Second Generation Inhibitors of HIV-1 Maturation

Alicia Regueiro-Ren,^{*,†} Ira B. Dicker,[§] Umesh Hanumegowda,[§] and Nicholas A. Meanwell^{*,†}[†]Department of Discovery Chemistry and Molecular Technologies Bristol-Myers Squibb Research and Development, PO Box 4000, Princeton, New Jersey 08543-4000, United States[‡]Department of Discovery Chemistry and Molecular Technologies Bristol-Myers Squibb Research and Development, 350 Carter Road, Room 126, Hopewell, New Jersey 08540, United States[§]ViiV Healthcare, 36 East Industrial Road, Branford, Connecticut 06405, United States

ABSTRACT: The strategy and tactics subtending the discovery and development of the second generation HIV-1 maturation inhibitor GSK-3532795/BMS-955176, a compound that exhibits a broader spectrum of antiviral effect in vitro and in clinical studies than the prototypical maturation inhibitor bevirimat, are described.

KEYWORDS: GSK3532785, BMS-955176, HIV-1, maturation inhibitor, triterpenoid

The development of the first HIV-1 protease inhibitor (PI) saquinavir (1), which was licensed by the FDA on December 6th, 1995, ushered in the era of drug combinations that have evolved into the highly active antiretroviral therapy (HAART) regimens that have become the contemporary standard for effectively controlling HIV-1 infection.^{1,2} In the ensuing 10 years, nine additional HIV-1 PIs were licensed, and this structural class of antiretroviral agent has become an important component of clinical therapy. HIV-1 PIs interfere with the cleavage of the viral Gag-Pol proteins, an essential and critical step in the release of both viral enzymes and the proteins that associate to form the virus capsid. The discovery of potent HIV-1 PIs was based on the design of transition state mimics that was facilitated by X-ray crystal structure data of the dimeric enzyme.¹ However, a phenotypic screen identified bevirimat (2), a relatively simple derivative of the naturally occurring triterpene betulinic acid (3), as a potent inhibitor of HIV-1 replication in cell culture that was subsequently determined to selectively interfere with cleavage of the spacer peptide 1 (SP1) from the C-terminus of the capsid protein (CA).^{3–5} This is the final step in the HIV-1 protease-mediated dissection of the Gag polyprotein that releases CA, which then engages in a complex sequence of events involving self-assembly into hexamers and pentamers that associate into higher order oligomers to produce the mature viral capsid. In contrast to the PIs, 2 appears to act by binding to the CA-SP1 substrate, thereby restricting access of HIV-1 protease to the site of cleavage, which occurs between Leu₃₆₃ and Ala₃₆₄ (Figure 1).^{6,7} This is the final and rate-determining cleavage step in Gag processing, and inhibition compromises the formation of the mature HIV-1 capsid, resulting in the production of virions that are noninfectious, with the defects affecting the uncoating process following viral entry. While the antiviral efficacy of 2 was established in several clinical trials, these studies also revealed that a significant (~50%) patient

Figure 1. Structure of the HIV-1 Gag-Pol protein and details associated with the sequence of the CA-SP1 cleavage site. The polymorphisms and deletions that attenuate the antiviral activity of 2 are highlighted in red.

population was unresponsive to the drug, ultimately traced to several naturally occurring polymorphisms in the Gag protein that were located proximal to the CA-SP1 cleavage site and that compromised the antiviral activity of the compound in vivo and in cell culture systems.^{8–10} More specifically, Val₃₆₂Ile, Gln₃₆₉His, Val₃₇₀Ala, Val₃₇₀Met, and Thr₃₇₁Ala mutations or deletions of Val₃₇₀ and Thr₃₇₁, which collectively amount to 49% of the subtype B virus sequences collated in the Los Alamos National Laboratory (LANL) database, resulted in significantly reduced sensitivity to 2. Further complicating the challenges of developing 2 as a clinically useful antiretroviral agent were its extensive binding to plasma proteins (>99%), which restricted the free fraction and contributed to high dosing requirements, and poor aqueous solubility (<1 μg/mL), which manifested as dissolution-limited oral bioavailability, requiring custom formulations that prevented use in fixed dose drug combinations.¹¹

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Table 1. Comparison of the Antiviral Properties of **2** and Benzoic Acid Derivatives **4–9**^a

Compd #	R	WT EC ₅₀ (nM)	WT EC ₅₀ (nM) in the presence of HSA (fold change)	Val ₃₇₀ Ala EC ₅₀ (nM)	ΔVal ₃₇₀ EC ₅₀ (nM)	CC ₅₀ (μM)	6 hour rat pharmacokinetic screening data AUC (nM.h)
2		10	974 (97x)	553	1,900	16.8	6,390
4		16	150 (9.4x)	233	>3,000	27.2	5,724
5		>8000	NT	NT	NT	14	NT
6		55	50 (1)	1400	NT	16	NT
7		114	117 (1)	>4,000	NT	17	NT
8		613	660 (1)	3,900	NT	7	NT
9		38	NT	212	NT	>20	NT

^aNT = not tested.

As part of a broader program directed toward the development of mechanistically novel antiretroviral agents designed to address the emergence of resistance to existing agents, we developed an interest in HIV-1 maturation inhibition based on the demonstrated clinical efficacy of **2** and the emerging understanding of its shortcomings.^{8–10} The identification of a compound suitable for both queue die (QD) dosing and formulation in fixed dose combinations that was capable of inhibiting >90% of B clade viruses was considered to be essential in order to provide a candidate with a broader clinical application that would not require prescreening of patients for virus susceptibility. At the outset of the program, no specific structure–activity studies had been

described toward optimizing the antiviral profile of **2** to capture the inherently resistant polymorphic viruses; indeed, there was no published evidence to suggest that structural manipulation of the compound would be able to achieve a broader spectrum of HIV-1 inhibition.^{3,12} Fortunately, this aspect of the profile of **2** was readily assessed in a preclinical setting by including the polymorphic Val₃₇₀Ala and ΔVal₃₇₀ viruses as sentinels in the initial profiling triage as a complement to wild-type (WT) virus. Against this backdrop, the burden of providing a solution to the problem fell to the medicinal chemistry team, which adopted an approach to compound design that sought to develop a broader and deeper understanding of the underlying pharmacophore and its topographical arrangements. It was hoped that the development of insights into new aspects of structure–activity relationships (SARs) would evolve into an approach to inhibit viruses harboring the clinically relevant polymorphs and deletions.

The carboxylic acid-containing C-3 side chain of **2** is a critical determinant of HIV-1 maturation inhibition, and early studies of this structural element had provided only limited insight into the boundaries associated with the expression of antiviral activity.^{3,12} All of the existing SAR studies had focused on compounds derived by esterification of the C-3 alcohol of **3** that collectively imparted little information on the preferred spatial relationship between the pharmacophoric CO₂H

moiety at C-3 and the triterpenoid core in **2**, although the presence of the gem-dimethyl moiety might be anticipated to confer a Thorpe–Ingold-type influence on the relative conformation of the terminus.¹³ Modification of the C-28 carboxylic acid element, the only other site of the molecule that offered convenience for structural manipulation, had also been examined only sparsely, while modest molecular editing of the iso-propenyl moiety was known to be compatible with HIV-1 inhibition.^{3,12} Interestingly, however, the E ring had been shown to be tolerant of more substantial structural variation.¹⁴ With this scenario as the background, the initial phase of the program was dedicated to exploring the effect on antiviral activity of structural motifs specifically designed to probe aspects of the critical C-3 substituent. Among several concepts given consideration, a series of carbon-linked, carboxylic acid-containing C-3 elements were conceived as potential targets from which the *para*-benzoic acid derivative **4** emerged as the first compound to be prepared in the program (Table 1).¹⁵ Remarkably, **4** demonstrated antiviral activity toward WT HIV-1 with potency that was comparable to **2** and, surprisingly given the then emerging beliefs around the effect of introducing phenyl rings on compound quality and developability, was much less affected by the presence of human serum, with just a modest 10-fold shift in the EC₅₀ value in contrast to the 900-fold shift measured for **2**.^{15,16} The meta-substituted isomer **5** was found to be a significantly less potent antiviral agent, a result that provided an important initial glimpse into the preferred topographical relationship between the C-3-based CO₂H moiety and the triterpenoid core. Additional informative insight was gleaned from screening the short series of analogues **6–8** in which the substitution pattern was elaborated at the aromatic ring carbon directly adjacent to the junction with the triterpene ring system. The antiviral potency of **4** and **6–8** correlated nicely with the A-value of the substituent, a relationship captured graphically in Figure 2.^{15,17} These results are consistent with

Figure 2. Plot of antiviral potency versus A-value for **4** and **6–8**.

allylic 1,3-strain influencing the conformation of **6–8** and suggested that the phenyl ring of **4** prefers to adopt an overall planar relationship with the core olefin rather than a conformation that approaches orthogonality. One additional interesting and perhaps surprising observation from this phase of the program was the antiviral activity associated with the diacid **9**, which hybridizes elements of **4** and **5** but largely preserves the potency of the former compound.¹⁵

The singular nature of the discovery of **4** was further underscored by the results of an extensive survey of structural variants around the benzoic acid moiety that explored modifications to the phenyl ring, replacement of both the CO₂H and phenyl ring with elements that might function as

bioisosteres, as well as alterations to the topographical relationships, all of which failed to identify more compelling scaffolds with advantageous properties that could be viewed as vehicles suitable for further structural manipulation.¹⁵

The pharmacokinetic (PK) profile of **4** in the rat compared favorably with that of **2** with a similar AUC (Table 1), a 2-fold lower C_{max} (1314 nM for **4** compared to 2587 nM for **2**) and lower clearance (1 mL/min/kg for **4** compared to 4 mL/min/kg for **2**) following oral dosing at 5 mpk and intravenous dosing at 1 mpk.¹⁵ This result reinforced the importance of the discovery, and as a consequence, the *para*-substituted benzoic acid motif was adopted as the vehicle for further study. The next phase of structural manipulation focused on modifications at the C-28 carboxylic acid moiety in an effort to broaden the antiviral activity toward encompassing the key polymorphic viruses that were not addressed by **4** (Table 1).¹⁸ The synthesis and evaluation of an extensive series of C-28 amides revealed that the introduction of basicity to this region of the molecule was associated with extending antiviral inhibitory activity to encompass the Val₃₇₀Ala Gag polymorphic virus while further helping to attenuate the often deleterious effects of the presence of human serum on antiviral potency. The SAR observations made with **10** and **11** that are presented synoptically in Figure 3 are illustrative, with the basic amine of **10** conferring enhanced potency toward WT, Val₃₇₀Ala, and ΔVal₃₇₀ viruses when compared to the nonbasic, matched carbon analogue **11**. However, the PK profile of **9** in the rat was found to be relatively poor, with a plasma AUC of 1623 nM·h measured over 6 h following an oral dose of 5 mg/kg, 4-fold lower than for **2**, and an oral bioavailability of 17%.¹⁸ By way of contrast, the β-alanine derivative **12** offered a much improved rat PK profile, with an oral bioavailability of 71% and a plasma AUC of 43 159 nM·h measured over 6 h, an almost 7-fold improvement when compared to **2**. Unfortunately, the antiviral profile of **12** was inferior to both **10** and **11**, with poor activity toward the Val₃₇₀Ala and ΔVal₃₇₀ viruses and a large deleterious effect of the presence of human serum on the EC₅₀ value (Figure 3). Fortunately, this SAR survey revealed that a basic amine was not an absolute requirement for inhibitory activity toward the polymorphic viruses since the bis-amide **13** offered an encouraging in vitro profile that was suggestive of opportunity for broader structural variation. The combination of both an amine and an acid component in the C-28 amide side chain was compatible with the targeted antiviral spectrum, as exemplified by **14**, although activity exhibits sensitivity to the pK_a of the amine since the more basic arrangement found in **15** is less potent. However, while the tactic of structural hybridization was promising with respect to expanding the antiviral effect, the PK profile of **14** in rats was very poor, with a plasma AUC of just 220 nM·h measured over 6 h following oral administration of a dose of 5 mg/kg, an exposure value that was 30-fold lower than for **2**.¹⁸

As the effort continued toward trying to resolve the conundrum posed by combining targeted antiviral activity with good oral exposure, the effect of incorporating basicity closer to the triterpenoid core was explored.¹⁹ This concept was evaluated initially in the context of a series of C-28-based amine derivatives, with a synopsis of the key compounds prepared captured in Figure 4. While the simple ethylene diamine derivative **16** conferred much of the sought after antiviral properties, it was incorporation of the thiomorpholine dioxide element in **17** and **18** that provided an important step forward for the program.¹⁹ This specific heterocycle was

Figure 3. SARs associated with the series of C-28-based amide derivatives **10–15**.

Figure 4. SARs associated with the series of C-28-based amine derivatives **16–20**.

selected as part of a focus on deploying amines with moderated basicity distal to the triterpenoid core that in the context of **17** and **18** provided high antiviral potency that was comparable to diamine **16**. The measured pK_a value of thiomorpholine 1,1-dioxide is 5.4, considerably lower than piperidine ($pK_a = 11.1$), 4,4-difluoropiperidine ($pK_a = 8.5$), and morpholine ($pK_a = 8.5$), the latter two being cyclic amine elements that are more commonly employed when attenuation of basicity is pursued.²⁰ The oral exposure of **18** in the rat was encouraging, with a plasma AUC of 1708 nM·h measured over 6 h following a 5 mg/kg dose, while that of the methylated homologue **19** was almost 3-fold higher at 4834 nM·h. Moreover, incorporation of thiomorpholine dioxide element in conjunction with a C-28 amide, compound **20**, also resulted in improved oral exposure, with a 6 h plasma AUC of 3986 nM·h that was almost 3-fold higher than that measured for the more basic dimethylamide analogue **5**. The antiviral and PK profiles associated with **19** and **20** were viewed through the lens of a structural compromise between the properties of the amine- and acid-containing series that married a modestly basic amine with a polar element that, while analogous to a carboxylic acid, was not burdened with an overt negative charge. This reflects a bioisosteric relationship between the thiomorpholine dioxide and β -alanine and glycine that is the basis of the conjecture presented in Figure 5, with overlap of the β -amino acid perhaps the more compelling structural metaphor.^{21,22}

Figure 5. Potential bioisosteric relationship between a thiomorpholine dioxide heterocycle and β -alanine and glycine.

However, while the antiviral activity of **19** and **20** was promising, the EC_{50} values of 26 and 60 nM, respectively, toward the ΔVal_{370} virus in the absence of human serum were higher than preferred; moreover, the oral exposure was considered to be less than optimal, with room for improvement based on exposure values that had been achieved with some of the earlier analogues in the series. Nevertheless, the observations with **19** provided an important insight that precipitated the concept implemented for the final round of molecular editing, a strategy that would lead to the identification of a compound that fulfilled the objectives of the program. Interpretation of the effects of the structural changes in **19** and **20** on their in vitro and in vivo profiles led to the suggestion that it would be beneficial to incorporate the amine element proximal to the core of the molecule so that the basic center would experience more effective lipophilic shielding. This led to the notion of attaching the amine directly to the triterpenoid core, a motif readily accessed synthetically by subjecting the C-28 carboxylic acid to a Curtius rearrangement.^{23,24} GSK-3532795/BMS-955176 (**21**)

emerged from this concept as a compound that met both the targeted virological and PK criteria, exhibiting a 6 h plasma AUC in rats of 14198 nM·h after a 5 mg/kg oral dose.^{23–25} The *ortho*-fluoro analogue **22** performed similarly to **21** in vitro although this molecule experienced a larger shift in the EC₅₀ value in the presence of human serum; however, this was offset by higher plasma exposure in the rat, with a plasma AUC of 25 465 nM·h over the 6 h postdosing period. Unfortunately, the plasma exposure of **22** in rats was subject to significant interanimal variability, attributed to dissolution problems rooted in the poor aqueous solubility of the molecule that diminished its potential to be given further consideration.^{23,24}

Development of GSK-3532795/BMS-955176 (21). A comprehensive evaluation of the PK profile of **21** in preclinical species is compiled in Table 2 and reveals a favorable

Table 2. Pharmacokinetic Parameters for 21 in Preclinical Species

species	Cl (mL/min/kg)	V _{ss} (L/kg)	t _{1/2} (h)	%F (solution)
mouse	1.1	0.82	8.9	27
rat	2.2	1.1	6.6	26
dog	0.13	0.43	31.7	9
cynomolgus monkey	1.9	0.36	8.6	4

pharmacokinetic disposition in the mouse, rat, dog, and cynomolgus monkey. Clearance was low across the species, ranging from 0.13 to 2.2 mL/min/kg, while the volume of distribution (0.36–1.1 L/kg) indicated adequate tissue distribution, parameters that contributed to a half-life ranging from 6.6 to 31.7 h.^{23,24} The low to moderate oral bioavailability of **21** (average = 16% in solution) is most likely associated with the poor aqueous solubility of the compound (<1 μg/mL), a persistently challenging physicochemical parameter in the program that was resistant to both manipulation of peripheral substituents and the introduction of polar elements into the triterpenoid core.^{23,24,26,27} However, **21** demonstrated adequate solubility in biologically relevant fluids that amounted to 0.28 mg/mL in fasted state simulated intestinal fluid (FaSSIF) and 1.02 mg/mL in fed state simulated intestinal fluid (FeSSIF). The combination of the C-3 benzoic acid element with the polar and a moderately basic C-28 amine resulted in much reduced binding to human serum proteins when compared with **2** (86.1% vs >99%), translating into a modest effect of the addition of human serum on antiviral potency in cell culture (Table 3). The data compiled in Table 3, which captures 96.5% of the HIV-1 subtype B viruses in the LANL database, demonstrates the

Table 3. Antiviral Profile of 21 Compared to 2

virus (HIV-1 NL _{4–3})	subtype B, % prevalence in LANL database ^a	EC ₅₀ (nM)	
		21	2
WT (NL _{4–3})	51	2	9
WT + serum ^b		10	1300
Val ₃₆₂ Ile	12	4	74
Gln ₃₆₉ His	2.4	2	8
Val ₃₇₀ Ala	15	3	553
Val ₃₇₀ Met	5	3	111
ΔVal ₃₇₀ ^c	0.6	13	>4000
ΔV ₃₇₀ /Thr ₃₇₁ Ala ^c	1.9	7	>4000
Thr ₃₇₁ Ala	5	3	10
ΔThr ₃₇₁	3	5	77

^aLos Alamos National Laboratory database, 2010. ^b40% human serum + 27 mg/mL human serum albumin. ^cSurrogate genotypes for subtype C. Data were determined using a reverse transcriptase-based assay.

improvements in the antiviral properties that are built into **21** compared to **2**. With EC₅₀ values of <15 nM toward all of the viruses compiled in Table 3, an efficacious human dose that would provide plasma levels at or above 3-fold the protein binding-adjusted EC₅₀ value for 24 h against the least sensitive virus (ΔVal₃₇₀) was predicted to be 120 mg QD using allometric scaling methodology.

The outcome of preclinical toxicological studies with **21** supported its advancement into clinical trials, where phase 1 studies conducted in normal healthy volunteers demonstrated a dose-related increase in drug exposure in plasma following oral dosing of a suspension formulation. This was a prelude to a phase 2a monotherapy study designed to assess the antiviral activity of **21** when administered once daily for 10 days at doses ranging from 5 to 120 mg to treatment-naïve and treatment-experienced HIV-1 infected patients.²⁸ At doses of 40 mg or more, plasma viral RNA in patients had declined by >1 log₁₀ by the end of the study period, and most importantly, the compound was effective in patients infected with both wild-type HIV-1 and viruses incorporating the polymorphisms that reduced sensitivity to **2**.²⁹ These data, which are compiled graphically in Figure 6, validated the preclinical screening strategy and reflect the predictive nature of the in vitro antiviral assays. In a 28 day trial in which **21** was administered to patients infected with HIV-1 subtypes B and C in conjunction with the HIV-1 protease inhibitor atazanavir (ATV), with or without the PK booster ritonavir (RTV), the 40–120 mg QD dose groups experienced viral load reductions that were comparable to patients treated with a combination of the nucleoside-based inhibitors tenofovir disoproxil fumarate (300 mg) and emtricitabine (200 mg) in combination with ATV/RTV (300 mg/100 mg).²⁸ The success of this trial was based, in part, on in vitro studies indicating that **21** maintains activity toward viruses that have developed resistance to HIV-1 protease inhibitors, with mutations emerging in response to exertion of selective pressure by **21** located in the Gag protein at the CA-SP1 junction.^{29,30} In addition, the results of the dual combination clinical trial of **21** and ATV over 28 days of therapy are also notable because efficacy is sustained by two drugs that target the same step in Gag polyprotein cleavage, although by orthogonal mechanisms.

The discovery and development of **21** relied upon several innovative elements that were critical to a successful outcome.

Figure 6. Plot of the maximum median change in HIV-1 RNA in plasma by baseline Gag polymorphism in HIV-1 infected patients after treatment with **21** for 10 days. *Baseline polymorphisms at Gag Val₃₆₂, Ala₃₆₄, Gln₃₆₉, and Val₃₇₀ were evaluated, but no baseline polymorphisms at position 364 were present in the study. Samples with polymorphisms at position 371 were susceptible to **21** in vitro and in study AI468002.

From a medicinal chemistry perspective, the approach adopted at the outset was to challenge the existing SAR beliefs around the C-3 carboxylic acid-containing substituent while not being constrained to simply derivatizing the C-3 alcohol and C-28 acid functionalities presented by the natural product. This strategy was rooted in an approach that sought to develop a fundamentally deeper understanding of the maturation inhibitor pharmacophore and its topography. The identification of the para-substituted benzoic acid that is a hallmark of **4** was remarkable not only for the rapidity of its discovery but also because of the properties that it conferred. This structural motif is preserved in **21** and its immediate discovery helped to propel the program toward candidate identification in the space of just 18 months. Notably, this was achieved despite having to deal with the inherently poor physicochemical properties associated with the highly lipophilic although sp³-rich triterpene core that presented persistent pharmaceutical challenges. The molecular weight (690) and cLog *P* values (8.3 (ChemDraw Professional V16.) and 11.7 (SciFinder, ACD Software V11.02)) for **21** are representative of a series of molecules that extend well beyond the rule-of-five guidelines for optimal oral bioavailability.^{16,31} In addition, the amphoteric properties of **21** strayed into a realm where phospholipidosis was a potential issue, assessed by the application of a newly developed in silico approach that takes the in vivo disposition of a molecule into consideration in the predictive algorithm.³² This methodology indicated that **21** would have a low propensity for causing phospholipidosis, a prediction that was confirmed with subsequent in vivo studies.

The origination of **2** in a phenotypic screen represents a direct contrast with the diametric philosophical and practical approach that identified HIV-1 protease inhibitors and which were optimized using the principles of structure-based drug design.^{1,3} The discovery of **2** provides a clear demonstration of the power of phenotypic screening to identify molecules with modes of action that might not be anticipated and, in some cases, cannot be recapitulated with a practical biochemical assay that faithfully reproduces the cellular context.³³ In the absence of a detailed understanding of the vulnerability of the CA-SP1 cleavage site toward therapeutic intervention, the design of a prospective assay would have presented several challenges. However, armed with that knowledge, a binding assay was developed that allowed biochemical profiling of the association of **2** and **21** with the HIV-1 Gag protein in virus-like particles and was useful in gleaning insight into the biochemical pharmacology of the compounds.^{7,34} The results

of these studies were concordant with cell culture data, with the polymorphic virus sequences exhibiting significantly reduced association with **2** and much attenuated dissociation half-lives in contrast to the performance of **21**.

The ability to implement a screening tier that triaged compounds based on activity toward WT virus, a prominent clinical polymorph, and the rare ΔVal₃₇₀ polymorph, which was utilized because it represented a stringent challenge, was critical to achieving compounds with the targeted antiviral profile. This approach was subsequently strengthened in the program seeking third generation inhibitors by including the Ala₃₆₄Val virus that arises as the primary resistance mutation to maturation inhibitors both in vitro and in the clinic.³⁰

The formation of a functional HIV-1 capsid depends on a carefully choreographed process of Gag protein cleavage and subsequent assembly, with the mature capsid comprising ~250 CA hexamers that are interspersed with exactly 12 CA pentamers, stoichiometry that confers both the unique conical shape and function to the virus capsid.^{35–37} Recently, structural and functional analyses have provided additional insight into the process of capsid formation, which appears to take advantage of inositol hexakisphosphate (IP₆) as an endogenous scaffolding element that binds to the center of the Gag hexamer, by sequentially engaging two circles of lysine residues in the protein.³⁸ The first glimpse of the molecular basis for the interaction of **2** with a construct of a portion of the Gag carboxy terminal domain that extends into the SP1 sequence has recently been gleaned from a microED structure, which indicates that the drug also binds in the center of the Gag hexamer, with the succinic acid moiety engaging some of the basic residues that interact with the phosphate moieties of IP₆.³⁹

The oligomeric nature of the HIV-1 capsid coupled with the precision of the assembly process imparts high sensitivity to therapeutic intervention since it has been shown that the incorporation of small numbers of improperly processed CA-SP1 proteins exerts a strong, dominant-negative effect on virus infectivity.^{40–42} Consequently, the HIV-1 capsid can be considered as a target of high vulnerability since the inhibitory effect of **21** on CA-SP1 cleavage is amplified by incorporation of a limited number of defective proteins into the oligomeric structure.⁴³ Target vulnerability has been described as the fractional target occupancy required to produce a pharmacodynamic (PD) effect and high susceptibility maybe a phenomenon of a more general nature when targeting oligomeric viral proteins.⁴³ This is most effectively exemplified

by hepatitis C virus NS5A replication complex inhibitors, which have been estimated to inhibit virus replication at a stoichiometry of >45 000 NS5A protein monomers (>22 500 of the dimeric species believed to be the functional target) to a single molecule of the inhibitor.⁴⁴

Compound **21** was advanced into a phase 2b clinical trial designed to assess the durability of HIV-1 suppression over 24 weeks in treatment-naïve patients at doses of 60, 120, and 180 mg QD in combination with the nucleoside analogues tenofovir (TDF) and emtricitabine (FTC), a prelude to selecting doses for the phase 3 trials. However, while the antiviral effect in the TDF/FTC/**21** arms was comparable to the TDF/FTC/efavirenz control group, the observation of gastrointestinal intolerance and the emergence of resistant virus led to the cessation of development activities for **21** in favor of compounds with improved antiviral profiles.^{30,45}

AUTHOR INFORMATION

Corresponding Authors

*E-mail: Alicia.Regueiroren@bms.com. Tel: +1 609 466 5035.

*E-mail: Nicholas.Meanwell@bms.com. Tel: +1 609 252 6195.

ORCID

Alicia Regueiro-Ren: 0000-0003-2991-719X

Nicholas A. Meanwell: 0000-0002-8857-1515

Notes

The authors declare no competing financial interest.

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DEDICATION

This article is dedicated to the memory of Beata Nowicka-Sans, who passed away on April 8, 2016, and made significant contributions to the discovery and development of GSK-3532795/BMS-955176.

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