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# The design, synthesis and structure-activity relationships associated with C28 amine-based betulinic acid derivatives as inhibitors of HIV-1 maturation

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ABSTRACT

The design and synthesis of a series of C28 amine-based betulinic acid derivatives as HIV-1 maturation inhibitors is described. This series represents a continuation of efforts following on from previous studies of C-3 benzoic acid-substituted betulinic acid derivatives as HIV-1 maturation inhibitors (MIs) that were explored in the context of C-28 amide substituents. Compared to the C-28 amide series, the C-28 amine derivatives exhibited further improvements in HIV-1 inhibitory activity toward polymorphisms in the Gag polyprotein as well as improved activity in the presence of human serum. However, plasma exposure of basic amines following oral administration to rats was generally low, leading to a focus on moderating the basicity of the amine moiety distal from the triterpene core. The thiomorpholine dioxide (TMD) **20** emerged from this study as a compound with the optimal antiviral activity and an acceptable pharmacokinetic profile in the C-28 amine series. Compared to the C-28 amide **3**, **20** offers a 2- to 4-fold improvement in potency towards the screening viruses, exhibits low shifts in the $EC_{50}$ values toward the V370A and ΔV370 viruses in the presence of human serum or human serum albumin, and demonstrates improved potency towards the polymorphic T371A and V362I virus variants.



The first effective therapy for the treatment of human immunodeficiency virus type 1 (HIV-1) infection was the nucleoside reverse transcriptase inhibitor (NRTI) zidovudine (AZT) approved in the US by the FDA in 1987.[1] Since then, more than 20 drugs that are used in 14 combinations for the treatment of HIV-1 infection have been approved that can be grouped into the following six mechanistic classes: NRTIs, non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTIs), fusion inhibitors (FIs) and chemokine receptor 5 antagonists (CCR5 antagonists). Each class of antiviral agent targets a unique step along the HIV-1 viral life cycle.[2] The current standard of care for HIV/AIDS patients is to combine multiple drugs from at least two different classes of these antiretroviral agents to suppress virus replication, a regimen termed combination antiretroviral therapy (cART).[3] While most of the regimens consist of three or more drugs, Juluca®, the first complete treatment regimen containing only two drugs (the INSTI dolutegravir and the NNRTI rilpirivine) was approved by FDA in November 2017 to treat certain adults with HIV-1 infection.[3] This approval marks the start of a new era in HIV treatment. Since the advent of potent combination therapy in 1995, cART has dramatically reduced HIV-1-associated morbidity and mortality by effectively suppressing viral replication, thus transforming HIV-1 infection from a life-threatening disease into a manageable, chronic condition.[4,5] However, despite the success of cART in recent years, HIV-1 continues to infect millions of people and remains one of the leading causes of death worldwide.[6] As a lifetime therapy, cART is associated with a variety of issues related to long term drug

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toxicity, intolerability, barriers to adherence, unfavorable drug-drug interactions and the eventual development of drug resistance. In addition, cross-resistance among existing antiretroviral agents also limits future treatment options, circumstances that collectively set the stage for the development of novel antiretroviral therapies that are endowed with distinct mechanisms in the HIV-1 viral life cycle.

Maturation inhibitors (MIs) represent a class of antiretroviral agents with a unique mechanism of action that act by binding to a region near the CA-SP1 segment of the Gag polyprotein and interfering with access of HIV-1 protease to one specific cleavage site within the Gag polyprotein.[7,8] Only the final and rate-limiting cleavage of the Gag protein between capsid p24 and spacer peptide 1 (SP1) into its structural components is blocked by MIs, leading to the production of immature and non-infectious viral particles. 3-*O*-(3′3′-Dimethylsuccinyl) betulinic acid (**1**, bevirimat, BVM, Fig. 1) represents the first-in-class HIV-1 MI that showed clinical efficacy and an acceptable safety profile in HIV-1 infected patients.[9–15] However, development of **1** was halted in 2010 after reaching Phase IIb clinical trials due to the finding that antiviral activity was sensitive to the presence of baseline polymorphisms at residues 369–371 within the CA-SP1 region of the Gag protein, the so-called glutamine-valine-threonine (QVT) motif, that compromised efficacy in ~50% of the patients in the study.[16,17] Residue 362 was subsequently shown to be important in the context of naturally-occurring reduced susceptibility to **1**.[18] In addition, **1** suffered from high human serum binding and was associated with formulation challenges, observations that have stimulated efforts to identify MIs with improved profiles.[19,20]

We have previously reported on the discovery of a C3 benzoic acid moiety that acts as an advantageous surrogate for the dimethyl succinate ester installed at the C3 position of **1**.[21a] Compound **2** (Fig. 1) demonstrates potency against wild-type (WT) HIV-1 that is comparable to **1** and is less affected by the presence of human serum, with a reduction in potency of only 10-fold compared to the nearly 100-fold change observed with **1**. Efforts to broaden the polymorphic coverage of **2** through modifications at the C28 position of the triterpenoid core were successful when it was discovered that the basic amine-containing C28 carboxylamide **3** (Fig. 1) demonstrated promising levels of antiviral activity toward the major polymorphic viruses, including V370A, ΔV370 and some of the more recalcitrant viruses that were not inhibited by first generation MIs.[21b,22] Moreover, the potency of **3** was reduced by only a modest 4-fold in the presence of human serum.[21b] Against this backdrop, attention was focused on further exploring the role that the basic amine moiety installed at C28 played in broadening MI activity toward polymorphisms in the Gag polyprotein.

In this iteration of compound optimization, attention was focused on modifications directed towards modulating the basicity and polarity of the C28 sidechain, with the objective of preserving the overall virology profile of **3** while improving the pharmacokinetic (PK) characteristics. One strategy examined was the incorporation of a second basic element into the molecule while maintaining the overall shape close to that of prototype **3**. This was accomplished by reducing the C28 amide moiety to the corresponding amine and resulted in the series of analogues **7**–**35** compiled in Table 1.

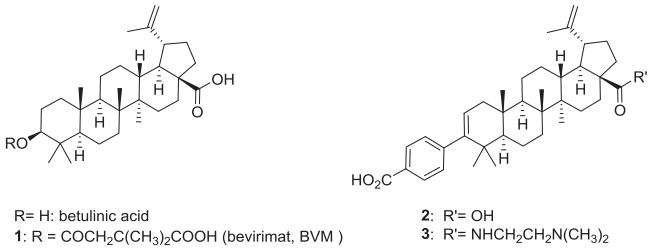


**Fig. 1.** Structures of HIV-1 maturation inhibitors.

The synthetic approach to the majority of the C28 amine analogues is described in Scheme 1. This three step procedure began with oxidation of **4** using PCC to afford aldehyde **5** in good yield, which was combined with a series of amines in a reductive amination process to give **6**.[21a] Unmasking of the *tert*-butyl ester of **6** or **6′** with $CF_3CO_2H$ (TFA) in $CH_2Cl_2$ at room temperature followed by HPLC purification afforded the target compounds **7**–**28** and **30**–**34** which were isolated as their TFA salts.

Following the same *in vitro* screening paradigm as described for the C28 amide series, novel C28 amine targets were screened for antiviral activity toward WT HIV-1 and two of the polymorphic viruses, V370A and ΔV370, that are known to reduce sensitivity to **1**.[16–18] The cytotoxicity of all of the compounds toward the host cell ($CC_50$ value) was also determined while select compounds were rescreened in the WT HIV-1 cell culture assay in the presence of human serum albumin (HSA), data that are presented in Table 1.[21b] Representative compounds were evaluated for their *in vivo* pharmacokinetic profile either in an abbreviated, 6 h snapshot rat PK screen, which was used as an expedited assay, or in a full 24 h rat PK study. The compounds were dosed at 5 mg/kg PO using a mixture of poly(ethylene glycol) 300 (PEG 300), ethanol and TW80 (tween 80, 89:10:1 v/v) as the vehicle unless otherwise noted, with the results presented in Table 1.

As shown in Table 1, compound **7**, the C-28 amine derived by reduction of amide **3**, exhibited comparable potency towards WT and the two polymorphic V370A and ΔV370 viruses when compared with **3**, and with a similar shift (~4-fold) in the presence of HSA. Homologation of the ethylene linker (**8**) resulted in an order of magnitude decline in potency towards all 3 viruses while the bulkier aliphatic dialkyl amines **9** and **10** retained the antiviral potency of **7** as did the primary amine **11**. The piperidine derivatives **12** and **13** exhibited disparate potency, with SARs that were the inverse of the acyclic analogues **7** and **8**, with the homologated **13** more potent than **12**. For the piperazines **14**–**16**, the propyl linker in **16** offered superior antiviral properties compared to the ethylene linker in **15** while the effect of dealkylation of the *N* atom was not significant (**14**). An *in vivo* rat PK study with **7** revealed that the plasma exposure (AUC) of the compound after oral dosing was 33% of that for the C-28 amide analogue **3** and 10-fold less than for **1** while plasma exposure for **8** was considerably lower. These results were broadly consistent with previous observations that had revealed that compounds incorporating basic side chains were generally associated with favorable antiviral properties while polar or negatively charged side chains conferred improved *in vivo* exposure.[21b] Thus, the belief was that a delicate balance of physicochemical properties would be required in order to identify a suitable compromise. Adding further to the burden associated with basic diamine-containing C-28 substituents was the specter of cytotoxicity that emerged with **11**, **13**, **15** and **16** which was more pronounced than for compounds **7**–**10** and, although not a consistent observation, was of sufficient persistence to be of concern.[23] Taken together, these observations led to a focus on moderating the basicity of the amine distal from the core by incorporating this element into ring systems containing electron withdrawing functionality (**17**–**23**) or by introducing or integrating into substituents that would either attenuate (**24**–**31**) or completely quench (**32**–**34**) the basicity.[24]

The two morpholine derivatives **17** and **18** ($pK_a$ = 8.2) demonstrated potent antiviral activity toward all 3 viruses that, in the case of **18** was not associated with a significant serum shift; however, this compound demonstrated a low plasma AUC over 6 h in