Expedited Articles

NMR-Based Discovery of Lead Inhibitors That Block DNA Binding of the Human Papillomavirus E2 Protein

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The E2 protein is required for the replication of human papillomaviruses (HPVs), which are responsible for anogenital warts and cervical carcinomas. Using an NMR-based screen, we tested compounds for binding to the DNA-binding domain of the HPV-E2 protein. Three classes of compounds were identified which bound to two distinct sites on the protein. Biphenyl and biphenyl ether compounds containing a carboxylic acid bind to a site near the DNA recognition helix and inhibit the binding of E2 to DNA. Benzophenone-containing compounds which lack a carboxylic acid group bind to the β-barrel formed by the dimer interface and exhibit negligible effects on the binding of E2 to DNA. Structure−activity relationships from the biphenyl and biphenyl ether compounds were combined to produce a compound [5-(3′-(3″,5″-dichlorophenoxy)-phenyl)-2,4-pentadienoic acid] with an IC_{50} value of approximately 10 μM. This compound represents a useful lead for the development of antiviral agents that interfere with HPV replication and further illustrates the usefulness of the SAR by NMR method in the drug discovery process.

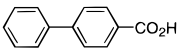
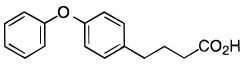
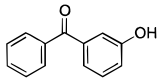
Introduction

Papilloma viruses are a group of small DNA tumor viruses that infect a wide variety of mammalian cells. They are the causative agents of benign proliferative lesions of the skin or mucosa,[1] and certain strains have been implicated in the development of cervical carcinomas and other malignancies.[2,3] All of the papillomaviruses encode a DNA-binding protein, E2, that regulates the transcription of several viral genes and, in conjunction with the E1 protein, is required for viral replication.[3−7]

Many approaches have targeted the E2 protein for developing antiviral agents against the human papillomaviruses (HPVs). One strategy is to block the expression of the HPV-E2 protein, which has been demonstrated using antisense oligonucleotides targeted at E2 mRNA.[8,9] Another approach is to target the E2 protein itself. Truncated forms of the HPV-E2 protein containing the DNA-binding domain (DBD) can act as trans-activating repressors by blocking the homo-dimerization of E2.[10,11] In addition, oligonucleotides which are recognized by the E2 protein can compete with DNA binding and inhibit viral cell growth.[12] These data suggest that the E2 protein can serve as a viable target for the development of therapeutics against HPVs.

The X-ray structure of the DNA-binding domain of bovine E2 when complexed to its cognate DNA has been reported,[13] and we have recently solved the NMR structure of the HPV-31 E2 DBD.[14] In order to aid in the discovery of small, novel therapeutic agents against the human papillomaviruses, we used an NMR-based approach called SAR by NMR (for structure−activity relationships by nuclear magnetic resonance)[15] to identify ligands for the DNA-binding domain of the E2 protein. Using this approach, small molecules which bind to proteins can be identified in an NMR-based screen by monitoring changes in the $^{15}N/^{1}H$ amide chemical shifts of the protein upon the addition of compounds. We have previously reported on the application of the SAR by NMR method for the discovery of high-affinity ligands for the FK506 binding protein[15] and the catalytic domain of the matrix metalloproteinase stromelysin.[16] Here we describe the application of the SAR by NMR technique to the discovery of com-

Table 1. Initial Ligands for the E2 Protein Discovered Using SAR by NMR

No.	Compound	K_D (mM)[a]	Binding Site[b]	Inhibition of DNA binding[c]
1		2.5	DNA recognition helix	+
2		1.9	DNA recognition helix	+
3		0.6	β-barrel	-

[a] Dissociation constants were derived from an analysis of the changes in amide chemical shifts as a function of added compound. [b] Binding sites were determined by mapping the observed chemical shift changes onto the structure of the protein as shown in Figure 2. [c] Inhibition of DNA binding was determined at compound concentrations of 1 mM using a filter-binding assay.

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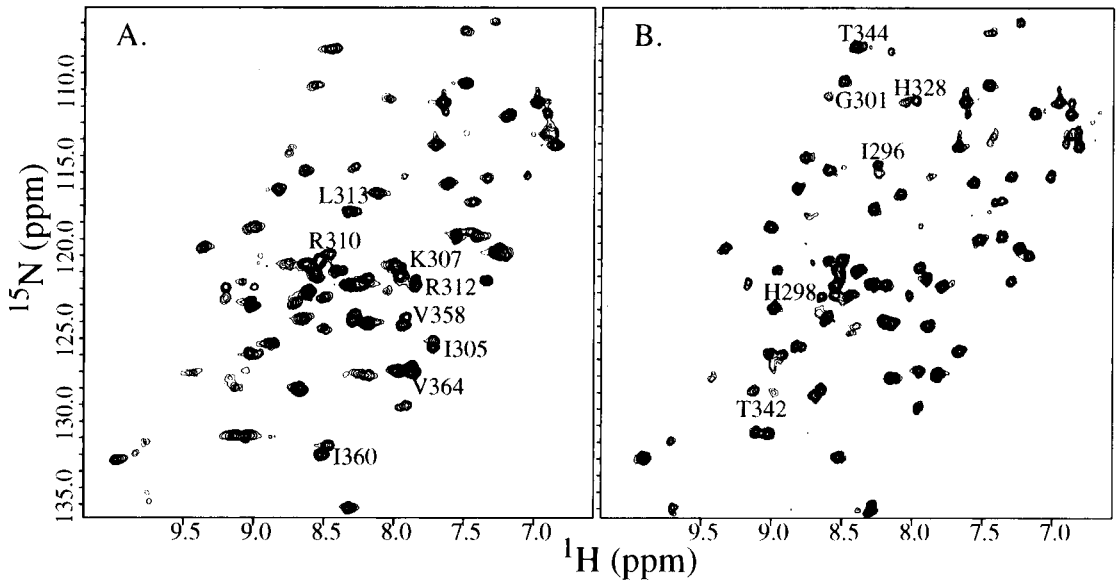


Figure 1. [^{15}N]HSQC spectra of E2 DBD in the absence of added ligand (black) and in the presence of **(**A**)** 3 mM **1** (red) and **(**B**)** 2 mM **3** (red). Some of the residues which exhibit significant chemical shift changes upon addition of ligand are labeled. Chemical shifts were considered significant if the average weighted ^{1}H/^{15}N chemical shift difference ($\Delta\delta(^{1}H/^{15}N) = \Delta\delta(^{1}H) + \Delta\delta(^{15}N)/5$) was greater than 0.04 ppm.

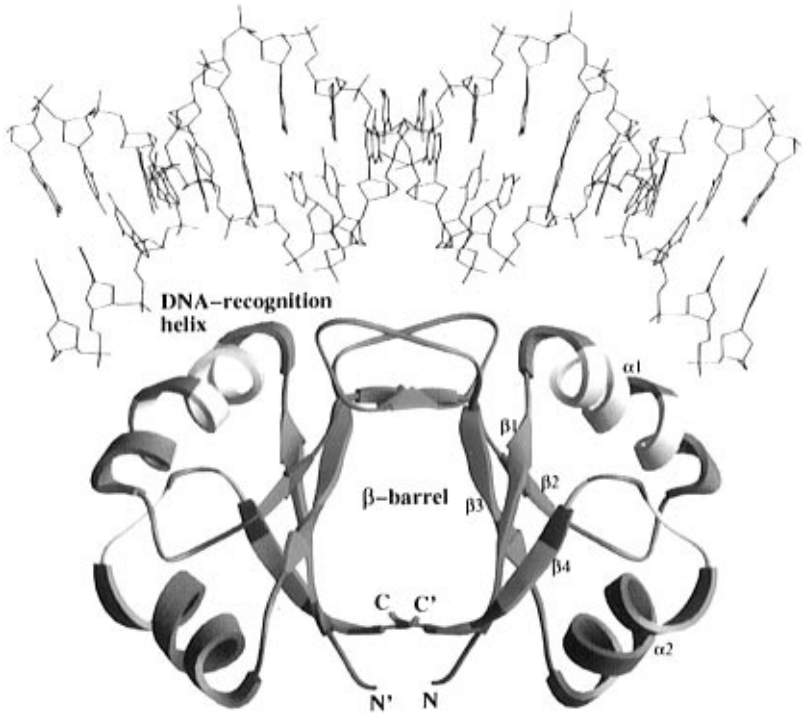


Figure 2. Ribbons[28] representation of the NMR structure of the DNA-binding domain of the E2 dimer from HPV-31.[14] DNA (top) is also depicted from the X-ray structure of bovine HPV-E2 complexed with DNA.[13] For clarity, the DNA was translated from its original position. Colored in yellow are those residues whose amide chemical shifts were significantly perturbed upon binding compound **1**, while those colored in magenta are those significantly perturbed upon binding compound **3**. Chemical shifts were considered significant if the average weighted ^{1}H/^{15}N chemical shift difference ($\Delta\delta(^{1}H/^{15}N) = \Delta\delta(^{1}H) + \Delta\delta(^{15}N)/5$) was greater than 0.04 ppm at compound concentrations of 3 and 2 mM for **1** and **3**, respectively.

pounds which bind to the DNA-binding domain of the E2 protein and inhibit its interaction with DNA.

Identification of Initial Lead Ligands

To identify compounds that bind to the E2 DBD from HPV strain 31b, we screened a library of small organic molecules[17] by recording two-dimensional ^{15}N-heteronuclear single-quantum correlation (^{15}N/^{1}H HSQC) spectra[18] of uniformly ^{15}N-labeled E2 DBD in the presence and absence of potential ligands. Three classes of compounds were identified that bind weakly to two distinct sites on the E2 protein (Table 1). Biphenylcarboxylic acids (e.g., **1**) and biphenyl ether carboxylic acids (e.g., **2**) caused significant chemical shift changes in the backbone amides of residues 305−310, 312, 313, 358−360, 364, and 371 of the protein. These residues are located in the DNA recognition helix or the adjacent loop (Figure 1A and yellow in Figure 2). The third class of

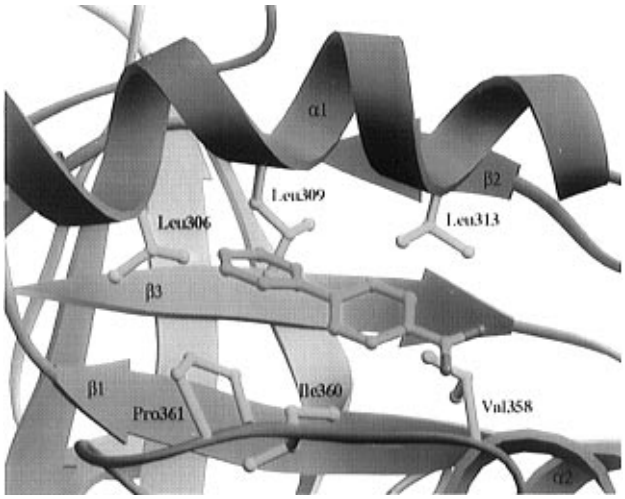


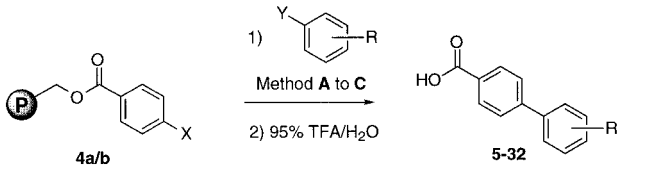
Figure 3. Ribbons[28] representation of the NOE-based model of the complex between E2 DBD and **1** (colored by atom type). Shown in blue are side chains of E2 DBD which make NOE contacts with the ligand. The secondary structural elements are labeled as in Figure 2 for aid in visualization.

compounds, which contain a benzophenone moiety and lack a carboxy functionality (e.g., **3**), caused significant changes in the amide chemical shifts of residues 296−301, 322, 328, 339−342, 344, 366, 367, 369, and 373 (Figure 1B and magenta in Figure 2). These compounds induced little or no changes in the chemical shifts of residues comprising the DNA recognition helix. Thus, the NMR data indicate that this third class of compounds bind to residues located near the β-barrel formed by the interface of the two monomer subunits (Figure 2).

The initial lead ligands from the three classes were further tested in a filter-binding assay to evaluate their ability to inhibit the binding of E2 to DNA. It was found that **1** and **2** inhibit the binding of E2 to DNA at concentrations of 1 mM (Table 1). These results were consistent with the observed chemical shift perturbations, since compounds which bind near the recognition helix may sterically occlude complex formation. In contrast, the compound (**3**) which binds near the β-barrel region did not affect E2/DNA complex formation. Thus, although this compound binds to the E2 DBD, it does not inhibit the binding of DNA either by directly blocking the DNA binding site or by disrupting the homodimerization of E2.

To confirm the ligand binding site and to provide a structural basis for the inhibition of DNA binding by the biphenyl and biphenyl ether carboxylates, structural studies were performed on the complex of E2 and **1** using two-dimensional isotope-edited NMR experiments. A model of the complex based on 23 intermolecular NOEs is shown in Figure 3. The compound binds to a hydrophobic groove between the DNA-recognition helix and the adjacent loop, and the biaryl moiety makes hydrophobic contacts with residues Leu306, Leu309, Leu313, Val358, Ile360, and Pro361 of the E2 protein. This location is consistent with the observed amide chemical shift perturbations (Figure 2) and indicates that the inhibition exhibited by this class of compounds is due an occlusion of the DNA-binding site on the E2 protein.

Scheme 1. General Synthesis of Biphenylcarboxylates



A: X = Br (**4a**), Y = $B(OH)_2$, $Pd(PPh_3)_4$, 2M Na_2CO_3, DME/EtOH, reflux, 20h

B: X =  (**4b**), Y = Br, $Pd(PPh_3)_4$, 2M K_2CO_3, DMF/H_2O, 80 °C, 2d

C: X =  (**4b**), Y = OTf, $Pd(OAc)_2$/dppf, 2M Na_2CO_3, toluene/EtOH, reflux, 2d

Table 2. Binding Data for Biphenylcarboxylate Compounds

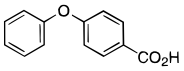
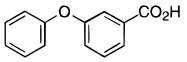
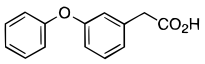
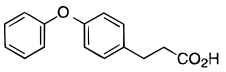
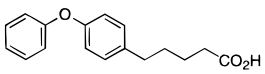
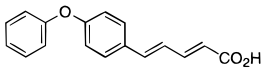
no.	R	K_D (mM)[a]
5	2′-CH_3	2.7
6	3′-CF_3	0.32
7	3′-NH_2	>10
8	3′-F	1.6
9	3′-Cl	1.2
10	3′-NO_2	8.9
11	4′-CH_3	1.5
12	4′-CF_3	1.5
13	4′-OCH_3	8.8
14	4′-*t*-Bu	>10
15	4′-OPh	2.1
16	4′-F	1.5
17	4′-Cl	1.6
18	2′,4′-Cl_2	3.5
19	3′-Cl,4′-F	3.4
20	3′,5′-CF_3	>10
21	3′,5′-Cl_2	0.06
22	2′-OCH_3	1.7
23	3′-CH_3	1.9
24	4′-$CONH_2$	>10
25	4′-CN	>10
26	4′-OH	>10
27	4′-NH_2	4.5
28	4′-$N(CH_3)_2$	6.8
29	4′-NO_2	>10
30	3′-Cl,4′-NO_2	>10
31	3′,4′-Cl_2	6.6
32	3′-$N(CH_3)_2$	>10

[a] Dissociation constants were derived from an analysis of the changes in amide chemical shifts as a function of added compound.

Optimization of the Biphenylcarboxylates

To explore the SAR of the biphenyl leads, a series of biphenylcarboxylate analogs were synthesized via Suzuki coupling using solid phase parallel synthesis[19] as shown in Scheme I. Since the number of commercially available boronic acids was very limited, the traditional strategy of attaching *p*-bromobenzoic acid to resin (method A) was switched to Guiles' method of reacting aryl halides with an immobilized aryl boronic acid (method B),[20] which had to be modified in the case of the 3′-NMe_2-substituted biphenylcarboxylate (method C). To obtain the maximum amount of SAR with a minimum number of reagents, the members of the Topliss scheme[21,22] for aromatic substitution were synthesized. This set of compounds is based on an optimum discrimination between hydrophobic (π), electronic (σ), and steric (E_S) effects of the substituents on the phenyl ring. The dissociation constants for a variety of biphenylcarboxylates were determined by NMR (Table 2). Biphenyls containing polar substituents (e.g., **7**, **24**−**26**) exhibited a significant decrease in binding affinity relative to the starting unsubstituted biphenylcarboxylic

Table 3. Binding Data for Biphenyl Ether Compounds

No.	Compound	K_D (mM)[a]
33		9.1
34		>10
35		>10
36		2.3
38		0.85
39		0.35

[a] Dissociation constants were derived from an analysis of the changes in amide chemical shifts as a function of added compound.

acid **1** (K_D = 2.5 mM). The most active compound in the series was the 3′,5′-dichloro-substituted compound **21** (K_D = 0.06 mM, IC_{50} = 0.15 mM), which exhibited more than a 40-fold enhancement in activity. The 3′-CF_3-substituted analogue **6** was the second most active compound found in the series, but the activity decreased by a factor of 15 when an additional CF_3 group in the 5′-position was added (**20**).

Optimization of Biphenyl Ether Leads

Analogs of the biphenyl ether compound **2** were also pursued and tested for binding to the DBD of the E2 protein. Compounds from commercial sources with shorter linkers between the biphenyl ether moiety and the carboxylic acid group (e.g. **33**−**36**) exhibited a reduced binding affinity for E2 DBD (Table 3). On the basis of these results, several compounds were synthesized (Scheme 2) which increased the distance between the biphenyl ether and carboxyl functionality. Increasing the length of the spacer to four methylene units (**38**) resulted in a 2-fold increase in affinity for the E2 DBD (Table 3). Further improvements were obtained with

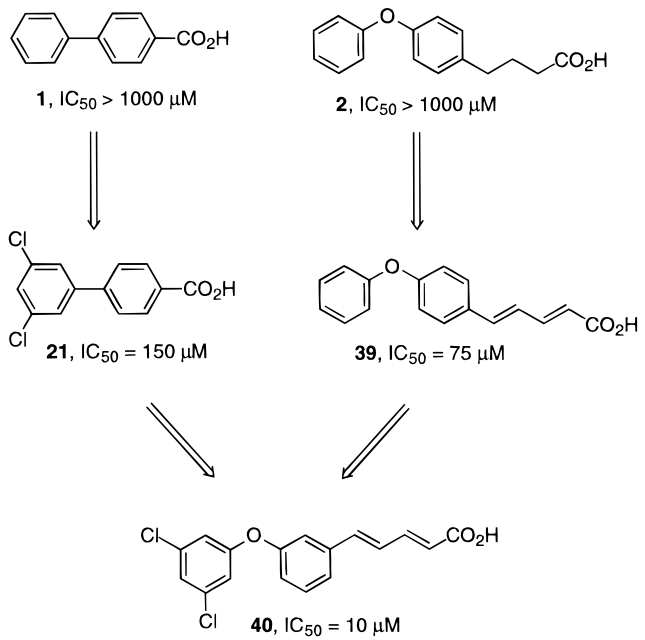


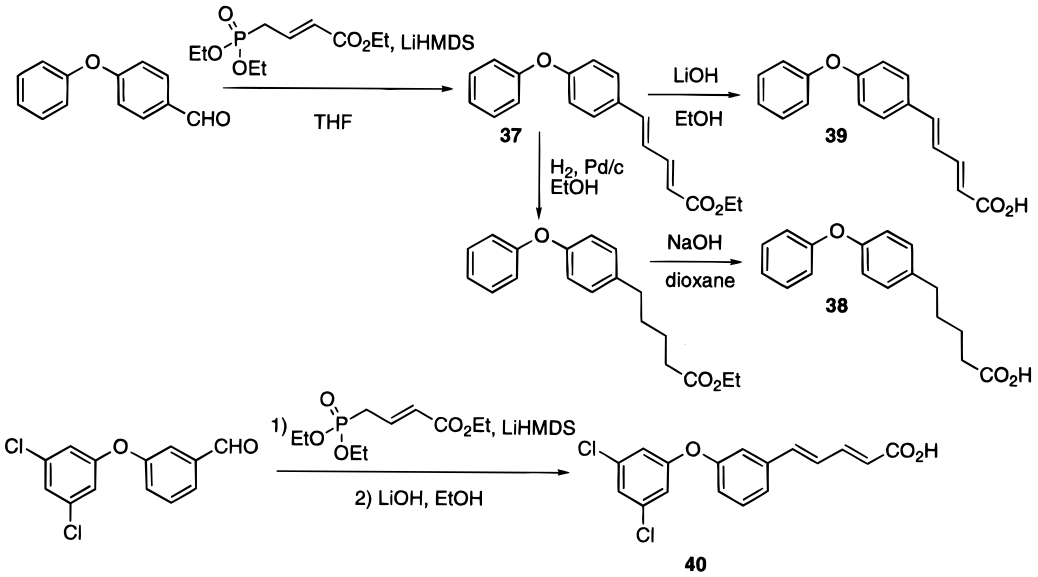
Figure 4. Summary of the discovery of a 10 μM inhibitor of HPV-E2 DBD starting from millimolar leads identified using SAR by NMR.

the rigid *trans,trans*-butadiene spacer (**39**), which exhibited a K_D of 0.35 mM and an IC_{50} of 75 μM in the filter binding assay.

Combining the SAR of Both Lead Ligands

When added to the E2 protein, the biphenyl and biphenyl ether leads caused the same set of amide crosspeaks to shift, indicating that both classes of compounds bind to the same location on E2 DBD. Thus, we reasoned that the SAR from both of these classes of compounds could be combined into a single molecule. Indeed, a compound (**40**) which incorporated both the butadiene spacer and the dichloro substituents, as synthesized according to Scheme 2, was found to be the most potent compound of the series with an IC_{50} value of 10 μM in a filter binding assay (Figure 4).[23]

Scheme 2. Synthesis of Biphenyl Ether Carboxylates **38**, **39**, and **40**



Concluding Remarks

An important aspect of drug discovery is the identification of lead compounds that can be used as starting points in the design of high-affinity ligands for proteins or other therapeutic targets. Using conventional high-throughput screening of large libraries of molecules, it is not always possible to find leads of suitable potency. In fact, using an E2-DNA binding assay, high-throughput screening of more than 100 000 compounds from our corporate library produced no inhibitors with activities better than 10 μM. As an alternative to finding high-affinity ligands from existing libraries, suitable lead compounds may be constructed from structure−activity relationships obtained on small, weakly binding molecules. A critical aspect of such an approach, however, is the ability to obtain reliable SAR for compounds which bind to the target with affinities in the millimolar range. Most conventional screening methods are severely limited for identifying compounds with such weak affinities due to background signals from the high concentrations of ligand required. However, the use of ^{15}N spectral editing allows the facile observation of the ^{15}N-labeled protein signals even in the presence of millimolar (or higher) compound concentrations. The use of [^{15}N]HSQC spectra allows not only the detection of weakly bound ligands, but also the ability to differentiate between multiple binding sites on the protein surface. In the case of E2 DBD, two distinct sites were observed, and independent structure−activity relationships could be developed at each location. Since ligand binding at only one of the sites (near the DNA-recognition helix) inhibited E2/DNA binding, we were able to focus our design efforts only on those compounds. One compound, which combined the SAR obtained on two lead series, inhibits the binding of the E2 protein to DNA at low micromolar concentrations and serves as a useful lead for an antiviral agent against the human papillomavirus.

Experimental Section

Solvents and other chemicals were reagent grade and used as received. ^{1}H-NMR spectra for analysis of synthesized compounds were recorded at 300 MHz and expressed as ppm downfield from tetramethylsilane (TMS) as an internal standard. Column chromatography was performed on Kiesel gel 60 silica (EM Science). Wang resin was obtained from Advanced ChemTech. Elemental analyses were performed by Robertson Microlit Laboratories, Inc. (Madison, NJ). Melting points were obtained using a Thomas Hoover UniMelt. Compounds **1** (Aldrich), **2**, **3** (Aldrich), **33** (Lancaster), **34** (Aldrich), **35** (Lancaster), and **36** (Maybridge) were obtained from our corporate library and used without further purification.

General Method for the Suzuki Coupling of Resin-Bound *p*-Bromobenzoic Acid 4a (Method A: 5−21). Wang resin-bound *p*-bromobenzoic acid **4a** (0.3 g, 0.83 mmol/g theoretical load), prepared by standard procedure (DCC, DMAP, CH_2Cl_2/THF (4:1), room temperature, 16 h), was preswollen in degassed DME (5.0 mL) for 10 min. $Pd(PPh_3)_4$ (29 mg, 0.025 mmol) was then added, and the mixture was agitated for 15 min under nitrogen. A solution of the appropriate boronic acid (0.5 mmol) in EtOH (1.0 mL) was added, and the mixture was agitated for further 10 min followed by treatment with 2 M aqueous Na_2CO_3 (0.5 mL). The resulting slurry was refluxed for 20 h, cooled, filtered, washed successively with DME/H_2O (1:1), H_2O, 2 N HOAc, H_2O, DME, EtOAc, EtOAc/MeOH (1:1), MeOH, 2 × [0.5% diethyldithiocarbamic acid, sodium salt trihydrate and 0.5% DIEA in DMF,[24] THF], and 3 × THF (10.0 mL each) and dried *in vacuo*. The product was cleaved from the resin by addition of 95% TFA/H_2O (5.0 mL) and agitation at room temperature for 2 h. The resin was filtered off and washed with CH_2Cl_2 (2.0 mL). The combined filtrates were concentrated in vacuo, and the crude material was recrystallized from EtOAc/*n*-hexane to give the products in >90% overall yield. MS and analytical reverse phase HPLC analyses showed that the products were obtained in purities > 90%.

General Method for the Coupling of Aryl Halides with Resin-Bound Arylboronic Acid 4b (Method B: 22−31). Benzoyldioxaborinane was prepared via the procedure of Takahashi,[23] loaded onto Wang resin, and coupled with a series of aryl bromides (method B).[20] The products were obtained as described above.

3′-(*N*,*N*-Dimethylamino)-4-biphenylcarboxylic Acid 32 (Method C). 1,1′-Bis(diphenylphosphino)ferrocene (67 mg, 0.12 mmol) and palladium(II)acetate (14 mg, 0.06 mmol) were stirred in degassed toluene at room temperature for 10 min. The solution was then added to a slurry of the phenyldioxaborinane on Wang resin **4b** (1.33 g, 0.75 mmol/g theoretical load), prepared via the procedure of Guiles,[20] and 3-(*N*,*N*-dimethylamino)phenyl trifluoromethanesulfonate[25] (0.29 g, 1.08 mmol) in toluene/EtOH (10:1, 11.0 mL). The mixture was stirred for further 10 min, then 2 M aqueous Na_2CO_3 (2.8 mL) was added, and the reaction mixture was heated to reflux for 2 d. After filtration, washing of the resin, cleavage of the product, and recrystallization as described above, 100 mg (44%) of the product was obtained as pale yellow crystals.

5-(4′-Phenoxyphenyl)-2,4-pentadienoate (37). LiHMDS (15 mL, 15.13 mmol, 2.0 equiv) was added dropwise to a 30 mL solution of triethyl 4-phosphonocrotonate (3.35 mL, 15.13 mmol, 2.0 equiv) under N_2 at 0 °C. The resulting solution was stirred at 0 °C for 30 min before adding 4-phenoxybenzaldehyde (1.5 g, 7.56 mmol. 1.0 equiv) in 30 mL of THF. After 3 h of stirring at 0 °C, the reaction was quenched by addition of 1 M HCl and extraction with ethyl acetate. The organic solution was washed with water and dried over $MgSO_4$. The solution was filtered, concentrated to a white solid, and carried on without further purification. Purification of the esters could be performed by flash column chromatography using 10−20% diethyl ether/hexanes. Recrystallization using ethyl acetate/petroleum ether afforded 1.8 g of colorless crystals: 85% yield; ^{1}H NMR ($CDCl_3$) δ 7.47−7.38 (5H, m), 7.15−7.09 (1H, m), 7.05−6.94 (4H, m), 6.90−6.73 (2H, m), 5.95 (1H, d, J = 15.3 Hz), 4.22 (2H, q, J = 7.1 Hz), 1.33 (3H, t, J = 7.1 Hz); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ 167.0 (C), 158.21 (C), 156.45 (C), 144.57 (CH), 139.51 (CH), 131.00 (C), 129.78 (CH), 128.63 (CH), 125.18 (CH), 123.74 (CH), 120.73 (CH), 119.29 (CH), 118.63 (CH), 60.12 (CH_2), 14.28 (CH_3); MS (M + H) 295, (M + NH_4^+) 312.

5-(4′-Phenoxyphenyl)-2,4-pentadienoic Acid (38). The ester **37** (1.0 g, 3.4 mmol, 1.0 equiv) was dissolved in 15 mL of 95% ethanol, and LiOH (81 mg, 3.4 mmol, 1.0 equiv) was added. The solution was stirred at room temperature for 12 h. The reaction was concentrated under vacuum and the residue taken up in water, acidified with 1 M HCl, and extracted with ethyl acetate. The organic solution was washed with water, dried over $MgSO_4$, filtered, and concentrated to a thick gum. Purification was carried out by flash column chromatography (2−10% methanol/chloroform + 0.5% acetic acid). Evaporation of the solvent afforded the acid as a white solid: recrystallized from ethyl acetate/petroleum ether; mp 176−178 °C; ^{1}H NMR (DMSO-d_6) δ 7.58 (2H, d, J = 8.5 Hz), 7.46−7.32 (3H, m), 7.28−7.19 (1H, m), 7.08−6.95 (6H, m), 6.00 (1H, d, J = 15.3 Hz); ^{13}C NMR (75.47 MHz, DMSO-d_6) δ 167.49 (C), 157.96 (C), 144.32 (CH), 138.99 (CH), 131.14 (C), 130.03 (CH), 128.88 (CH), 125.57 (CH), 123.83 (CH), 121.62 (CH), 119.02, (CH), 118.41 (CH); MS (M + H) 267, (M + NH_4^+) 284. Anal. Calcd for $C_{17}H_{14}O_3$: C, 76.67; H, 5.29. Found: C, 76.95; H, 5.28.

5-(4′-Phenoxyphenyl)pentanoic Acid (39). The dieneoic ester **37** (500 mg, 1.7 mmol, 1.0 equiv) was dissolved in 20 mL of ethanol, and 10% Pd/C (50 mg) was added. The ester was hydrogenated under atmospheric pressure (i.e. balloon) for 3 h. After the mixture was purged with N_2, Celite was added, and the solution was filtered and concentrated to a thick oil/glass. The crude ester was taken up in 15 mL of

dioxane, and 3 M NaOH (680 mL, 2.04 mmol, 1.2 equiv) was added. The solution was stirred at room temperature overnight. The solution was concentrated, and the residue was taken up in water, acidified with 1 M HCl, extracted with ethyl acetate, washed with water, dried over $MgSO_4$, filtered, and concentrated to a white solid. The solid was further purified by flash column (2−10% methanol/chloroform + 0.5% acetic acid). Recrystallization from ethyl acetate/petroleum ether afforded colorless needles: mp 58−59 °C; ^{1}H NMR (DMSO-d_6) δ 7.38 (2H, t, J = 7.4 Hz), 7.19 (2H, d, J = 8.5 Hz), 7.11 (1H, t, J = 7.4 Hz), 6.97 (2H, t, J = 7.46 Hz), 6.92 (2H, d, J = 8.5 Hz); 2.57 (2H, t, J = 7.1 Hz), 2.24 (2H, t, J = 7.1 Hz), 1.64−1.5 (4H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.38 (C), 157.06 (C), 154.41 (C), 137.20 (C), 129.89 (CH), 129.66 (CH), 123.02 (CH), 118.68 (CH), 118.17 (CH), 34.05 (CH_2), 33.50 (CH_2), 30.45 (CH_2), 24.08 (CH_2); MS (M + H) 271, (M + NH_4^+) 288. Anal. Calcd for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71. Found: C, 75.56; H, 6.90.

5-(3′-(3″,5″-Dichlorophenoxy)phenyl)-2,4-pentadienoic Acid (40). LHMDS (7.5 mL, 7.48 mmol, 2.0 equiv) was added dropwise to a 20 mL solution of triethyl 4-phosphonocrotonate (1.7 mL, 7.48 mmol, 2.0 equiv) under N_2 at 0 °C. The resulting solution was stirred at 0 °C for 30 min before adding 3-(3′,5′-dichlorophenoxy)benzaldehyde (1.0 g, 3.74 mmol, 1.0 equiv) in 20 mL of THF. After 3 h of stirring at 0 °C, the reaction was quenched by addition of 1 M HCl and extraction with ethyl acetate. The organic solution was washed with water and dried over $MgSO_4$. The reaction was filtered, concentrated to a white solid, and carried on without further purification. Purification of the esters could be performed by flash column chromatography using 10−20% diethyl ether/hexanes. Recrystallization using diethyl ether/hexane afforded 1.1 g, 80% yield. The resulting ester (700 mg, 1.9 mmol, 1.0 equiv) was dissolved in 15 mL of dioxane, and LiOH (51 mg, 2.3 mmol, 1.2 equiv) was added. The solution was stirred at room temperature for 12 h. The reaction was concentrated under vacuum, and the residue taken up in water, acidified with 1 M HCl, and extracted with ethyl acetate. The organic solution was washed with water, dried over $MgSO_4$, filtered, and concentrated to a thick gum. Purification was carried out by flash column chromatography (2−10% methanol/chloroform + 0.5% acetic acid). Evaporation of the solvent afforded the acid as a white solid. Recrystallization from ethyl acetate/petroleum ether afforded 581 mg of a white solid: 89% yield; mp 133−139 °C; ^{1}H NMR (DMSO-d_6) δ 7.38−7.32 (3H, m), 7.21−7.02 (5H, m), 7.49−7.44 (2H, m), 6.04 (1H, d, J = 15.3 Hz); ^{13}C NMR (75.47 MHz, DMSO-d_6) δ 167.38 (C), 158.53 (C), 155.38 (C), 143.83 (CH), 138.53 (CH), 138.44 (C), 134.92 (C), 130.71 (CH), 127.83 (CH), 123.79 (CH), 122.97 (CH), 122.91 (CH), 119.99 (CH), 117.98 (CH), 116.88 (CH); MS (M + NH_4^+) 352. Anal. Calcd for $C_{17}H_{12}O_3Cl_2$: C, 60.91; H, 3.60. Found: C, 61.19; H, 3.73.

Detection of Ligand Binding Using NMR. The DNA-binding domain of the HPV-31b E2 protein was expressed and purified as previously described.[14] NMR samples were composed of uniformly ^{15}N-labeled E2 DBD at 0.3 mM in a H_2O/D_2O (9:1) solution containing 20 mM phosphate, 10 mM DTT, pH 6.5. Ligand binding was detected at 25 °C by acquiring sensitivity-enhanced [^{15}N]HSQC spectra[18] on 400 μL of 0.3 mM HPV-E2 DBD in the presence and absence of added compound. Compounds were added as solutions in perdeuterated DMSO. A Bruker sample changer was used on a Bruker AMX500 spectrometer. Compounds were initially tested at 1.0 mM each, and binding was determined by monitoring changes in the [^{15}N]HSQC spectra. Dissociation constants were obtained for selected compounds by monitoring the chemical shift changes as a function of ligand concentration. Data were fit using a single binding site model. A least-squares grid search was performed by varying the values of K_D and the chemical shift of the fully saturated protein.

NOE-Based Model of the E2/1 Complex. NMR experiments were performed at 30 °C on a Bruker AMX500 spectrometer. NMR samples were composed of uniformly ^{15}N/^{13}C-labeled E2 DBD at 1.0 mM in a 100% D_2O solution containing 2.0 mM **1**, 20 mM phosphate, 1 mM perdeuterated DTT, pH 6.5 (uncorrected). Backbone and side chain assignments for E2 DBD were known,[14] and assignments for the protein in the complexed form were obtained by titrating **1** from 0.0 to 2.0 mM into a sample of ^{15}N/^{13}C-labeled E2 DBD and following the changes in [^{13}C]HSQC spectra as a function of added ligand. NOEs between **1** and E2 were obtained from 2D ^{13}C-filtered[26] NOESY spectra with mixing times ranging from 80 to 350 ms. A total of 23 intermolecular NOEs between the ligand and the protein were unambiguously assigned.

A model of the complex was obtained by manually docking **1** onto the NMR-derived structure of the E2 DBD dimer[14] followed by energy minimizations with the XPLOR 3.1 program[27] on a Silicon Graphics computer. The XPLOR F_{repel} function was used to simulate van der Waals interactions with a force constant of 4.0 kcal mol^{-1} and with atomic radii set to 0.8 times their CHARMM values. A total of 23 intermolecular distance restraints were were employed with a square well potential (F_{NOE} = 50 kcal mol^{-1} Å^{-2}) and given lower and upper bounds of 1.8 and 5.0 Å, respectively. Experimental hydrogen bonds and dihedral angle restraints from the NMR structure of E2 DBD[14] were included in the calculations. After minimization, the energetic penalty from the intermolecular distance violations was less than 0.2 kcal/mol, and only minor changes were observed in the structure of E2 DBD (rmsd of 0.60 Å for the C_α trace).

Inhibition Assays. Inhibition of binding of the E2 DBD to DNA was assayed using a nitrocellulose filter binding assay. Plasmid p1, containing the HPV11 genome (four E2 binding sites) inserted into the *Bam*H1 site of pSP65, was linearized with *Eco*R1 and end-labeled with polynucleotide kinase to a specific activity of approximately 2 × 10^5 dpm/fmol. Binding reactions were performed at room temperature for 10 min in 25 mL of binding buffer (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 5 mM DTT, 1 mM EDTA, 50 mg/mL BSA) containing 4 nM DNA, 400 nM E2 DBD, and various concentrations of compounds added from DMSO stocks. DNA fragments bound by E2 were captured by filtration through a MultiSCreen-HA 96-well nitrocellulose plate (Millipore) and washed once with 100 μL of binding buffer lacking BSA. Next, 30 μL of Microscint O (Packard) was added to each well, and plates were counted in a TopCount scintillation counter (Packard). IC_{50} values were determined by plotting the compound concentration versus percent inhibition to determine the concentration required for 50% inhibition.

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