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Highly potent metallopeptide analogues of luteinizing hormone-releasing hormone

(targeted chemotherapeutic agents/platinum/copper/nickel/receptor binding)

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ABSTRACT **Metal complexes related to the cytotoxic complexes cisplatin [*cis*-diamminedichloroplatinum(II)] and *trans*-bis(salicylaldoximato)copper(II) were incorporated into suitably modified luteinizing hormone-releasing hormone (LH-RH) analogues containing D-lysine at position 6. Some of the metallopeptides thus obtained proved to be highly active LH-RH agonists or antagonists. For instance, SB-40, a $PtCl_2$-containing metallopeptide in which platinum is coordinated to an N^{ε}-(DL-2,3-diaminopropionyl)-D-lysine residue [D-Lys(DL-A_2pr] at position 6, showed 50 times higher LH-releasing potency than the native hormone. SB-95, [Ac-D-Nal(2)^1,D-Phe(pCl)^2,D-Pal(3)^2,Arg^5,D-Lys{DL-A_2pr(Sal$_2$Cu)}^{6},D-Ala^{10}]LH-RH, where Nal(2) is 3-(2-naphthyl)alanine, Pal(3) is 3-(3-pyridyl)alanine, and copper(II) is coordinated to the salicylideneimino moieties resulting from condensation of salicylaldehyde with D-Lys(DL-A_2pr)^6, caused 100% inhibition of ovulation at a dose of 3 μg in rats. Most metallopeptide analogues of LH-RH showed high affinities for the membrane receptors of rat pituitary and human breast cancer cells. Some of these metallopeptides had cytotoxic activity against human breast cancer and prostate cancer cell lines *in vitro* (this will be the subject of a separate paper on cytotoxicity evaluation). Such cytostatic metallopeptides could be envisioned as targeted chemotherapeutic agents in cancers that contain receptors for LH-RH-like peptides.**

Superagonist analogues of luteinizing hormone (LH)-releasing hormone (LH-RH), <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (where <Glu is pyroglutamic acid), have been reported to be of value in treating patients with certain sex hormone-dependent tumors, such as breast and prostatic cancers (1–3). Inhibition of breast or prostate tumor growth during therapy with LH-RH analogs is mediated mainly by the suppression of gonadal steroid secretion (3–5), but direct effects of these peptides on tumor cells may also be involved (6). Human breast cancer specimens and some breast cancer cell lines contain binding sites for LH-RH (6–10), and the growth of some breast cancer cell lines can be inhibited by an LH-RH agonist (7) or antagonist (8). Sharoni *et al.* (11) recently reported that LH-RH antagonists inhibited [^{3}H]thymidine incorporation and cell growth in cultures of MDA-MB-231 human mammary tumor cells. Binding of the agonist [D-Trp^6]LH-RH to the cell membranes of human prostate cancers has also been demonstrated (12). Nevertheless, clinical studies have shown that the duration of remission of tumor growth may be limited, as hormonal manipulations do not prevent the ultimate tumor growth of hormone-independent cells (13). It has been suggested that a combination of hormonal therapy with chemotherapy could forestall this phenomenon and prolong survival (14).

In addition to the use of the combination of LH-RH analogues and cytotoxic agents, such combined therapy could be provided by treatment with highly potent agonistic or antagonistic analogues of LH-RH that contain cytotoxic moieties. Such analogues could exert the effect of LH-RH agonists or antagonists and, at the same time, act as chemotherapeutic agents targeted to the tumor cells by their peptide portions for which binding sites are present on the cell membranes. In the accompanying paper (15) we report the synthesis of LH-RH analogues containing D-melphalan. Here we describe highly potent agonistic and antagonistic analogues of LH-RH that contain moieties related to the cytotoxic complexes cisplatin [*cis*-diamminedichloroplatinum-(II); ref. 16] and *trans*-bis(salicylaldoximato)copper(II) (17).

MATERIALS AND METHODS

Amino Acid Derivatives. Boc-D-Ala, Boc-Arg(Tos), Boc-Gly, Boc-His(Tos), Boc-Leu, Boc-D-Nal(2), Boc-D-Phe(pCl), Boc-Pro, Boc-Ser(Bzl), Boc-Trp, Boc-D-Trp, and Boc-Tyr(Cl_2Bzl) were purchased from Bachem [Boc, *tert*-butoxycarbonyl; Tos, p-toluenesulfonyl; Bzl, benzyl; Cl_2Bzl, 2,6-dichlorobenzyl; Nal(2), 3-(2-naphthyl)alanine; Phe(pCl), p-chlorophenylalanine]. Boc-protected 3-(3-pyridyl)-D-alanine [Boc-D-Pal(3)] was prepared according to ref. 18. The methyl ester of 6-aminohexanoic acid (Ahx-OMe) was purchased from American Dynamics (South Plainfield, NJ). L-2,3-Diaminopropionic acid (A_2pr) was obtained from Calbiochem–Behring, DL-A_2pr, DL- and L-2,4-diaminobutyric acid (A_2bu), <Glu, salicylaldehyde, 5-chlorosalicylaldehyde, pyridoxal, pyridoxal 5′-phosphate, $Cu(OAc)_2$, $Ni(OAc)_2$, and K_2PtCl_4 were purchased from Aldrich. Diamino acids were derivatized (Boc_2A_2pr, Boc_2A_2bu, Z_2A_2pr, and Z_2A_2bu, where Z is benzyloxycarbonyl) by published methods (19).

Metal Complexes of Hydroxy Oxo Compounds. Bis(salicylaldehydato)nickel(II), nickel(II) complexes of related hydroxy oxo compounds, and the corresponding copper(II) complexes were prepared according to the methods described previously (20).

Dipeptides. Boc_2A_2pr-Ahx and Boc_2A_2bu-Ahx were obtained from the appropriate di-Boc-amino acid and Ahx-OMe by mixed anhydride coupling followed by saponification.

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Abbreviations: LH, luteinizing hormone (lutropin); LH-RH, LH-releasing hormone (luliberin); <Glu, pyroglutamic acid; Nal(2), 3-(2-naphthyl)alanine; Pal(3), 3-(3-pyridyl)alanine; A_2pr, 2,3-diaminopropionic acid; A_2bu, 2,4-diaminobutyric acid; Ahx, 6-aminohexanoic acid; Boc, *tert*-butoxycarbonyl; Z, benzyloxycarbonyl; Sal, 2-oxidobenzylidene (salicylidene); ClSal, 5-chlorosalicylidene; Pxd, 2-methyl-5-hydroxymethyl-3-oxido-4-picolylidene (pyridoxylidene); P-Pxd, 5′-phosphopyridoxylidene.

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Boc-D-Lys(Z_2A_2pr) and Boc-D-Lys(Z_2A_2bu) were similarly prepared from Boc-D-Lys and Z-protected diamino acids.

Decapeptides Containing D-Lysine at Position 6. D-Lys^6 peptides were prepared by standard solid-phase synthesis (21) starting with a benzhydrylamine resin containing about 1 mmol of amino groups per gram (Advanced ChemTech). Coupling was achieved with preformed 1-hydroxybenzotriazole esters (22). Deblocking was performed with trifluoroacetic acid/dichloromethane (1:1). Acetylation of the N terminus was done with a 10-fold excess of acetic anhydride in the presence of imidazole. Peptide-resins were treated with HF (15 ml/g of peptide-resin) at 0°C for 45 min in the presence of 10% anisole. The HF was removed under vacuum, and the residue was triturated with anhydrous diethyl ether and filtered. Peptides were extracted from the resin with aqueous 50% acetic acid. The crude D-Lys^6 peptides were purified by HPLC and then acylated with the appropriate Boc-protected diamino acid, deblocked, and purified to yield the desired N^{ε}-diaminoacyl-D-Lys^6 analogues of LH-RH. In an alternative approach, Boc-D-Lys(Z_2A_2pr) or Boc-D-Lys(Z_2A_2bu) was introduced into position 6 of the growing peptide chain, and so the N^{ε}-diaminoacyl-D-Lys-containing LH-RH analogues were obtained directly.

Metallopeptides. To obtain metallopeptides containing $PtCl_2$, intermediate peptides having the A_2pr or A_2bu side chains were reacted with K_2PtCl_4 in aqueous dimethylformamide. Metallopeptides comprising the Schiff base of a hydroxy oxo compound (e.g., salicylaldehyde) and copper(II) or nickel(II) were prepared by reacting A_2pr/A_2bu-containing peptides in aqueous dimethylformamide with an aldehydato complex [e.g., bis(salicylaldehydato)copper(II)]. Alternatively, the intermediate peptides were treated first with the hydroxy oxo compound (e.g., pyridoxal 5′-phosphate) and then with $Cu(OAc)_2$ or $Ni(OAc)_2$ in aqueous dimethylformamide at pH 7–8. In both cases products were isolated by HPLC.

HPLC. Purification of crude products was carried out on a Beckman HPLC system (type 142) using a Dynamax macro column (21.2 × 250 mm) packed with spherical C_{18} silica gel (300-Å pore size, 12-μm particle size) or a Vydac protein and peptide C_{18} column (10 × 250 mm, 300-Å pore size, 5-μm particle size). Elution was with solvent systems *i–iv* consisting of components A and B as follows. System *i*: A, 0.1% trifluoroacetic acid; B, 0.1% trifluoroacetic acid in aqueous 70% acetonitrile. System *ii*: A, 0.2% acetic acid; B, 0.2% acetic acid in aqueous 70% acetonitrile. System *iii*: A, 0.1 M ammonium acetate (pH 7.0); B, 0.1 M ammonium acetate in aqueous 65% acetonitrile. System *iv*: A, 0.1 M ammonium acetate (pH 7.0); B, 2-propanol. Gradient elution was used.

Analytical HPLC was run on a Hewlett–Packard model HP-1090 liquid chromatograph using a 4.6 × 250-mm W-Porex 5-μm C_{18} column (Phenomenex, Rancho Palos Verdes, CA). Elution was at 1.2 ml/min with solvent system *i*, for intermediate peptides and metallopeptides containing $PtCl_2$, systems *ii* and *iii*, for agonistic metallopeptides with copper(II) or nickel(II), or system *iv*, for the corresponding antagonistic analogues.

Amino Acid Analysis. Samples were hydrolyzed at 110°C for 20 hr in evacuated sealed tubes containing 4 M methanesulfonic acid and 0.2% 3-(2-aminoethyl)indole. Analyses were performed with a Beckman 6300 amino acid analyzer.

Agonist/Antagonist Activity. LH-releasing and LH-RH-inhibiting activities were assayed in a dispersed rat pituitary cell superfusion system (22, 23). The *in vivo* antiovulatory assay was carried out in 4-day cycling rats as described (22).

Receptor Binding. Affinity for the receptors of rat pituitary and human breast cancer cell membranes was determined as described (9, 10) by using ^{125}I-labeled [D-Trp^6]LH-RH as the radioactive ligand.

RESULTS

LH-RH analogues chelating platinum, copper, or nickel were prepared. The ligand portion of these metallopeptides was obtained by incorporation of an N^{ε}-diaminoacyl-D-lysine, D-Lys(A_2xy), or an N^{ε}-[6-(diaminoacyl)aminohexanoyl]-D-lysine, D-Lys(A_2xy-Ahx), into position 6 of LH-RH and some of its antagonistic analogues as illustrated by formulas **A–C**:

A <Glu-His-Trp-Ser-Tyr-D-Lys(A_2xy)-Leu-Arg-Pro-Gly-NH_2

B Ac-R^1-D-Phe(*p*Cl)-R^3-Ser-Arg-D-Lys(A_2xy)-Leu-Arg-Pro-D-Ala-NH_2

C <Glu-His-Trp-Ser-Tyr-D-Lys(A_2xy-Ahx)-Leu-Arg-Pro-Gly-NH_2,

wherein A_2xy stands for A_2pr or A_2bu, R^1 is D-Nal(2) or D-Phe(*p*Cl), and R^3 is D-Pal(3) or D-Trp.

Linear decapeptides, obtained by the solid-phase method, were derivatized by linking A_2xy to the ε-amino group of the D-Lys^6 residue. A direct synthesis of **A** and **B** peptides on solid support could also be performed, by incorporating the D-Lys(A_2xy) as a single unit, i.e., Boc-D-Lys(Z_2A_2xy).

In the metallopeptides containing $PtCl_2$, platinum was chelated by the two amino groups of the A_2pr side chain as illustrated in Fig. 1*A*. Copper and nickel were chelated by two —N=C< groups and two negatively charged oxygens originating from the condensation of the two amino groups of A_2pr or A_2bu with a 3-hydroxy oxo compound, as exemplified by the copper(II) complex with the 2,3-bis(2-oxidobenzylidenimino)propionyl moiety in Fig. 1*B*. The hydroxy oxo compounds used, and the corresponding residues incorporated into peptides **A–C** were as follows: salicylaldehyde, 2-oxidobenzylidene (Sal); 5-chlorosalicylaldehyde, 5-chloro-2-oxidobenzylidene (ClSal); pyridoxal, 2-methyl-5-hydroxymethyl-3-oxido-4-picolylidene (Pxd); pyridoxal 5′-phosphate, 2-methyl-5-phosphooxymethyl-3-oxido-4-picolylidene (*P*-Pxd).

HPLC proved to be the method of choice for following the formation of metallopeptides and for the purification of intermediates and end products. After purification by preparative HPLC, the purity of the peptides was found to be >95%. Amino acid analyses and metal contents were consistent with expected values.

The metallopeptides were tested *in vitro* for their ability to stimulate LH release from dispersed rat pituitary cells and for their affinity for membrane receptors in pituitary of male rats and human breast cancers by using ^{125}I-labeled [D-Trp^6]LH-RH as labeled ligand. Table 1 presents data on the LH-releasing activity of agonistic metallopeptide analogues (**I–XI**) and the corresponding peptide ligands (**A1**, **A2**, and **C1**) and on their binding properties. Most of the metallopeptide analogues had similar activity (**V**, **VI**, **IX**, **XI**) as their carrier peptides or were 2–10 times more potent (**I–IV**, **VIII**). Like-

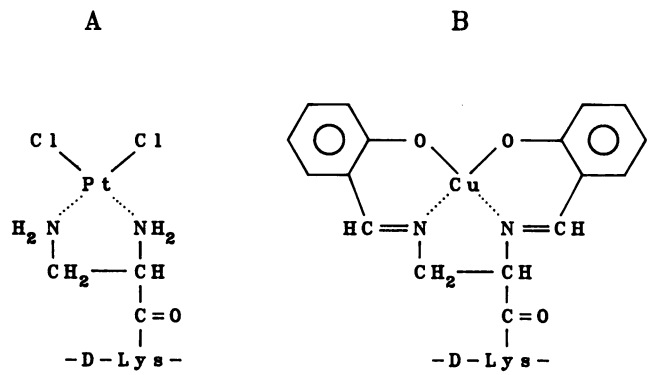

FIG. 1. (*A*) $PtCl_2$ complex of the D-Lys(A_2pr) residue. (*B*) *cis*-Bis(salicylideneiminato)copper(II) complex of the D-Lys(A_2pr) residue.

Table 1. LH-releasing activity and receptor binding of <Glu-His-Trp-Ser-Tyr-D-Lys{A_2xy(B_2Q)}-Leu-Arg-Pro-Gly-NH_2 metallopeptides and their metal-free parent compounds

Peptide					Relative activity*	Affinity constant (K_{a1}),† nM^{-1}	
No.	Code	Q	B	A_2xy		Pituitary	Cancer cells
A1	SB-120	–	–	A_2pr	5.1	9.908	6.018
I	SB-40	$PtCl_2$	–	DL-A_2pr	55	3.285	1.743
II	SB-91	Cu	Sal	A_2pr	10	7.968	3.589
III	SB-101	Ni	Sal	A_2pr	12	9.710	5.234
IV	SB-105	Cu	ClSal	DL-A_2pr	10	8.625	3.995
V	SB-106	Ni	ClSal	DL-A_2pr	4	4.513	2.236
VI	SB-108	Cu	Pxd	DL-A_2pr	9	5.817	3.005
VII	SB-111	Cu	*P*-Pxd	A_2pr	0.3	8.676	4.528
A2	SB-119	–	–	DL-A_2bu	6.2	5.770	0.322
VIII	SB-109	Cu	Sal	DL-A_2bu	25	5.589	1.628
IX	SB-118	Cu	Pxd	DL-A_2bu	5.5	6.405	3.141
X	SB-113	Cu	*P*-Pxd	DL-A_2bu	1.5	9.751	5.038
CI	SB-121	–	–	DL-A_2bu-Ahx	7.4	10.409	4.937
XI	SB-122	Cu	Sal	DL-A_2bu-Ahx	9.2	2.180	7.258

*LH-releasing activity was determined in a perfused rat pituitary cell system. Each peptide was superfused for 3 min with peptide at 1 pM–5 nM. LH release (determined by radioimmunoassay) was compared to that produced by 3 nM LH-RH.
†Affinity constants of the peptides for the first receptor class (i.e., for high-affinity binding sites) of male rat pituitary and human breast cancer cells were determined by using ^{125}I-labeled [D-Trp^6]LH-RH.

wise, the binding properties of the metallopeptides did not differ markedly from those of the parent compounds.

Table 2 shows the potency of antagonistic metallopeptides (**XII–XIX**) and of their peptide portions (**B1** and **B2**). Of these compounds, **XIII**, **XIV**, and **XIX** exerted particularly high, long-lasting inhibitory action, much greater than the potency of their respective peptide carrier precursors **B1** and **B2**.

Table 3 indicates that no binding was observed with one of the most potent inhibitory analogues, **XIII**, a D-Pal(3)^3 peptide having a DL-A_2pr(Sal_2Cu) moiety, while its nickel-containing congener (**XII**) showed affinity for both receptors. The highest K_{a1} values were obtained for the corresponding D-Trp^3 peptide (**XVI**).

In vivo antiovulatory activity of the antagonistic metallopeptides is shown in Table 4. Metallopeptides **XII** and **XIII** proved to have high antagonistic potency, whereas the others were only moderately active.

DISCUSSION

There is much evidence that highly potent LH-RH agonists are of value in the treatment of certain sex hormone-dependent tumors, such as prostatic and breast cancer (1–3, 9–12). However, hormonal manipulation may not prevent the ultimate tumor growth of hormone-independent cells (13). A combination of hormonal therapy with chemotherapy could forestall this phenomenon (14). Treatment with LH-RH analogues that contain cytotoxic moieties might provide such combined therapy.

In the present study metal complexes related to the cytotoxic complexes cisplatin (16) and *trans*-bis(salicylaldoximato)copper(II) (17) were incorporated into the LH-RH molecule and some of its antagonists to yield metallopeptide analogues of this hormone. Diamino acids (A_2pr and A_2bu) linked to LH-RH analogues provided coordination compounds with $PtCl_2$ (Fig. 1*A*) or gave bis(salicylideneiminato)-copper(II) complexes (Fig. 1*B*) through their amino groups. The copper complex is composed of the Schiff base of the diamino compound, a hydroxy oxo compound (salicylaldehyde), and copper(II). In addition to copper(II) complexes, nickel(II) chelates were also prepared. These metal chelates, being hydrophobic moieties, should preferably reside at the side-chain terminus of a D residue at position 6—e.g., at the ε-amino group of the D-Lys^6 of LH-RH analogues. Hydrophobic D residues at position 6 of LH-RH greatly increase the LH-releasing activity of the parent hormone. Such residues can also be favorable in antagonistic analogues, provided that

Table 2. LH-RH-inhibiting activity of Ac-D-Nal(2)-D-Phe(*p*Cl)-R^3-Ser-Arg-D-Lys{DL-A_2pr(B_2Q)}-Leu-Arg-Pro-D-Ala-NH_2 metallopeptides and their metal-free parent compounds

Peptide				% inhibition of LH response			
No.	Code	Q	B	0 min	30 min	60 min	90 min
			Peptides with D-Pal(3)^3				
B1	SB-93	–	–	90	61	37	33
XII	SB-94	Ni	Sal	90	46	4	0
XIII	SB-95	Cu	Sal	86	60	50	48
XIV	SB-117	Cu	Pxd	91	61	52	51
XV	SB-114	Cu	*P*-Pxd	32	38	40	49
			Peptides with D-Trp^3				
B2	SB-58	–	–	33	0	0	0
XVI†	SB-104	Cu	Sal	77	13	14	14
XVII	SB-116	Cu	Pxd	37	25	12	24
XVIII	SB-115	Cu	*P*-Pxd	10	0	0	0
XIX	SB-57	$PtCl_2$	–	64	53	38	33

Rat pituitary cells were exposed to the analogues (3 nM) for 12 min. During the last 3 min 3 nM LH-RH was also given (0-min response). LH-RH (3 nM) was also administered 30, 60, and 90 min later for 3 min.
†Contains D-Lys(L-A_2pr)^6 residue.

Table 3. Affinity of Ac-D-Nal(2)-D-Phe(*p*Cl)-R^3-Ser-Arg-D-Lys{DL-A_2pr(B_2Q)}-Leu-Arg-Pro-D-Ala-NH_2 metallopeptides and their metal-free parent compounds for membrane receptors of rat pituitary and human breast cancer

Peptide			Affinity constant (K_{a1}), nM^{-1}	
No.	Q	B	Pituitary	Cancer cells
Peptides with D-Pal(3)^3				
B1	–	–	7.117	4.616
XII	Ni	Sal	4.025	0.500
XIII	Cu	Sal	2.272	ND
XIV	Cu	Pxd	4.286	2.311
XV	Cu	*P*-Pxd	5.250	2.436
Peptides with D-Trp^3				
B2	–	–	—	12.286
XVI*	Cu	Sal	9.183	4.951
XVII	Cu	Pxd	7.460	3.259
XVIII	Cu	*P*-Pxd	2.901	1.337
XIX	$PtCl_2$	–	3.135	6.062

Affinity constants were determined by using ^{125}I-labeled [D-Trp^6]LH-RH; ND, no displacement.
*Contains D-Lys(L-A_2pr)^6 residue.

they are paired with proper substitutions in the N-terminal tripeptide fragment.

Most of the agonistic analogues (Table 1) showed higher potency than LH-RH, with metallopeptide **I**, containing $PtCl_2$, having an activity 55 times higher than LH-RH. Of the bis(salicylideneiminato)copper(II) complexes, compound **VIII**, containing DL-A_2bu, was the most potent (25 times the activity of LH-RH). The use of pyridoxal instead of salicylaldehyde (or its 5-chloro derivative) as Schiff base-forming agent led to weaker compounds. Even less active metallopeptides were produced when pyridoxal 5′-phosphate was incorporated. The low potency of **VII** and **X**, which contain phosphate, is in accord with a report (24) that D-Glu^6 (an acidic D residue) substitution in an agonist has deleterious effects on bioactivity. Compounds **VII** and **X** showed high affinity for both pituitary and breast cancer receptors. This suggests that structural requirements for binding to the pituitary receptors found in dispersed cells and in membrane preparations, respectively, are not identical (22). Since K_{a1} values for the membrane receptor of breast cancer cells were similar to those of the rat pituitary (10), this indicates that the structures of the high-affinity LH-RH binding sites of rat pituitary and human breast cancer cells are closely related.

Table 4. Antiovulatory activities of Ac-D-Nal(2)-D-Phe(*p*Cl)-R^3-Ser-Arg-D-Lys{DL-A_2pr(B_2Q)}-Leu-Arg-Pro-D-Ala-NH_2 metallopeptides and their metal-free parent compounds in rats

Peptide			% blockade of ovulation*			
No.	Q	B	25 μg	10 μg	3 μg	1.5 μg
Peptides with D-Pal(3)^3						
B1	–	–		100	17	0
XII	Ni	Sal			100	75
XIII	Cu	Sal			100	30
XIV	Cu	Pxd		100	50	
XV	Cu	*P*-Pxd		100	80	
Peptides with D-Trp^3						
B2	–	–	100	17		
XVI†	Cu	Sal		100	20	
XVII	Cu	Pxd	100	40		
XVIII	Cu	*P*-Pxd	100	60		
XIX	$PtCl_2$	–	100	0		

Peptides were tested at doses of 1.5, 3, 10, and 25 μg per rat.
*No. of rats not ovulating/no. tested (n = 6–10).
†Contains D-Lys(L-A_2pr)^6 residue.

The antagonistic metallopeptide analogues of LH-RH were derived from the so-called transposition antagonists (25), which have, besides Arg^5, a hydrophobic D residue at position 6. Of these analogs, **XIII** and **XIV** exhibited very strong and prolonged antagonistic effects *in vitro* (Table 2). It is also apparent that in this series, D-Trp^3 substitution is less favorable than D-Pal(3)^3. With respect to binding to pituitary receptors (Table 3), no such advantage of the D-Pal(3)^3 substitution was observed (*cf.* **XIII** with **XVI**, **XIV** with **XVII**, and **XV** with **XVIII**). The acidic phosphate group seemed to improve the binding of antagonists to a lesser extent (**XIV** vs. **XV**; **XVII** vs. **XVIII**) than that of agonists (**VI** vs. **VII**; **IX** vs. **X**). For binding to cancer receptors D-Trp^3 is more favorable than D-Pal(3)^3. No displacement was found with the D-Pal(3)^3 peptide **XIII**, whereas the corresponding D-Trp^3 analogue, **XVI**, had a high K_{a1} value.

Antiovulatory activities (Table 4) show that conversion of LH-RH analogues **B1** and **B2** into metallopeptides (**XII–XIX**) led in most cases to an increase in potency. In the case of the D-Pal(3)^3 peptides (**XII–XV**), the salicylideneiminato and pyridoxylideneiminato copper and nickel complexes caused 50–100% blockade of ovulation at doses of 3 μg. Interestingly, the deleterious effects of the acidic phosphate on the bioactivity could not be demonstrated in this assay (*cf.* **XIV** with **XV** and **XVII** with **XVIII**).

Some of these metallopeptides were evaluated *in vitro* for cytotoxicity. We were able to demonstrate significant cytotoxic activity (73% inhibition of [^{3}H]thymidine incorporation) in cultures of human mammary cancer line T-47D and human prostate adenocarcinoma line PC-3 for copper-containing agonistic analogue **II** (SB-91). The present study provides evidence that incorporation of cytotoxic metal complexes into suitably designed LH-RH analogs can result in metallopeptides having high LH-RH agonist or antagonist activity. Continued work along these lines may eventually lead to LH-RH analogues containing cytotoxic moieties that can be targeted to tumors that have receptors for LH-RH-like peptides.

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