9, 10]. The binding of these proteins to platinum-DNA adducts modulates the cytotoxicity of cisplatin [11–13] and offers new strategies for improving the chemotherapeutic potential of platinum-based anticancer drugs [14].

Recent work from our laboratory revealed that estrogen receptor-positive, or ER(+), cells treated with estrogen are sensitized to cisplatin [15]; estrogen induces overexpression of HMGB1, a protein that shields cisplatin-DNA adducts from nucleotide excision repair (NER) [11, 15, 16]. This finding suggested to us that estrogen-tethered platinum(IV) complexes might prove valuable as novel anticancer drug candidates. The proposed mechanism is illustrated in Figure 1. Upon entering the reducing environment of the cell, the platinum(IV) complexes will be reduced to platinum(II) and simultaneously release one equivalent of cisplatin and two equivalents of a linker-modified estrogen. Subsequent binding of cisplatin to DNA and hydrolysis of the ester moieties should lead to HMGB1 upregulation, as described above, as well as sensitization of the cells as a result of repair shielding of the cisplatin-DNA adducts.

In the present article, we describe the application of this strategy through the design, synthesis, and characterization of a series of compounds, **BEP1–BEP5** (Figure 2). In these molecules, estradiol is tethered to the terminal carboxylate groups of *cis, cis, trans*-diamminedichlorodisuccinatoplatinum(IV) through polymethylene chains of varying lengths. We programmed this variability into our study to allow for the best match between the kinetics of estrogen-mediated HMGB1 overproduction and cisplatin-DNA adduct formation in the cancer cell. Here we report and discuss the ability of the BEP_n (*bis*-estrogen-*cis*-diamminedichloroplatinum(IV) compounds containing *n* methylene linker chain groups) complexes to upregulate HMGB1, as well as their cytotoxic profile in both ER(+) and ER(−) cells.

Results and Discussion

Synthesis and Characterization of BEP1–BEP5

The preparation of several steroid-tethered platinum complexes has been reported [17–21]. None of these compounds enables the release of unmodified estrogen inside cells, nor are any expected to affect the expression of HMGB1 because of their low affinity for the estrogen receptor (ER). Previously synthesized platinum-estrogen conjugates were designed to take advantage of known steroid transport mechanisms to accumulate specifically in ER(+) cells [17–19]. The present study describes the first report of a hormone-conjugated platinum(IV) complex linked at the 17β position. We chose this particular strategy for several reasons. Platinum(IV) complexes are relatively inert to ligand substitution, which allows for oral administration and differentiates their pharmacokinetic properties from those of cisplatin [22, 23]. In addition, the designed platinum(IV) complexes facilitate simultaneous delivery of cisplatin and

*Correspondence: lippard@lippard.mit.edu

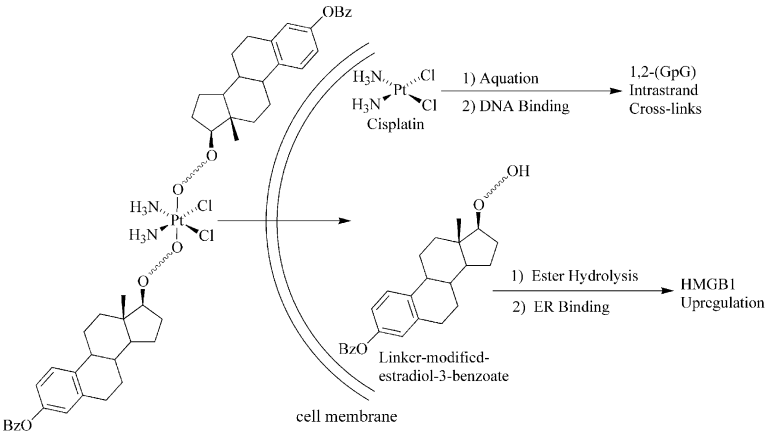

Figure 1. Proposed Mechanism of Action for Estrogen-Tethered Platinum(IV) Complexes

The reducing environment of the cell will convert platinum(IV) to platinum(II) and thereby release cisplatin and two equivalents of a modified estrogen. Upregulation of HMGB1 will shield cisplatin-DNA crosslinks from repair and enhance cell death.

estrogen to the same population of cells. Furthermore, release of cisplatin and unmodified estradiol can readily be achieved by use of the platinum(IV) platform. The concurrent delivery of cisplatin and estrogen allows for the selective targeting of ER(+) cells by conferring both DNA damage and HMGB1-induced repair shielding. Upon entering the cell, **BEP$_n$** will be readily reduced by glutathione or other intracellular agents to afford cisplatin and two equivalents of the linker-modified estrogen [22, 24–26]. The linkers were designed to be susceptible to hydrolysis by intracellular esterases because the estrogen receptor does not recognize estrogens modified at the 17β position [27, 28]. Although the relative amounts of platinum and estradiol delivered to the cell cannot be optimized, by varying length of the estrogen-linker we can control the kinetics of estrogen hydrolysis and release.

Initial attempts to prepare the estrogen-tethered complexes involved the synthesis of an ester-linked compound. We tried a variety of coupling methodologies in an attempt to attach *cis, cis, trans*-diamminedichlorodisuccinatoplatinum(IV) directly to estradiol-3-benzoate; however, the desired compound could not be obtained. Next, 17-hemisuccinate-estradiol-3-benzoate was synthesized, and numerous attempts were made to couple it to *cis, cis, trans*-diamminedichlorodihydroxyplatinum(IV), but without success [29]. Ultimately, the first member of the **BEP$_n$** family of estrogen-tethered platinum(IV) complexes, **BEP1**, was obtained through coupling of a linker-modified estrogen with *cis, cis, trans*-diamminedichlorodisuccinatoplatinum(IV) (Figure 2).

Cisplatin is readily oxidized by hydrogen peroxide to produce *cis, cis, trans*-diamminedichlorodihydroxyplatinum(IV) [23]. This *trans*-dihydroxyplatinum(IV) complex can be further modified by reaction with succinic anhydride to yield a *trans*-dicarboxylatoplatinum(IV) complex that is amenable to additional derivatization. The preparation of the *trans*-dicarboxylatoplatinum(IV) complex

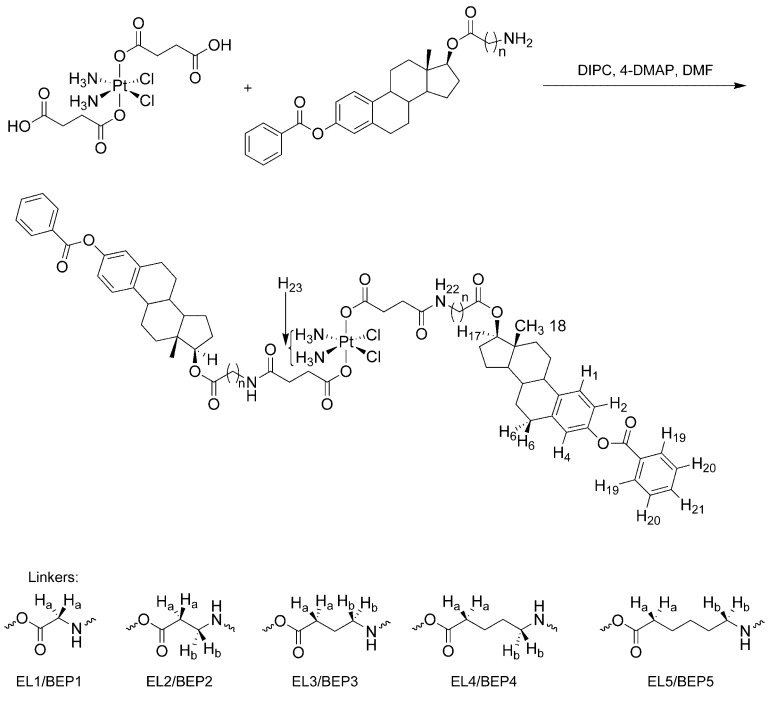

Figure 2. Route for the Preparation of the BEP$_n$ Complexes

Table 1. ^{1}H NMR Data for Estrogen Ligands and Platinum Complexes in d_6-DMSO at 25°C

	H_1	H_2	H_4	H_6	H_{17}	H_{18}	H_{19}	H_{20}	H_{21}	H_{22}	H_{23}	H_a	H_b
EL1	7.34	7.01	6.96	2.83	4.66	0.796	8.12	7.59	7.74	1.75		3.83	
	d, 1H	d, 1H	s, 1H	m, 2H	t, 1H	s, 3H	d, 2H	t, 2H	t, 1H	bs, 2H		d, 2H	
EL2	7.32	7.00	6.94	2.83	4.67	0.814	8.08	7.58	7.73	1.75		3.04	2.68
	d, 1H	d, 1H	s, 1H	m, 2H	t, 1H	s, 3H	d, 2H	t, 2H	t, 1H	bs, 2H		t, 2H	m, 2H
EL3	7.33	7.00	6.95	2.83	4.65	0.813	8.09	7.59	7.73	1.70		2.83	2.42
	d, 1H	d, 1H	s, 1H	m, 2H	t, 1H	s, 3H	d, 2H	t, 2H	t, 1H	bs, 2H		m, 2H	m, 2H
EL4	7.32	7.00	6.95	2.83	4.65	0.815	8.09	7.59	7.73	1.70		2.83	2.36
	d, 1H	d, 1H	s, 1H	m, 2H	t, 1H	s, 3H	d, 2H	t, 2H	t, 1H	bs, 2H		m, 2H	m, 2H
EL5	7.32	7.00	6.93	2.82	4.62	0.798	8.08	7.58	7.72	1.70		2.78	2.31
	d, 1H	d, 1H	s, 1H	m, 2H	t, 1H	s, 3H	d, 2H	t, 2H	t, 1H	bs, 2H		m, 2H	m, 2H
BEP1	7.34	7.01	6.95	2.83	4.65	0.785	8.10	7.59	7.74	8.33	6.56	3.80	
	d, 2H	d, 2H	s, 2H	m, 4H	t, 2H	s, 6H	d, 4H	t, 4H	t, 2H	t, 2H	bs, 6H	d, 4H	
BEP2	7.35	7.00	6.95	2.83	4.65	0.799	8.10	7.60	7.74	7.95	6.49	3.23	2.43
	d, 2H	d, 2H	s, 2H	m, 4H	t, 2H	s, 6H	d, 4H	t, 4H	t, 2H	t, 2H	bs, 6H	t, 4H	m, 4H
BEP3	7.35	7.00	6.96	2.83	4.69	0.805	8.12	7.59	7.74	7.85	6.50	3.04	2.47
	d, 2H	d, 2H	s, 2H	m, 4H	t, 2H	s, 6H	d, 4H	t, 4H	t, 2H	t, 2H	bs, 6H	t, 4H	m, 4H
BEP4	7.35	7.00	6.97	2.84	4.64	0.804	8.10	7.60	7.74	7.85	6.51	3.01	2.44
	d, 2H	d, 2H	s, 2H	m, 4H	t, 2H	s, 6H	d, 4H	t, 4H	t, 2H	t, 2H	bs, 6H	t, 4H	m, 4H
BEP5	7.32	6.98	6.94	2.82	4.62	0.799	8.09	7.57	7.71	7.81	6.49	2.98	2.40
	d, 2H	d, 2H	s, 2H	m, 4H	t, 2H	s, 6H	d, 4H	t, 4H	t, 2H	t, 2H	bs, 6H	t, 4H	m, 4H

^{1}H NMR data are given in ppm. See Figure 2 for an atom-labeling diagram.

presented here is a variation of a previously reported synthesis [30] that affords a similar yield, requires less stringent conditions, and leads to a product in 15 hr versus 24–48 hr. The resulting *cis, cis, trans*-diamminedichlorodisuccinatoplatinum(IV) complex was characterized by ESI-MS, ^{1}H NMR, and ^{195}Pt NMR spectroscopy. The platinum ammine proton resonances appear as a broad singlet at 6.51 ppm, a value that is consistent with the ammine chemical shifts of other *trans*-dicarboxylatoplatinum(IV) complexes [22]. The succinato protons and ammine protons integrate in a 4:3 ratio, indicating the presence of two succinato ligands per platinum center. The ^{195}Pt NMR resonance at 1226 ppm is consistent with other known platinum(IV) carboxylates [31, 32].

The preparation of the BOC-protected aminoalkyl carboxylic acids (**L_n**) was based on previously published methodology, and their ^{1}H NMR spectra agreed with data reported in the literature [33]. For obtaining the series of linker-modified estrogens (**EL_n**), estradiol-3-benzoate was coupled with **L_n** by the use of diisopropylcarbodiimide, followed by removal of the BOC-protecting group. The free amine provides a suitable handle for coupling to the *trans*-disuccinatoplatinum(IV) complex. Because of their poor solubility, the **EL** compounds were used without purification. Formation of the desired linker-modified estrogens was confirmed by ESI-MS and ^{1}H NMR spectroscopy (Table 1). A significant downfield shift of the H_{17} proton resonance is observed upon formation of the new ester linkage. Furthermore, the integrated intensity of the H_{22} amine protons is the same as those of the H_{19} and H_{20} aromatic protons.

The amine-modified estrogens are easily coupled to *trans*-dicarboxylatoplatinum(IV) by the use of diisopropylcarbodiimide, a common peptide coupling reagent. The ESI-MS and ^{1}H NMR spectroscopic data (Table 1) confirm the presence of the desired estrogen-tethered platinum(IV) complexes. Coupling of the series of amino-modified estrogens to *cis, cis, trans*-diamminedichlorodisuccinatoplatinum(IV) yielded the desired amide-linked compounds. Formation of the amide bond is evident from the loss of the free amino NH_2 proton resonance at 1.70–1.75 ppm and the appearance of an amide proton resonance at 7.80–8.33 ppm (H_{22}). The ^{1}H NMR data provide quantitative evidence for the presence of two estrogen moieties for every platinum center. The integrated intensity of the methyl protons (H_{18}) of the two estrogen groups matches that of the platinum-ammine protons (H_{23}). In addition, there are an equal number of amide (H_{22}) and estrogen 17-α protons (H_{17}). The experimentally determined masses for **BEP1–BEP5** are in excellent agreement with the calculated values ($\pm$0.003%). The synthetic methodology presented provides a convenient method for preparing a variety of platinum(IV) compounds for testing as anticancer drugs and has the potential to target such complexes to specific tissue or cell types.

Overexpression of HMGB1 in MCF-7 Cells after BEP$_n$ Treatment

The ability of compounds **BEP1–BEP5** to upregulate HMGB1 levels was investigated by immunofluorescence microscopy. As shown in Figure 3, 4 hr incubation with 200 nM **BEP1–BEP5** induces the overexpression of HMGB1 in MCF-7 cells. Treatment with **BEP1, BEP2, BEP3,** or **BEP4** increases HMGB1 expression to a similar degree as treatment with an equal amount of estradiol, whereas **BEP5** treatment induces considerably less protein expression. The ability of these estrogen-tethered platinum(IV) compounds to upregulate HMGB1 implies that all are taken into the cell and reduced to a Pt(II) species with concomitant release of the linker-modified estradiol. Moreover, hydrolysis of the linker-modified estradiol ester group must occur to allow for interaction of free-estradiol with the ER. Although hydrolysis of the estrogenic moiety alone could result in upregulation of HMGB1, reduction of Pt(IV) is required for DNA binding activity [24]. From the known kinetics associated with

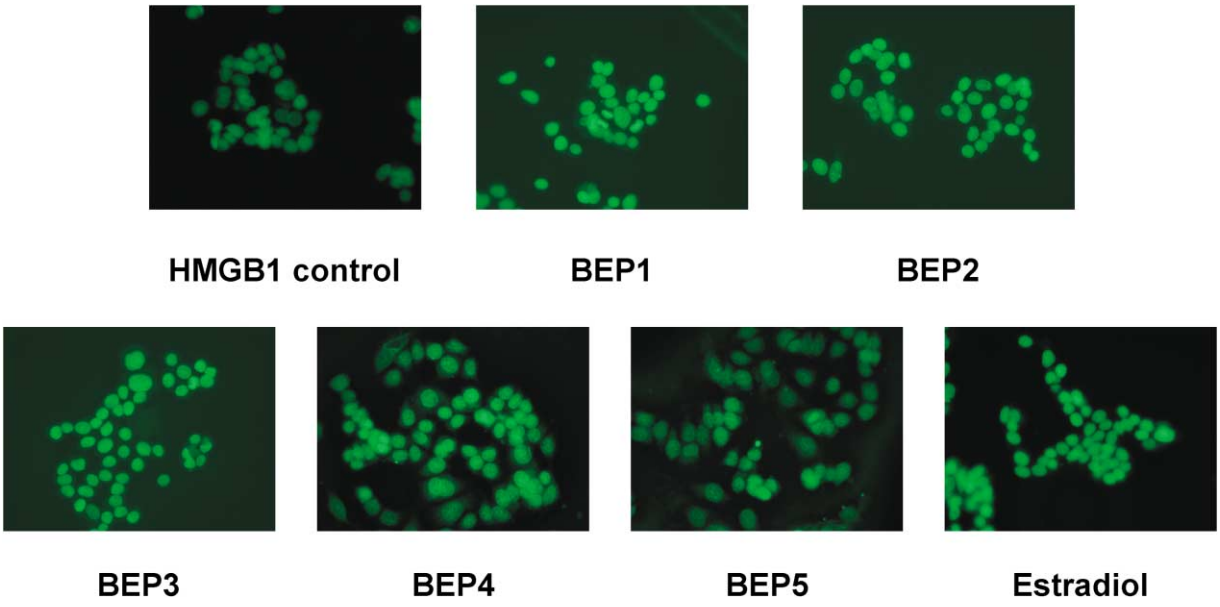

Figure 3. Expression of HMGB1 in MCF-7 Cells as Monitored by Immunofluorescence Microscopy

MCF-7 cells were grown on cover slips and subsequently incubated with 200 nM **BEP**$_n$ or estradiol for 4 hr. The cell membrane was permeabilized, and the cells were treated with anti-HMGB1 primary antibody and subsequently with a FITC-conjugated secondary antibody to allow for HMGB1 visualization.

these processes [22, 27], it is likely that ester hydrolysis will precede platinum(IV) reduction.

Estrogen interaction with the hormone binding domain induces the formation of ER homodimers, which then associate with the estrogen response element (ERE) [34]. The exact mechanism by which estrogen binding to the ER induces overexpression of HMGB1 is not known; however, the presence of HMGB1 is required for ER interaction with the ERE [35, 36]. HMGB1 may facilitate ER interaction with the ERE either by bending the element to provide a binding site for the ER or by stabilizing the distorted DNA of the ER-ERE complex [34, 36]. The ability of the ER to bind to the ERE may also be affected by the intracellular levels of HMGB1 or by the number of free HMGB1 binding sites [36]. In addition, HMGB1 increases the transcriptional activity of the ER [37].

Selective Cytotoxic Behavior of BEP1–BEP5 in ER(+) versus ER(−) Cells

The ability of **BEP1–BEP5** to stimulate upregulation of HMGB1 suggests that ER(+) cells will be more sensitive than ER(−) cells toward these compounds. The cytotoxic behavior of **BEP1–BEP5** and *cis, cis, trans*-[$Pt(NH_3)_2Cl_2(succinato)_2$] was evaluated in human breast cancer cell lines MCF-7 and HCC-1937, which are ER(+) and ER(−), respectively. Cell viability was evaluated by both the sulforhodamine B (SRB) and colony-counting assays. The two methods provided comparable dose response curves, with the SRB assay resulting in less variance in the results. The colony-counting method afforded more reliable data for **BEP5**, however. Because, in a control experiment, the sensitivities of the MCF-7 and HCC-1937 cells (IC_{50} = 4.0 μM) to *cis, cis, trans*-[$Pt(NH_3)_2Cl_2(succinato)_2$], the **BEP**$_n$ family synthetic precursor that carries no steroid appendage, were nearly identical, any difference in the ability of the derivatized complexes to kill the two different cell lines can be attributed to the release of estrogen and subsequent upregulation of HMGB1. The induction of HMGB1 overexpression after treatment with the estrogen-tethered platinum(IV) complexes did not always translate to sensitization of MCF-7 cells. Instead, as shown in Table 2, the sensitivity of these cells to compounds **BEP1–BEP5** varies with linker length. The cytotoxicities of **BEP1** and **BEP2** were quite comparable in MCF-7 and HCC-1937 cells, whereas **BEP5** was more cytotoxic to the HCC-1937 cells. One possible explanation for these results is that the elevated levels of HMGB1 produced by these complexes do not lead to a significant amount of platinum-DNA adduct repair shielding. The cytotoxic profile of **BEP5** may be explained by the relatively low level of HMGB1 overexpression induced upon treatment of the MCF-7 cells. As shown in Figure 4, **BEP3** is significantly more cytotoxic in the MCF-7 cells. Based on IC_{50} values, MCF-7 cells were 1.8- and 1.3-fold more sensitive toward **BEP3** and **BEP4** treatment, respectively, than they were to HCC-1937 cells. The differential toxicity toward MCF-7 and HCC-1937 cells observed with **BEP3** and **BEP4** suggest that these compounds can upregulate

Table 2. IC_{50} values, in μM, for **BEP1–BEP5**

Compound	MCF-7	HCC-1937	Cytotoxicity Ratio (HCC-1937:MCF-7)
BEP1	3.2 ± 0.1	3.4 ± 0.4	1.1
BEP2	3.0 ± 0.2	3.6 ± 1.2	1.2
BEP3	2.1 ± 0.4	3.7 ± 0.9	1.8
BEP4	3.7 ± 0.5	4.8 ± 0.5	1.3
BEP5	5.5 ± 1.7	3.0 ± 0.2	0.55

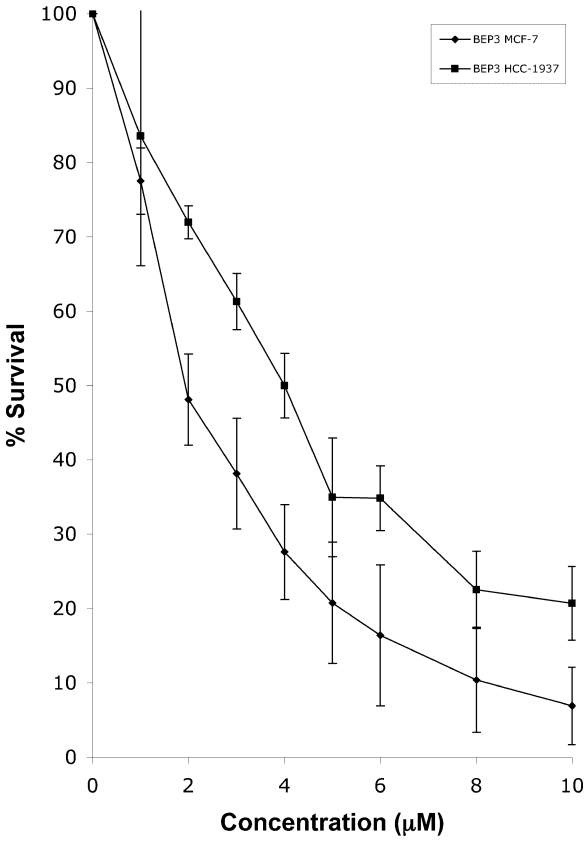

Figure 4. Effect of **BEP3** Treatment on MCF-7 and HCC-1937 Cell Survival, as Monitored by the SRB Assay (± SD)

Cells were treated continuously for 72–96 hr. Experiments were carried out on at least three separate days with triplicate cultures for each drug concentration.

HMGB1 in a manner that is kinetically competent to shield cisplatin-DNA adducts from repair and sensitize the cells. It is significant that **BEP3** is nearly 2-fold more active in MCF-7 cells because an identical degree of differential cytoxicity was achieved by independent administration of cisplatin and estradiol [15].

The importance of the kinetics of HMGB1 upregulation in determining the toxicities of compounds **BEP1–BEP5** can be understood in the light of our laboratory's previous work demonstrating that estrogen-induced cisplatin sensitization was only achieved when the two compounds were coadministered [15]. In addition, sensitization to carboplatin was maximized when MCF-7 cells were pretreated for 24 hr with hormone. These observations suggest that the kinetics of HMGB1 overexpression and platinum-DNA adduct formation are critical for achieving an optimal level of repair shielding. The rate of ester hydrolysis depends upon the length of the substituent attached at the estrogen 17β position [28]. The timing of hormone interaction with the ER and subsequent HMGB1 overexpression are therefore also expected to depend upon the length of the ester linker chain. The inability of **BEP5** to induce significant HMGB1 upregulation after a 4 hr treatment may be a consequence of the kinetics of ester hydrolysis. It is possible that compound **BEP3** stimulates HMGB1 overexpression at such a time that repair shielding of the platinum DNA adducts is maximized, whereas **BEP1** and **BEP2** induce HMGB1 overexpression but the timing is insufficient to sensitize the cells to cisplatin-DNA lesions.

Despite the sensitization of ER(+) cells to **BEP3** treatment, the degree of cytotoxicity achieved with cisplatin/estrogen cotreatment was not attained (IC_{50} = 1.0 μM). The diminished cytotoxicity of **BEP3** compared to cisplatin/estrogen coadministration may be a consequence of reduced uptake of the different pharmacokinetics of the estrogen-tethered platinum(IV) complex. The concentration of estrogen delivered to the cell may also be a factor. When cells were treated with cisplatin and estrogen alone, 200 nM concentrations of estradiol were used, whereas BEP_n complexes deliver micromolar concentrations of the hormone. It is also possible that the estrogen dose administered by BEP_n treatment itself induces a degree of cell proliferation. Alternatively, the micromolar concentration of estrogen could induce increased HMGB1 overexpression, which may actually inhibit cell death instead of leading to enhanced apoptosis [38]. Despite these caveats, the BEP_n compounds provide a new model for the development of platinum(IV) complexes designed to target and treat a variety of cancers in a specific manner, based on known cellular pathways. Animal studies are in progress for further investigation of the potential utility of **BEP3** as an anticancer agent.

Significance

Several novel estrogen-tethered platinum(IV) complexes were synthesized, evaluated for their ability to upregulate HMGB1, and screened for cytotoxicity against breast cancer cell lines. All BEP_n complexes induced the overexpression of HMGB1 in ER(+) MCF-7 cells. BEP3 was nearly 2-fold more cytotoxic in ER(+) MCF-7 cells than in ER(−) HCC-1937 cells. This result suggests the possibility of using compounds of this class specifically to target ER(+) malignancies, such as breast and ovarian cancers. In addition, these BEP_n compounds provide an example of a novel strategy—namely, using mechanistic insights to aid in the rational design of new complexes—in the development of platinum-containing anticancer agents. The chemistry used to construct the BEP_n complexes is of potential utility for attaching other moieties to direct platinum complexes to cancer cells and improve their efficacy.

Experimental Procedures

General Considerations

Potassium tetrachloroplatinate(II) was a gift from Engelhard. Cisplatin and *cis*, *cis*, *trans*-diamminedichlorodihydroxyplatinum(IV) were prepared as described in the literature [23, 39]. The preparation of compounds **L2, L3, L4,** and **L5** was based on previously reported methodology [33]. All chemicals and solvents were purchased from commercial sources unless specified otherwise. ^{1}H and ^{195}Pt NMR spectra were recorded on either a Varian 300 MHz or a 500 MHz spectrometer at the MIT Department of Chemistry Instrumentation Facility (DCIF). High-resolution mass spectral analysis was carried out at the MIT DCIF.

Synthesis of *cis, cis, trans*-Diamminedichlorodisuccinatoplatinum(IV) (1)

Succinic anhydride (4.1 g, 41 mmol) and *cis, cis, trans*-diamminedichlorodihydroxyplatinum(IV) (3.3 g, 10 mmol) were dissolved in 5 ml of DMSO. The solution was heated to 70°C for 15 hr with constant stirring, cooled to room temperature, and filtered. The DMSO solvent was removed from the filtrate by lyophilization to yield a yellow solid. Recrystallization from acetone at −20°C afforded a pale yellow solid (3.7 g, 6.9 mmol, 69% yield). ^{1}H NMR (d_6-acetone, 300 MHz): δ 2.45 (m, 4H, CH_2), 2.53 (m, 4H, CH_2), 6.511 (broad singlet, 6H, NH_3). ^{195}Pt NMR (500 MHz): δ 1226.531. ERMS (ESI) calculated for $[M + H]^+$ 534.0004 amu, found 534.0001 amu.

Synthesis of 3-*tert*-Butoxycarbonylaminopropionic Acid (L2)

Triethylamine (6.2 ml, 45 mmol) was added to a solution of 3-aminopropionic acid (2.67 g, 30.0 mmol) in 50% aqueous dioxane (30 ml). BOC-ON (8.15 g, 33.1 mmol) was added, and the reaction was stirred for 3 hr at room temperature. The reaction solution was diluted with H_2O (40 ml) and ethyl acetate (60 ml). The aqueous layer was isolated, washed with ethyl acetate, and acidified with a 5% citric-acid solution. The aqueous layer was subsequently extracted with ethyl acetate. The organic fractions were combined and evaporated to yield **L2** as a cream solid (3.6 g, 19 mmol, 63% yield). ^{1}H NMR (d_6-DMSO, 300 MHz): δ 1.35 (s, 9H, CH_3), 2.33 (t, 2H, CH_2), 3.11 (q, 2H, CH_2), 6.67 (t, 1H, NH). HRMS (ESI) calculated for $[M - H]^-$ 188.0928 amu, found 188.0922 amu.

Synthesis of 4-*tert*-Butoxycarbonylaminobutyric Acid (L3)

This compound was prepared as described for **L2** from stirring 4-aminobutyric acid (3.9 g, 38 mmol) in 50% aqueous dioxane with triethylamine (8.0 ml, 57 mmol) and BOC-ON (10.4 g, 42 mmol). A yellow oil that solidified upon addition of hexanes was obtained, and cooling to –20°C yielded a cream solid (4.0 g, 19 mmol, 52% yield). ^{1}H NMR (d_6-DMSO, 300 MHz): δ 1.36 (s, 9H, CH_3), 1.57 (m, 2H, CH_2), 2.17 (t, 2H, CH_2), 2.90 (m, 2H, CH_2), 6.80 (t, 1H, NH). HRMS (ESI) calculated for $[M - H]^-$ 202.1085 amu, found 202.1075 amu.

Synthesis of 5-*tert*-Butoxycarbonylaminopentanoic Acid (L4)

This compound was prepared as described for **L2** from 5-aminopentanoic acid (3.4 g, 29 mmol), triethylamine (6.0 ml, 43 mmol), and BOC-ON (7.9 g, 32 mmol). A cream solid (3.1 g, 14 mmol) was isolated in 49% yield. ^{1}H NMR (d_6-DMSO, 300 MHz): δ 1.36 (s, 9H, CH_3), 1.43 (m, 4H, $2CH_2$), 2.17 (t, 2H, CH_2), 2.88 (m, 2H, CH_2), 6.73 (t, 1H, NH). HRMS (ESI) calculated for $[M - H]^-$ 216.1241 amu, found 216.1244 amu.

Synthesis of 6-*tert*-Butoxycarbonylaminohexanoic Acid (L5)

This compound was prepared as described for **L2** from 6-aminohexanoic acid (3.5 g, 27 mmol), triethylamine (5.5 ml, 40 mmol), and BOC-ON (7.5 g, 30 mmol). A pale yellow solid (3.0 g, 13 mmol) was isolated in 48% yield. ^{1}H NMR (d_6-DMSO, 300 MHz): δ 1.20 (m, 2H, CH_2), 1.30 (m, 2H, CH_2), 1.36 (s, 9H, CH_3), 1.46 (m, 2H, CH_2), 2.16 (t, 2H, CH_2), 2.87 (m, 2H, CH_2), 6.74 (t, 1H, NH). HRMS (ESI) calculated for $[M - H]^-$ 230.1398 amu, found 230.1395 amu.

Synthesis of 17-(Aminoacetoxy)-Estradiol-3-Benzoate (EL1)

Diisopropylcarbodiimide (3.7 ml, 24 mmol) was added to a solution of N-*tert*-butoxycarbonylglycine (4.2 g, 24 mmol) and 4-dimethylaminopyridine (4-DMAP; 3.0 g, 24 mmol) in THF (200 mL). The solution was stirred for 10 min before the addition of estradiol-3-benzoate (5.0 g, 13 mmol). After being stirred overnight, the solution was filtered, and the solvent was removed by rotary evaporation. The solid residue was dissolved in a 1 M HCl/dioxane solution (265 ml) and stirred for an additional 6 hr. After evaporation of dioxane, the residue was suspended in 100 ml H_2O, and the pH was adjusted to 10 with ammonium hydroxide. The solution was stirred for several hours and filtered to yield a white solid (4.3 g, 9.9 mmol, 75%). ^{1}H NMR (d_6-DMSO, 300 MHz): δ 8.12 (d, 2H, ArH), 7.74 (t, 1H, ArH), 7.59 (t, 2H, ArH), 7.34 (d, 1H, ArH), 7.01 (d, 1H, ArH), 6.96 (s, 1H, ArH), 4.66 (t, 1H, CH), 3.83 (d, 1H, CH), 2.83 (m, 2H, CH), 1.75 (bs, 2H, NH_2); 2.3–1.2 (m, 13H, CH_2), 0.796 (s, 3H, CH_3). HRMS (ESI) calculated for $[M + H]^+$ 434.2326 amu, found 434.2319 amu.

Synthesis of 17-(3-Aminopropionate)-Estradiol-3-Benzoate (EL2)

A solution of **L2** (2.1 g, 11 mmol) and 4-DMAP (1.3 g, 11 mmol) was prepared in 100 ml of DMF. Diisopropylcarbodiimide (1.7 mL, 11 mmol) was added, and the solution was stirred for 10 min. Estradiol-3-benzoate (2.6 g, 6.9 mmol) was added, and the reaction was allowed to stir for 7 hr. The solution was filtered, diluted with water, and extracted with ether. The organic fractions were combined and evaporated to dryness to yield a pink residue. The residue was dissolved in 800 ml of methylene chloride; 120 ml TFA was added, and the solution was stirred for 2 hr. The methylene chloride was evaporated to yield a pink oil, which was dissolved in H_2O and had its pH adjusted to 10 with ammonium hydroxide. A white solid began to precipitate, and the slurry was stirred for an additional hour. Filtering the solution caused a white solid (approximately 3 g of crude material) to collect. ^{1}H NMR (d_6-DMSO, 300 MHz): δ 8.08 (d, 2H, ArH), 7.73 (t, 1H, ArH), 7.58 (t, 2H, ArH), 7.32 (d, 1H, ArH), 7.00 (d, 1H, ArH), 6.94 (s, 1H, ArH), 4.67 (t, 1H, CH), 3.04 (t, 2H, CH_2), 2.83 (m, 2H, CH), 2.68 (m, 2H, CH_2), 2.30–1.20 (m, 13H, CH/CH_2), 1.70 (bs, 2H, NH_2), 0.814 (s, 3H, CH_3). HRMS (ESI) calculated for $[M + H]^+$ 448.2482 amu, found 448.2481.

Synthesis of 17-(4-Aminobutanoate)-Estradiol-3-Benzoate (EL3)

This compound was prepared as described for **EL2** with L3 (1.8 g, 8.9 mmol), 4-DMAP (1.1 g, 9.1 mmol), diisopropylcarbodiimide (1.4 ml, 8.9 mmol), and estradiol-3-benzoate (2.2 g, 5.7 mmol). For removal of the BOC-protecting group, 700 ml methylene chloride and 100 ml TFA were stirred into the solution. A white solid was isolated (2.56 g crude) and used without purification. ^{1}H NMR (d_6-DMSO, 300 MHz): δ 8.09 (d, 2H, ArH), 7.73 (t, 1H, ArH), 7.59 (t, 2H, ArH), 7.33 (d, 1H, ArH), 7.00 (d, 1H, ArH), 6.95 (s, 1H, ArH), 4.65 (t, 1H, CH), 2.83 (m, 4H, CH_2), 2.42 (m, 2H, CH_2), 2.40–2.00 (m, 3H, CH_2), 1.81 (m, 4H, CH_2), 1.70 (bs, 2H, NH_2), 1.60–1.20 (m, 8H, CH), 0.813 (s, 3H, CH_3). HRMS (ESI) calculated for $[M + H]^+$ 462.2639 amu, found 462.2622 amu.

Synthesis of 17-(5-Aminopentanoate)-Estradiol-3-Benzoate (EL4)

This compound was prepared as described for **EL2** with **L4** (1.3 g, 6.6 mmol), 4-DMAP (0.82 g, 6.7 mmol), diisopropylcarbodiimide (1.0 ml, 6.4 mmol), and estradiol-3-benzoate (1.6 g, 4.1 mmol). For deprotection of the amine, 480 ml of methylene chloride and 72 ml of TFA were stirred into the solution. A white solid was isolated (1.89 g crude). ^{1}H NMR (d_6-DMSO, 300 MHz): δ 8.09 (d, 2H, ArH), 7.73 (t, 1H, ArH), 7.59 (t, 2H, ArH), 7.32 (d, 1H, ArH), 7.00 (d, 1H, ArH), 6.95 (s, 1H, ArH), 4.65 (t, 1H, CH), 2.83 (m, 4H, CH_2), 2.36 (m, 2H, CH_2), 2.30–2.00 (m, 3H, CH), 1.90–1.72 (m, 4H, CH) 1.70 (bs, 2H, NH_2), 1.57 (m, 4H, CH_2), 1.42–1.20 (m, 6H, CH), 0.815 (s, 3H, CH_3). HRMS (ESI) calculated for $[M + H]^+$ 476.2795 amu, found 476.2800 amu.

Synthesis of 17-(6-Aminohexanoate)-Estradiol-3-Benzoate (EL5)

This compound was prepared as described for **EL2** with **L5** (1.1 g, 4.6 mmol), 4-DMAP (0.58 g, 4.8 mmol), diisopropylcarbodiimide (730 μl, 4.7 mmol), and estradiol-3-benzoate (0.51 g, 1.4 mmol). For removal of the BOC group, a solution of TFA (25 ml) in methylene chloride (240 ml) was added. A white solid was isolated by filtration (0.6 g crude). ^{1}H NMR (d_6-DMSO, 300 MHz): δ 8.08 (d, 2H, ArH), 7.72 (t, 1H, ArH), 7.58 (t, 2H, ArH), 7.32 (d, 1H, ArH), 7.00 (d, 1H, ArH), 6.93 (s, 1H, ArH), 4.62 (t, 1H, CH), 2.82 (m, 2H, CH_2), 2.78–2.77 (m, 4H, CH_2), 2.31 (m, 2H, CH_2), 2.23–1.72 (m, 6H, CH), 1.70 (bs, 2H, NH_2), 1.57–1.20 (m, 10H, CH/CH_2), 0.798 (s, 3H, CH_3). HRMS (ESI) calculated for $[M + H]^+$ 490.2952 amu, found 490.2954 amu.

Synthesis of *cis, cis, trans*-Diamminedichloro-*bis*-(17-(N-Carbonylmethylsuccinato)-Estradiol-3-Benzoate)platinum(IV) (BEP1)

Diisopropylcarbodiimide (0.57 ml, 3.7 mmol) was added to a solution of **1** (0.81 g, 1.5 mmol) and 4-DMAP (0.48 g, 3.9 mmol) in DMF (100 ml). The solution was allowed to stir for 10 min at room temperature before the addition of **EL1** (1.6 g, 3.7 mmol). The solution was stirred for 15 hr at room temperature and then filtered, and the filtrate was diluted with 200 ml of ether. Cooling the resultant solution at −20°C

for 10 hr facilitated precipitation. The crude product was filtered, triturated with ethanol (30 ml), and centrifuged (five times). The tan solid was then triturated with boiling water (10 ml) five times and centrifuged to yield a pale yellow solid (0.72 g, 0.53 mmol, 35% yield). ^{1}H NMR (d_6-DMSO, 300 MHz): δ 8.33 (t, 2H, NH), 8.10 (d, 4H, ArH), 7.74 (t, 2H, ArH), 7.59 (t, 4H, ArH), 7.34 (d, 2H, ArH), 7.01 (d, 2H, ArH), 6.95 (s, 2H, ArH), 6.56 (bs, 6H, NH_3), 4.65 (t, 2H, CH), 3.80 (d, 4H, CH_2), 2.83 (m, 4H, CH_2), 2.60–1.20 (m, 34H, CH/CH_2), 0.785 (s, 6H, CH_3). HRMS calculated for [M + H]$^+$ 1364.4299 amu, found 1364.4253 amu.

Synthesis of *cis*, *cis, trans*-Diamminedichloro-*bis*-(17-(N-(2-Carboxy-Ethyl)-Succinato)-Estradiol-3-Benzoate)platinum(IV) (BEP2)

This compound was prepared as described for **BEP1** with **1** (0.37 g, 0.69 mmol), 4-DMAP (0.36 g, 2.9 mmol), diisopropylcarbodiimide (430 μl, 2.7 mmol), and **EL2** (1.2 g, 2.8 mmol). Crude **BEP2** was purified as described for **BEP1** to yield a pale yellow solid (0.13 g, 0.093 mmol, 14% yield). ^{1}H NMR (d_6-DMSO, 300 MHz): δ 8.10 (d, 4H, ArH), 7.95 (t, 2H, NH), 7.74 (t, 2H, ArH) 7.60 (t, 4H, ArH), 7.35 (d, 2H, ArH), 7.00 (d, 2H, ArH), 6.95 (s, 2H, ArH), 6.49 (bs, 6H, NH_3), 4.65 (t, 2H, CH), 3.23 (t, 4H, CH_2), 2.83 (m, 4H, CH), 2.43 (m, 4H, CH_2), 2.43–1.20 (m, 34H, CH/CH_2), 0.799 (s, 6H, CH_3). HRMS (ESI) calculated for [M + H]$^+$ 1391.4591 amu, found 1391.4562 amu.

Synthesis of *cis*, *cis*, *trans*-Diamminedichloro-*bis*-(17-(N-(3-Carboxy-Propyl)-Succinato)-Estradiol-3-Benzoate)platinum(IV) (BEP3)

This compound was prepared as described for **BEP1** with **1** (0.65 g, 1.2 mmol), 4-DMAP (0.61 g, 5.0 mmol), diisopropylcarbodiimide (760 μl, 4.9 mmol), and **EL3** (2.3 g, 5.0 mmol). The crude material was purified as described for **BEP1** to yield a pale yellow solid (0.12 g, 0.084 mmol, 7.0% yield). ^{1}H NMR (d_6-DMSO, 300 MHz): δ 8.12 (d, 4H, ArH), 7.85 (t, 2H, NH), 7.74 (t, 2H, ArH) 7.59 (t, 4H, ArH), 7.35 (d, 2H, ArH), 7.00 (d, 2H, ArH), 6.96 (s, 2H, ArH), 6.50 (bs, 6H, NH_3), 4.69 (t, 2H, CH), 3.26 (t, 4H, CH_2), 3.04 (t, 4H, CH_2), 2.83 (m, 4H, CH_2), 2.47 (m, 4H, CH_2), 2.40–1.20 (m, 34H, CH_2), 0.805 (s, 6H, CH_3). HRMS (ESI) calculated for [M + H]$^+$ 1419.4904, found 1419.4890 amu.

Synthesis of *cis*, *cis, trans*-Diamminedichloro-*bis*-(17-(N-(4-Carboxybutyl)-Succinato)-Estradiol-3-Benzoate)platinum(IV) (BEP4)

This compound was prepared as described for **BEP1** with **1** (0.22 g, 0.40 mmol), 4-DMAP (0.15 g, 1.2 mmol), diisopropylcarbodiimide (189 μl, 1.2 mmol), and **EL4** (0.62 g, 1.3 mmol). The crude material was purified as described for **BEP1** to yield a pale yellow solid (0.25 g, 0.17 mmol, 43%). ^{1}H NMR (d_6-DMSO, 300 MHz): δ 8.10 (d, 4H, ArH), 7.85 (t, 2H, NH), 7.74 (t, 2H, ArH) 7.60 (t, 4H, ArH), 7.35 (d, 2H, ArH), 7.00 (d, 2H, ArH), 6.97 (s, 2H, ArH), 6.51 (bs, 6H, NH_3), 4.64 (t, 2H, CH), 3.01 (t, 4H, CH_2), 2.84 (m, 4H, CH_2), 2.44 (m, 4H, CH_2), 2.30–2.21 (m, 8H, CH/CH_2), 2.20–1.20 (m, 34H, CH/CH_2), 0.804 (s, 6H, CH_3). HRMS (ESI) calculated for [M + Na]$^+$ 1469.5036, found 1469.5006 amu.

Synthesis of *cis*, *cis*, *trans*-Diamminedichloro-*bis*-(17-(N-(5-Carboxy-Pentyl)-Succinato)-Estradiol-3-Benzoate)platinum(IV) (BEP5)

This compound was prepared as described for **BEP1** with **1** (0.14 g, 0.26 mmol), 4-DMAP (0.10 g, 0.83 mmol), diisopropylcarbodiimide (127 μl, 0.81 mmol), and **EL5** (0.40 g, 0.82 mmol). The crude product was purified as described for **BEP1** to yield a pale yellow solid (0.085 g, 0.057 mmol, 22%). ^{1}H NMR (d_6-DMSO, 300 MHz): δ 8.09 (d, 4H, ArH), 7.81 (t, 2H, NH), 7.71 (t, 2H, ArH) 7.57 (t, 4H, ArH), 7.32 (d, 2H, ArH), 6.98 (d, 2H, ArH), 6.94 (s, 2H, ArH), 6.49 (bs, 6H, NH_3), 4.62 (t, 2H, CH), 2.98 (t, 4H, CH_2), 2.82 (m, 4H, CH_2), 2.40 (m, 4H, CH_2), 2.30–2.20 (m, 12H, CH/CH_2), 2.10–1.20 (m, 34H, CH/CH_2), 0.799 (s, 6H, CH_3). ESI-MS [M + H]$^+$: 1475.5530 (calculated); 1475.5551 (observed).

Cell Culture Studies

MCF-7 cells were grown in DMEM (GIBCO/BRL) supplemented with 10% FBS, 1× antibiotic/antimycotic solution (GIBCO), and 2 mM L-glutamine. HCC-1937 cells were grown in RPMI-1640 media (ATCC) containing 10% FBS and 1× antibiotic/antimycotic solution. All cells were incubated at 37°C under a 5% CO_2 atmosphere.

Overexpression of HMGB1 Induced by BEP_n Complexes

MCF-7 cells were grown to 70% confluence on 12 mm glass cover slips in 24-well plates. The cells were treated with either estrogen or BEP_n and incubated for 4 hr. The cells were then permeabilized with 25% acetic acid in methanol for 10 min at room temperature, washed with PBS, and incubated with a 1:100 dilution of anti-HMGB1 polyclonal antibody (PharMingen) for 1 hr at 37°C. The cells were subsequently incubated with a 1:200 dilution of goat anti-rabbit IgG conjugated to FITC (Biosource International) for 1 hr at 37°C. The cover slips were then placed on microscope slides, fixed with gelvatol, and incubated at 4°C for 12 hr. HMGB1 levels were then visualized under a fluorescent-light microscope (Nikon) equipped with a digital camera (Diagnostic Instruments).

Cytotoxic Profile of BEP_n

The cytotoxicities of the BEP_n compounds were evaluated by two methodologies, SRB and colony-counting assays. For the SRB assay, MCF-7 and HCC-1937 cells were seeded onto 96-well plates at a density of 1000 cells per well and allowed to grow for 24 hr. Cells were treated with compounds **BEP1–BEP5** at the following concentrations: 1, 2, 3, 4, 5, 6, 8, and 10 μM. The 96-well plates were covered with Breathe-Easy gas-permeable membranes (Diversified Biotech), and the cells incubated at 37°C for 96 hr. After the incubation period, the cells were fixed by the addition of 25 μl of 50% TCA and subsequent incubation at 4°C for 30 min. The viable cells were then stained by the addition of 100 μl of sulforhodamine B in 0.1% acetic acid followed by incubation for 30 min at room temperature. The cells were washed with 1% acetic acid and allowed to dry overnight. The SRB was solubilized by the addition of 100 μl of 10 mM Tris (pH 10.5) and subsequent shaking for 5 min. The number of viable cells was then quantified by measurement of the absorbance at 492 nm.

For the colony-counting assay, MCF-7 and HCC-1937 cells were seeded onto 6-well plates at a density of 1000 cells per well in 2 ml media and allowed to grow for 24 hr. Cells were then treated for 72 hr with the BEP_n complexes at the following concentrations: 0, 2, 4 6, 8, and 10 μM. After 72 hr, cells were washed with PBS, and fresh medium was added. Staining the cells with a 1% methylene blue/50% ethanol (vol/vol) solution after 7 days allowed the colonies to be counted.

Acknowledgments

This work was supported by grants from the National Cancer Institute, CA34992 and T32-CA009112. The Massachusetts Institute of Technology Department of Chemistry Instrument Facility is funded through the National Science Foundation (CHE-9808061, CHE-9808063, and DBI-9729592). We thank Olga Burenkova and Caroline Saouma for technical assistance.

Received: December 10, 2003
Revised: January 25, 2004
Accepted: January 30, 2004
Published: April 16, 2004

References

1. Rosenberg, B., Van Camp, L., and Krigas, T. (1965). Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. Nature *205*, 698–699.
2. Wong, E., and Giandomenico, C.M. (1999). Current status of platinum-based antitumor drugs. Chem. Rev. *99*, 2451–2466.
3. Trimmer, E.E., and Essigmann, J.M. (1999). Cisplatin. Essays Biochem. *34*, 191–211.
4. Loehrer, P.J., and Einhorn, L.H. (1984). Cisplatin. Ann. Intern. Med. *100*, 704–713.
5. Reedijk, J. (1987). The mechanism of action of platinum antitumor drugs. Pure Appl. Chem. *59*, 181–192.
6. Jamieson, E.R., and Lippard, S.J. (1999). Structure, recognition,

and processing of cisplatin-DNA adducts. Chem. Rev. *99*, 2467–2498.

7. Cohen, S.M., and Lippard, S.J. (2001). Cisplatin: from DNA damage to cancer chemotherapy. Prog. Nucleic Acid Res. Mol. Biol. *67*, 93–130.
8. Pil, P., and Lippard, S.J. (1997). Cisplatin and Related Drugs. In Encyclopedia of Cancer, Vol. 1, J.R. Bertino, Ed. (San Diego: Academic Press), pp. 392–410.
9. Takahara, P.M., Frederick, C.A., and Lippard, S.J. (1996). Crystal structure of the anticancer drug cisplatin bound to duplex DNA. J. Am. Chem. Soc. *118*, 12309–12321.
10. Kartalou, M., and Essigmann, J.M. (2001). Recognition of cisplatin adducts by cellular proteins. Mutat. Res. *478*, 1–21.
11. Huang, J.-C., Zamble, D.B., Reardon, J.T., Lippard, S.J., and Sancar, A. (1994). HMG-domain proteins specifically inhibit the repair of the major DNA adduct of the anticancer drug cisplatin by human excision nuclease. Proc. Natl. Acad. Sci. USA *91*, 10394–10398.
12. Brown, S.J., Kellett, P.J., and Lippard, S.J. (1993). Ixr1, a yeast protein that binds to platinated DNA and confers sensitivity to cisplatin. Science *261*, 603–605.
13. Cohen, S.M., Jamieson, E.R., and Lippard, S.J. (2000). Enhanced binding of the TATA-binding protein to TATA boxes containing flanking cisplatin 1,2-cross-links. Biochemistry *39*, 8259–8265.
14. Barnes, K.R., and Lippard, S.J. (2003). Cisplatin and related anticancer drugs: recent advances and insights. Met. Ions Biol. Syst., in press.
15. He, Q., Liang, C.H., and Lippard, S.J. (2000). Steroid hormones induce HMG1 overexpression and sensitize breast cancer cells to cisplatin and carboplatin. Proc. Natl. Acad. Sci. USA *97*, 5768–5772.
16. Chau, K.Y., Lam, H.Y.P., and Lee, K.L.D. (1998). Estrogen treatment induces elevated expression of HMG1 in MCF-7 cells. Exp. Cell Res. *241*, 269–272.
17. Altman, J., Castrillo, T., Beck, W., Bernhardt, G., and Schonenberger, H. (1991). Metal complexes with biologically important ligands. 62. Platinum(II) complexes of 3-(2-aminoethoxy)estrone and -estradiol. Inorg. Chem. *30*, 4085–4088.
18. Jackson, A., Davis, J., Pither, R.J., Rodger, A., and Hannon, M.J. (2001). Estrogen-derived steroidal metal complexes: agents for cellular delivery of metal centers to estrogen receptor-positive cells. Inorg. Chem. *40*, 3964–3973.
19. Gandolfi, O., Apfelbaum, H.C., Migron, Y., and Blum, J. (1989). Syntheses of *cis*-dichlorodiammineplatinum analogs having steroidal hormones bound to the metal atom via malonato bridges. Inorg. Chim. Acta *161*, 113–123.
20. Ehrenstorfer-Schafers, E.-M., Steiner, N., Altman, J., and Beck, W. (1990). Metal complexes of biologically important ligands, LV. Binding of steroidal hormones through α-amino acid ligands to platinum(II) and palladium(II). Z. Naturforsch. *45b*, 817–827.
21. Descoteaux, C., Provencher-Mandeville, J., Mathieu, I., Perron, V., Mandal, S.K., Asselin, E., and Berube, G. (2003). Synthesis of 17β-estradiol platinum(II) complexes: biological evaluation on breast cancer cell lines. Bioorg. Med. Chem. Lett. *13*, 3927–3931.
22. Hambley, T.W., Battle, A.R., Deacon, G.B., Lawrenz, E.T., Fallon, G.D., Gatehouse, B.M., Webster, L.K., and Rainone, S. (1999). Modifying the properties of platinum(IV) complexes in order to increase biological effectiveness. J. Inorg. Biochem. *77*, 3–12.
23. Giandomenico, C.M., Abrams, M.J., Murrer, B.A., Vollano, J.F., Rheinheimer, M.I., Wyer, S.B., Bossard, G.E., and Higgins, J.D. (1995). Carboxylation of kinetically inert platinum(IV) hydroxy complexes. An entree into orally active platinum(IV) antitumor agents. Inorg. Chem. *34*, 1015–1021.
24. Choi, S., Filotto, C., Bisanzo, M., Delaney, S., Lagasee, D., Whitworth, J., Jusko, A., Li, C., Wood, N.A., Willingham, J., et al. (1998). Reduction and anticancer activity of platinum(IV) complexes. Inorg. Chem. *37*, 2500–2504.
25. Lemma, K., Sargeson, A.M., and Elding, L.I. (2000). Kinetics and mechanism for reduction of oral anticancer platinum(IV) dicarboxylate compounds by L-ascorbate ions. J. Chem. Soc. Dalton Trans. 1167–1172.
26. Lemma, K., Shi, T., and Elding, L.I. (2000). Kinetics and mechanism for reduction of the anticancer prodrug *trans, trans, trans*-$[PtCl_2(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$ (JM335) by thiols. Inorg. Chem. *39*, 1728–1734.
27. Larner, J.M., MacLusky, N.J., and Hochberg, R.B. (1985). The naturally occurring C-17 fatty acid esters of estradiol are long-acting estrogens. J. Steroid Biochem. *22*, 407–413.
28. MacLusky, N.J., Larner, J.M., and Hochberg, R.B. (1989). Actions of an estradiol-17-fatty acid ester in estrogen target tissues of the rat: comparison with other C-17 metabolites and a pharmacological C-17 ester. Endocrinology *124*, 318–324.
29. Orme, M.W., and Labroo, V.M. (1994). Synthesis of β-estradiol-3-benzoate-17-(succinyl-12A-tetracycline): a potential bone-seeking estrogen. Bioorg. Med. Chem. Lett. *4*, 1375–1380.
30. Alvarez-Valdes, A., Perez, J.M., Lopez-Solera, I., Lannegrand, R., Continente, J.M., Amo-Ochoa, P., Camazon, M.J., Solans, X., Font-Bardia, M., and Navarro-Ranninger, C. (2002). Preparation and characterization of platinum(II) and (IV) complexes of 1,3-diaminepropane and 1,4-diaminebutane: circumvention of cisplatin resistance and DNA interstrand cross-link formation in CH1cisR ovarian tumor cells. J. Med. Chem. *45*, 1835–1844.
31. Khan, S.R.A., Huang, S., Shamsuddin, S., Inutsuka, S., Whitmire, K.H., Siddik, Z.H., and Khokhar, A.R. (2000). Synthesis, characterization and cytotoxicity of new platinum(IV) axial carboxylate complexes: crystal structure of potential antitumor agent. Bioorg. Med. Chem. *8*, 515–521.
32. Khokhar, A.R., Deng, Y., Kido, Y., and Siddik, Z.H. (1993). Preparation, characterization, and antitumor activity of new ethylenediamine platinum(IV) complexes containing mixed carboxylate ligands. J. Inorg. Biochem. *50*, 79–87.
33. Salmon-Chemin, L., Buisine, E., Yardley, V., Kohler, S., Debreu, M.-A., Landry, V., Sergheraert, C., Croft, S.L., Krauth-Siegel, R.L., and Davioud-Charvet, E. (2001). 2- and 3-substituted 1,4-napthoquinone derivatives as subversive substrates of trypanothione reductase and lipoamide dehydrogenase from *Trypanosoma cruzi*: synthesis and correlation between redox cycling activities and in vitro cytotoxicity. J. Med. Chem. *44*, 548–565.
34. Verrier, C.S., Roodi, N., Yee, C.J., Bailey, L.R., Jensen, R.A., Bustin, M., and Parl, F.F. (1997). High-mobility group (HMG) protein HMG-1 and TATA-binding protein-associated factor $TAF_{II}30$ affect estrogen receptor-mediated transcriptional activation. Mol. Endocrinol. *11*, 1009–1019.
35. Romine, L.E., Wood, J.R., Lamia, L.A., Prendergast, P., Edwards, D.P., and Nardulli, A.M. (1998). The high mobility group protein 1 enhances binding of the estrogen receptor DNA binding domain to the estrogen response element. Mol. Endocrinol. *12*, 664–674.
36. Zhang, C.C., Krieg, S., and Shapiro, D.J. (1999). HMG-1 stimulated estrogen response element binding by estrogen receptor from stably transfected HeLa cells. Mol. Endocrinol. *13*, 632–643.
37. Boonyaratanakornkit, V., Melvin, V., Prendergast, P., Altmann, M., Ronfani, L., Bianchi, M.E., Taraseviciene, L., Nordeen, S.K., Allegretto, E.A., and Edwards, D.P. (1998). High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding in vitro and transcriptional activity in mammalian cells. Mol. Cell. Biol. *18*, 4471–4487.
38. Brezniceanu, M.-L., Volp, K., Bosser, S., Solbach, C., Lichter, P., Joos, S., and Zornig, M. (2003). HMGB1 inhibits cell death in yeast and mammalian cells and is abundantly expressed in human breast carcinoma. FASEB J. *17*, 1295–1297.
39. Dhara, S.C. (1970). A rapid method for the synthesis of *cis*-$[Pt(NH_3)_2Cl_2]$. Indian J. Chem. *8*, 193–194.