Discovery of Small-Molecule Inhibitors of the ATPase Activity of Human Papillomavirus E1 Helicase

Anne-Marie Faucher,* Peter W. White, Christian Brochu, Chantal Grand-Maître, Jean Rancourt, and Gulrez Fazal

Boehringer Ingelheim (Canada) Ltd., Research and Development, 2100 Cunard Street, Laval (Québec), Canada, H7S 2G5

Received September 30, 2003

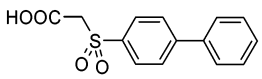
Abstract: The Boehringer Ingelheim compound collection was screened for inhibitors of the ATPase activity of human papillomavirus E1 helicase to develop antiviral agents that inhibit human papillomavirus (HPV) DNA replication. This screen led to the discovery of (biphenyl-4-sulfonyl)acetic acid **1**, which inhibits the ATPase activity of HPV type 6 E1 helicase with a low micromolar IC_{50} value. A hit-to-lead exercise rapidly converted **1** into a low nanomolar lead series.

Introduction. Papillomaviruses are small, nonenveloped DNA viruses that infect and replicate in the cutaneous or mucosal epithelia of human and other mammals.[1] There are over 80 types of human papillomavirus (HPV), which cause conditions ranging from plantar (HPV1) and genital warts (HPV6 and -11) to cervical cancer (HPV16, -18, and -31). HPV6 and -11 are also responsible for laryngeal papillomatosis, a rare but very serious infection of the respiratory tract. HPV6 is responsible for the majority of cases of genital warts.[2] Antiviral agents capable of specifically inhibiting PV replication could play an important role in the treatment of these diseases, but unfortunately no such antiviral agent exists at the present time.

Despite the host and tissue specificity of PV types, they all share a common genomic organization. The circular DNA genome of ~8000 base pairs codes for only 10 proteins: eight early proteins termed E1−E8 and two late proteins L1 and L2, which make up the viral capsid. E1, a DNA helicase, is the only PV protein that possesses enzymatic activity[3] and is the most highly conserved of the PV proteins. For these reasons, the E1 helicase has been considered the most attractive molecular target for the development of antiviral agents.

This paper reports the discovery of a small-molecule series inhibiting the ATPase activity of HPV6-E1 helicase and the hit-to-lead exercise that was performed to evaluate its value and potential for further optimization.

Results and Discussion. With the aim of discovering anti-HPV agents, high-throughput screening of the Boehringer Ingelheim compound collection was performed using a novel scintillation proximity assay measuring the ATPase activity of recombinant HPV6 E1 helicase.[4,5] This screen revealed a leadlike hit,[6] (biphenyl-4-sulfonyl)acetic acid **1**, which binds reversibly to E1 through a hyperbolic competitive kinetic mechanism[7] such that binding of the compound decreases the affinity of the enzyme for ATP but binding of the inhibitor and ATP are not mutually exclusive. The specificity of this hit was demonstrated by the fact that it does not inhibit the ATPase activity of the unrelated herpes simplex virus helicase primase[8] or the activities of other unrelated enzymes (data not shown).



1

IC_{50} (HPV6) = 2.0 μM
IC_{50} (HSV) > 80 μM
K_i (HPV6) = 2.0 μM

Unfortunately, to date, there are no structural data available on HPV E1.[9] Therefore, to evaluate the potential of **1** for further optimization, we decided to rely on a two-step hit-to-lead exercise. The first step involved making a minimal number of meaningful structural modifications to prospect the entire structure. The second step involved a more in-depth analysis of the most permissive areas to reveal exploitable binding pockets or sites for further optimization.

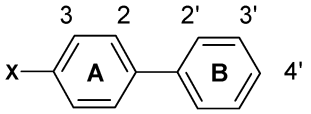
In the prospecting exercise (Table 1), we surveyed two regions of **1**: the sulfonylacetic acid (X) and the biphenyl (Ar). Starting with the sulfonylacetic acid moiety, truncation of the carboxyl group (**2a**, entry 2) or replacement by a primary amide (**2b**, entry 3) suppressed the activity. Replacement of the carboxylic group by other isosters such as tetrazolyl (**2c**, entry 4), nitro (**2d**, entry 5), sulfonate (**2e**, entry 6), or phosphonate (**2f**, entry 7) significantly reduced potency. The sulfone moiety was found to be as important as the carboxylic acid group. Altering the oxidation state at sulfur was detrimental to potency; sulfoxide **2g** and thioether **2h** analogues were both inactive (entries 8 and 9). Substitution at the α carbon with a fluoride (**2i**, entry 10) or a methyl group (**2j**, entry 11) reduced potency while *gem*-dimethyl substitution gave an inactive analogue (**2k**, entry 12). Spacing the sulfone and the carboxylate group with an extra methylene (**2l**, entry 13) also reduced potency. In summary, the sulfonylacetic acid moiety was not tolerant to modifications and did not offer the expected handle for further optimization.

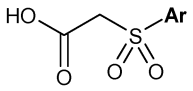
Next, modification of the biphenyl system was undertaken. Phenyl ring B was replaced by five-membered heterocyclic rings such as 2- and 4-thiazolyl (**3a** and **3b**, entries 14 and 15), but these compounds had significantly reduced potency. Spacing phenyl ring B with various tethers disturbed binding to E1 significantly (**3c**−**f**, entries 16−19). Changing the position of the linkage of ring B to ring A was detrimental (**3g**, entry 20), and fusing the two rings into a 2-naphthyl (**3h**, entry 21) also significantly reduced potency. Attempts at replacing phenyl ring A with heterocycles were unsuccessful because of the lability of the resulting sulfonylacetic acid compounds.[10] Conservative substitutions of ring B such as those presented in **3i**−**l** (entries 22−25) led to compounds that were more or less equipotent to our initial hit and revealed an area in the 3′ and 4′ positions that was permissive to substitution and may potentially lead to more interactions with E1.

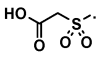
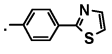
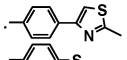
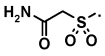
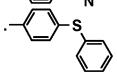
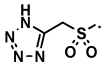
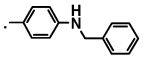
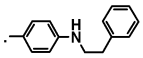
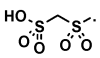
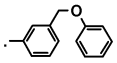
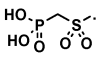
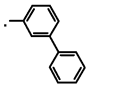
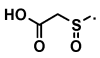
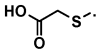
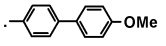
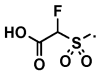
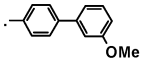
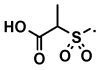
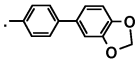
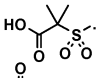
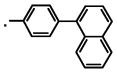
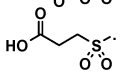
* To whom correspondence should be addressed. Phone: (450) 682-4640. Fax: (450) 682-4189. E-mail: amfaucher@lav.boehringer-ingelheim.com.

10.1021/jm034206x CCC: $27.50
Published on Web 12/09/2003

Table 1. Preliminary SAR Study

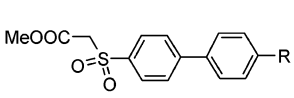




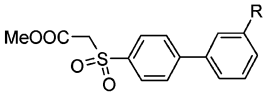
Entry	Compd no.	X	IC_{50} (μM)[a]	Entry	Compd no.	Aryl	IC_{50} (μM)
1	**1**		2.0	14	**3a**		20
2	**2a**		>80	15	**3b**		30
3	**2b**		>80	16	**3c**		>80
4	**2c**		63	17	**3d**		69
5	**2d**		65	18	**3e**		67
6	**2e**		32	19	**3f**		>80
7	**2f**		30	20	**3g**		>80
8	**2g**		>80	21	**3h**		40
9	**2h**		>80	22	**3i**		1.3
10	**2i**		6.5	23	**3j**		4.0
11	**2j**		17	24	**3k**		1.3
12	**2k**		>80	25	**3l**		5.4
13	**2l**		72				

[a] For procedure for IC_{50} determination, see ref 4.

At the completion of the first step of this hit-to-lead exercise, preliminary SAR had been demonstrated and the existence of permissive sites had been revealed by the fact that substituents were tolerated at the 3′ and 4′ positions. In the second step, a parallel synthesis approach was used to rapidly verify the presence of a putative binding pocket with the hope of finding a more potent lead series.



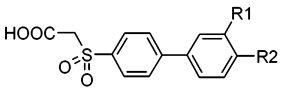
4 R = COOH
5 R = CH_2COOH

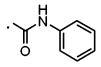
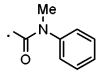
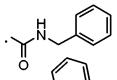
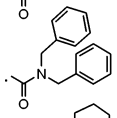
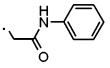
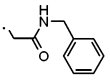
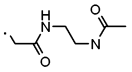
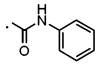
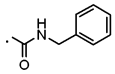
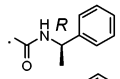
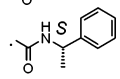
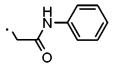
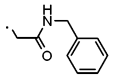
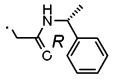
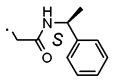


6 R = COOH
7 R = CH_2COOH

In preparation for our library, various possible functional groups for the 3′ and 4′ positions were considered. We favored functional groups that could be introduced via a short synthesis and for which the diversification step would be late in the sequence of reactions. Ultimately, we selected the 3′- and 4′-carboxamide and acetamide functionalities. Amide bond formation is easily amenable to parallel synthesis, and a large number of structurally diverse amine building blocks are readily available. A set of commercially available diverse amines were selected.[11] Four building blocks bearing an acid function were prepared (**4**−**7**) and four libraries of 25−50 amides were generated to provide over 100 diverse analogues with IC_{50} values ranging from 0.04 to >80 μM. Table 2 shows a sample of the results for each library. An increased potency was achieved with many members of these prospecting libraries. In general, amides derived from primary amines bearing an aromatic lypophilic substituent such as a phenyl or a benzyl group gave the best results. The most potent analogues belonged to the 3′-substituted series **6** and **7**. The SAR observed within these two series of compounds (entries 10−18), particularly where enantiomers show striking differences in IC_{50} (compare **6d**, **6e**, **7c**, and **7d**, entries 13, 14, 17, and 18), provided a convincing argument in favor of the existence of a well-defined binding pocket. To further follow-up on the best results, an additional set of amine building blocks was selected to make two focused libraries of about 75 members each in the **6** and **7** series. Most of the inhibitors in these libraries had submicromolar IC_{50} values, a few of them being over 400-fold more potent than **1** (Table 3). The most gratifying results were obtained with the 3′-carboxamide series **6** where 1-(*R*)-naphthalenylethyl **6f** and *N*-1-naphthalenylmethyl **6g**

Table 2. Sample of Diverse Libraries



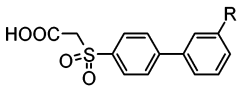
Entry	Compd no.	R1	R2	IC_{50} (μM)[a]
1	**1**	H	H	2.0
2	**4a**	H		1.3
3	**4b**	H		4.8
4	**4c**	H		0.51
5	**4d**	H		1.1
6	**4e**	H		3.3
7	**5a**	H		0.16
8	**5b**	H		0.34
9	**5c**	H		1.9
10	**6a**		H	22
11	**6b**		H	0.42
12	**6c**		H	0.087
13	**6d**		H	0.043
14	**6e**		H	1.7
15	**7a**		H	0.11
16	**7b**		H	0.30
17	**7c**		H	0.32
18	**7d**		H	0.087

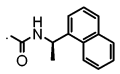
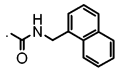
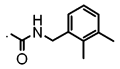
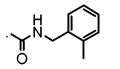
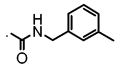
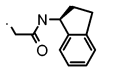
[a] For procedure for IC_{50} determination, see ref 4.

had over a log reduction in IC_{50} compared to their corresponding analogues **6d** and **6c**.

It was clear from these SAR results that this series of inhibitors could be optimized for potency against the in vitro ATPase activity of HPV6 E1. However, prior to any further optimization in potency, we set out to address two liabilities of this series that we associated with the sulfonylacetic acid pharmacophore. The first liability was the absence of activity in a cell-based assay of HPV DNA replication.[12] This is presumably in part

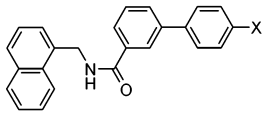
Table 3. Best Results from Focused Libraries

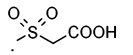
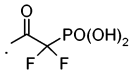
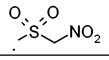


Entry	Compd no.	R	IC_{50} (μM)[a]
1	**6f**		0.0045
2	**6g**		0.0043
3	**6h**		0.0092
4	**6i**		0.032
5	**6j**		0.027
6	**7e**		0.020

[a] For procedure for IC_{50} determination, see ref 4.

Table 4. Sulfonacetic Acid Surrogates



Entry	Compd no.	X	IC_{50} (μM)[a]
1	**6g**		0.0043
2	**8a**		>80
3	**8b**		0.74
4	**8c**		0.63

[a] For procedure for IC_{50} determination, see ref 4.

because of the highly polar negatively charged group that could limit the permeability of these inhibitors.[13] Alternatively or in addition, the lack of cellular activity could also be attributed to a higher effective intracellular concentration of ATP compared to that in the ATPase assay.[5,7c] The second liability that needed to be addressed was the instability of the sulfonylacetic acid group, which under certain conditions undergoes decarboxylation (see chemistry section of Supporting Information) to afford the corresponding inactive methyl sulfone (e.g., **2a** (entry 2) in Table 1 and **8a** (entry 2) in Table 4). In the context of drug discovery, this last finding seriously compromised the future of this series. Therefore, a search for a more acceptable surrogate to the sulfonylacetic acid group was initiated with the primary goal of improving stability while maintaining IC_{50} values at the submicromolar level. We reasoned that improvement in cellular permeability could be achieved either by identifying a more cell-permeable pharmacophore or later by subsequent modifications of a polar but stable pharmacophore using a prodrug approach.

To replace the sulfonylacetic acid moiety, several known carboxylate and phosphate isosteres were screened. Two surrogates, ketodifluoromethylphosphonic acid derivative **8b** and nitromethyl sulfone **8c**, exhibited the desired level of potency (Table 4, entries 3 and 4). One of the two, the nitromethyl sulfone **8c**, was moderately permeable[14] but not potent enough to show cell-based activity. Nevertheless, **8b** and **8c** constitute excellent starting points for lead optimization.

Conclusion. The hit-to-lead exercise presented herein led to the discovery of low nanomolar inhibitors of the ATPase activity of HPV6 E1 helicase. Preliminary SAR studies have revealed a permissive area of inhibitor **1**, which suggested the existence of an exploitable binding site or pocket in HPV6 E1. Parallel synthesis of diverse libraries produced analogues with improved potency compared to the initial hit **1** supporting the hypothesis of an additional binding site. Subsequent focused libraries were generated, producing analogues such as **6g**, which is nearly 500-fold more potent than **1**. In addition, the sulfonacetic acid pharmacophore could be replaced with more stable surrogates such as a ketodifluoromethylphosphonic acid or a nitromethyl sulfone. Future work should focus on further optimization of the new surrogates for binding to E1 and improvement of the overall biopharmaceutical profile of this lead series.

Acknowledgment. The authors are grateful to the following colleagues for their support of the project, their help in the preparation of the manuscript, and their valuable assistance in the preparation and characterization of analogues: Jacques Archambault, Paul Anderson, Michael Bös, Michael Cordingley, Pierre Lavallée, Jianmin Duan, Nathalie Goudreau, Araz Jakalian, Marc-André Poupart, Kimberley Thompson, Norman Aubry, Marie-Eve Beaudoin, Sylvain Bordeleau, Colette Boucher, Karine Brault, Michel Garneau, Michael Little, Miguel Nantel, Serge Valois, Ewald Welchner, and Dominik Wernick.

Supporting Information Available: Figures showing a sample list of amine building blocks used in libraries and additional sulfonylacetic acid replacements tested; chemistry section containing synthetic description, schemes, and additional references; experimental procedures and characterization of all compounds described in this paper. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Howley, P. M. Papillomavirinae: The Viruses and Their Replication. In *Fields Virology*, 3rd ed.; Field, B. N., Knipe, D. M., Howley, P. M., Eds. Lippincott-Raven: Philadelphia, PA, 1996; pp 2045−2076 and references therein. (b) Shah, K. V.; Howley, P. M. Papillomaviruses. In *Fields Virology*, 3rd ed.; Field, B. N., Knipe, D. M., Howley, P. M., Eds.; Lippincott-Raven: Philadelphia, PA, 1996; pp 2077−2109 and references therein.

(2) Brown, D. R.; Bryan, J. T.; Cramer, H.; Fife, K. H. Analysis of Human Papillomavirus Types in Exophytic Condylomata Acuminata by Hybrid Capture and Southern Blot Techniques. *J. Clin. Microbiol.* **1993**, 2667−2673.

(3) Yang, L.; Mohr, I.; Fouts, E.; Lim, D. A.; Nohaile, M.; Botchan, M. The E1 Protein of Bovine Papillomavirus 1 Is an ATP-Dependent DNA Helicase. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5086−5090.

(4) IC_{50} values were determined at room temperature using 3.5 nM HPV6 E1 and serial 2-fold dilutions of inhibitors in a buffer containing 1 μM ATP with a trace amount of [γ-^{33}P]ATP and 500 μM $Mg(OAc)_2$ at pH 7.5. (a) Jeffery, J. A.; Sharom, J. R.; Fazekas, M.; Rudd, P.; Welchner, E.; Thauvette, L.; White, P. W. An ATPase Assay Using Scintillation Proximity Beads for High-Throughput Screening or Kinetic Analysis. *Anal. Biochem.* **2002**, *304* (1), 55−62. (b) White, P. W.; Pelletier, A.; Brault, K.; Titolo, S.; Welchner, E.; Thauvette, L.; Fazeka, M.; Cordingley, M.; Archambault, J. Characterization of Recombinant HPV6 and 11 E1 Helicases. *J. Biol. Chem.* **2001**, *276* (25), 22426−22438.

(5) The ATPase assay used in the screen and for the determination of IC_{50} presented in this report is carried out using an ATP concentration (1 μM) well below the K_m (12 μM)[4b] to maximize its sensitivity to inhibitors.

(6) (a) Rishton, G. M. Nonleadlikeness and Leadlikeness in Biochemical Screening. *Drug Discovery Today* **2003**, *8* (2), 86−96. (b) Teague, S. J.; Davis, A. M.; Leeson, P. D.; Oprea, T. The Design of Leadlike Combinatorial Libraries. *Angew. Chem., Int. Ed.* **1999**, *38* (24), 3743−3747.

(7) (a) A detailed account of the experiments performed to clarify the mechanism of inhibition of the compounds in this series will be published separately. White, P. W.; et al. Manuscript in preparation. (b) Segel, I. H. *Enzyme Kinetics*; John Wiley & Sons: New York, 1975; pp 161−226. (c) As a consequence of the hyperbolic competitive mechanism, compounds in this series are less active at higher ATP concentration. For example, IC_{50} values are approximately 10-fold higher at 50 μM ATP.

(8) Crute, J.; Grygon, C. A.; Hargrave, K. D.; Simoneau, B.; Faucher, A.-M.; Bolger, G.; Kibler, P.; Liuzzi, M.; Cordingley, M. G. Herpes Simplex Virus Helicase-Primase Inhibitors are Active in Animal Models of Human Disease. *Nat. Med.* **2002**, *8* (4), 386-391.

(9) Recently, an X-ray structure of the helicase domain of SV40 large T antigen (a helicase belonging to the same superfamily as HPV E1 but with very low homology) has been published: Li, D.; Zhao, R.; Lilyestrom, W.; Gai, D.; Zhang, R.; DeCaprio, J. A.; Fanning, E.; Jochimiak, A.; Szakonyi, G.; Chen, X. Structure of the Replicative Helicase of the Oncoprotein SV40 Large Tumor Antigen. *Nature* **2003**, *423*, 512−518.

(10) Presumably due to a fragmentation reaction analogous to that of the Julia coupling of alkylsulfonylbenzothiazolyl to aldehydes. Baudin, J. B.; Hareau, G.; Julia, S. A. A Direct Synthesis of Olefins by Reaction of Carbonyl Compounds with Lithio Derivatives of 2-[Alkyl- or (2′-Alkenyl)- or Benzyl-sulfonyl]-benzothiazoles. *Tetrahedron Lett.* **1991**, *32* (9), 1175−1178.

(11) Diverse amines were selected using the "Molecular Operating Environment" MOE software available from Chemical Computing Group Inc., Montréal, Canada: http\www.chemcorp.com. See Supporting Information for a sample of the amine building blocks used in this library.

(12) Assay described in the following. White, P. W.; Titolo, S.; Brault, K.; Thauvette, L.; Pelletier, A., Welchner, E.; Bourgon, L.; Doyon, L.; Ogilvie, W. W.; Yoakim, C.; Cordingley, M. G.; Archambault, J. Inhibition of Human Papillomavirus DNA Replication by Small Molecules Antagonists of the E1−E2 Protein Interaction. *J. Biol. Chem.* **2003**, *278* (29), 26765−26772.

(13) The sulfonylacetic acid methyl ester prodrugs were inactive in the cell-based assay, but their low aqueous solubility limited the concentration at which they could be tested to 1−10 μM.

(14) A to B permeability of 5×10^{-6} cm/s in Caco2 monolayer assay modified (cell density of 125 000/cm^2 in 12-well plates from Costar no. 3401) from the following. Liang, E.; Proudfoot, J.; Yazdanian, M. Mechanisms of Transport and Structure−Permeability Relationship of Sulfasalazine and Its Analogs in Caco-2 Cell Monolayers. *Pharm. Res.* **2000**, *17* (10), 1168−1174.

JM034206X