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Bioorganic & Medicinal Chemistry Letters 13 (2003) 2539–2541

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Discovery of the First Series of Inhibitors of Human Papillomavirus Type 11: Inhibition of the Assembly of the E1–E2–Origin DNA Complex

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Received 6 March 2003; accepted 2 May 2003

Abstract—We have discovered a series of inhibitors of the assembly of the HPV11 E1–E2–origin DNA complex, which incorporate an indandione fused to a substituted tetrahydrofuran.

Infection with human papillomaviruses (HPV) is widespread in the population and is associated with a broad spectrum of disease ranging from benign warts to malignant lesions.[1] More than 25 types of HPV infect the anogenital region. The two types most often detected in benign genital warts (condyloma acuminata) are HPV6 and 11, known as 'low-risk' types whereas precancerous cervical lesions contain mostly the 'high-risk' HPV types 16, 18, 31 and 45. Current therapies of HPV-induced lesions rely on surgical removal of the infected tissues or their destruction with cytotoxic agents or by cryotherapy.[2] The immunomodulator imiquimod (Aldara, 3M) is also widely used for the treatment of external genital warts.[3] However, there is a need for virus-specific drug therapy.[4]

HPV are small, non-enveloped viruses containing a double-stranded covalently closed circular DNA genome (approximately 8 kb in length) that encodes five well-characterized early gene products and two late capsid proteins.[5] Only two virally encoded proteins, E1 and E2, are required for replication of the genome.[6] E2 is a sequence-specific DNA-binding protein that is required for transcription and replication of the viral genome as well as its segregation during mitosis.[7] E1 is the replicative helicase of papillomaviruses.[8] E2 binds with high affinity to specific sites in the viral origin of DNA replication and, by forming a complex with E1, enhances binding of E1 specifically to the origin. Formation of this complex between E1, E2 and origin DNA is an essential step in HPV DNA replication as is needed for the assembly of larger oligomeric E1 complexes that have helicase activity. This activity is required during the initiation and elongation phases of DNA replication to unwind the origin as well as the DNA ahead of the replication fork.[6] Once bound to the origin, E1 and E2 also interact with components of the host DNA replication machinery, including the polymerase α primase complex and human single-stranded binding protein (hSSB/RPA), to promote replication.[6] The essential roles of the E1 and E2 in HPV DNA replication make them attractive targets for antiviral therapies.[4]

The lack of structural information on the E1 and E2 proteins from HPV type 11 led us to rely on high throughput screening for the identification of inhibitors. During the HTS campaign, we screened 140,000 compounds from our corporate collection, which includes combinatorial libraries, compounds from medicinal chemistry programs, as well as external acquired compounds. Approximately 1200 initial hits were obtained, however, after characterization and further profiling for selectivity we have identified only one lead structure. This paper will outline the initial discovery and early SAR optimization of the first inhibitors of the assembly

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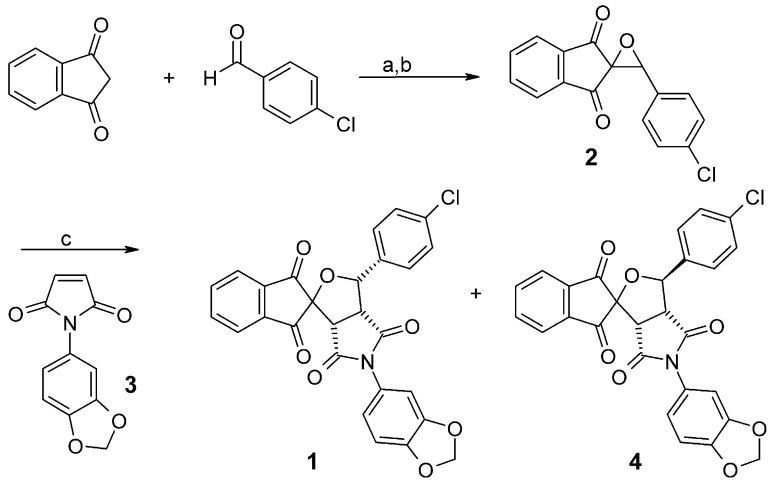
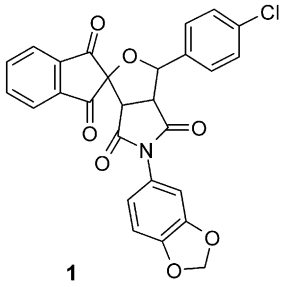
doi:10.1016/S0960-894X(03)00510-9



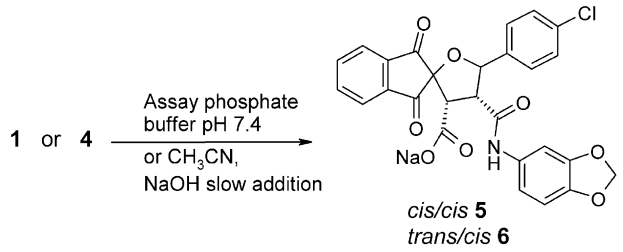
Scheme 1. (a) EtOH, piperidine (cat) (91%); (b) H_2O_2, NaOH, MeOH (95%); (c) toluene, reflux.

of the HPV11 E1–E2–origin ternary complex. Compound **1** represents our initial hit showing activity in an E2-dependent E1/DNA binding assay ($IC_{50} = 11$ μM).[9]



1

Confirmation of our initial hit structure as well as the relative stereochemistry of the substituent on the furan ring was accomplished via an independent synthesis. Synthesis of **1** was achieved based on a literature procedure[10] and is outlined in Scheme 1. Commercially available indan-1,3-dione was condensed with *p*-chlorobenzaldehyde in the presence of a catalytic amount of piperidine to give the corresponding benzylidene. Base-catalyzed oxidation with peroxide afforded epoxide **2**.[11] Epoxide **2** undergoes thermal 1,3-dipolar cycloaddition in the presence of the maleimide **3** to give, after purification, a racemic 1:1 mixture of the *cis*/*cis* isomer (**1**), and the *trans*/*cis* isomer (**2**). Comparison with these two synthetic isomers allowed us to confirm by NMR that the initial hit has the all *cis* relative stereochemistry. In addition, the *trans*/*cis* isomer **4** was found not to be an inhibitor ($IC_{50} > 80$ μM).



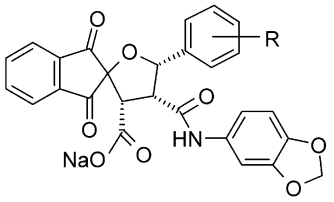
Scheme 2.

In early studies to address the stability of our hit in the assay buffer, it was found that compound **1** is rapidly converted to a single product. This product was identified as the hydrolyzed compound **5**, which was also obtained after treatment of **1** with sodium hydroxide under slow addition conditions. Similarly, the *trans*/*cis* isomer (**4**) was converted to the corresponding sodium salt under dilute conditions as shown in Scheme 2.

The syntheses of all compounds were carried out according to Schemes 1 and 2. Satisfactory MS, ^1H NMR, IR, and homogeneity data (>95% by HPLC) were obtained for all inhibitors. The biochemical assay used for screening was reported previously.[9] All IC_{50} values reported are the average of at least three separate determinations.

Having confirmed that the active form of our hit was indeed represented by compound **5**, with a 7.8 μM activity, and that the relative stereochemistry was important as shown by the inactivity of **6** (IC_{50} >80 μM), we undertook our initial SAR study. We first turned our attention to the phenyl bearing the chlorine atom. As shown in Table 1, removal of the chlorine atom resulted in a loss of approximately 10-fold in potency (compound **7** vs **5**). A further decrease in activity was observed when the chlorine is at the *ortho*

Table 1.



Compd	R	IC_{50} (μM)
5	4-Cl	7.8
7	H	71
8	2-Cl	>80
9	3-Cl	2.6
10	3,4-Cl_2	0.35

position (compound **8**, $IC_{50} > 80$ μM). However a 3-fold increase in potency was observed with a *meta*-chloro substituent (compound **9**). Furthermore, it was gratifying to observe that the beneficial effects were additive. Indeed, the 3,4-dichlorocompound (**10**) shows a 10-fold improvement in activity over our original hit ($IC_{50} = 0.35$ μM).

In studies to be published elsewhere,[12] this series of compounds was found to be devoid of any DNA intercalating activity and to be inactive in unrelated biochemical assays of protein–DNA interactions, suggesting that they are specific inhibitors of HPV11 E1 and/or E2. It was also found that our best compound is able to inhibit HPV DNA replication in a surrogate cellular assay.[12] However, because HPV or other papillomaviruses can not be propagated easily in vitro, there is no assay to evaluate the antiviral activity of this series of compounds.

In summary, the first series of inhibitors capable of antagonizing the assembly of the HPV11 E1–E2–origin DNA complex have been described. They incorporate an indandione system spiro-fused to a substituted tetrahydrofuran ring. Future publications will describe the full SAR of this series of inhibitors and will address their exact mechanism of action.

Acknowledgements

We are grateful to C. Boucher and S. Valois for their excellent analytical support. We extend our gratitude to Michael Bös for his support to the program.

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