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# Inhibitors of HIV-1 maturation: Development of structure–activity relationship for C-28 amides based on C-3 benzoic acid-modified triterpenoids

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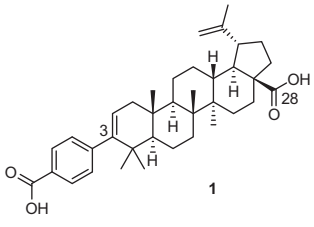
ABSTRACT

We have recently reported on the discovery of a C-3 benzoic acid (**1**) as a suitable replacement for the dimethyl succinate side chain of bevirimat (**2**), an HIV-1 maturation inhibitor that reached Phase II clinical trials before being discontinued. Recent SAR studies aimed at improving the antiviral properties of **2** have shown that the benzoic acid moiety conferred topographical constraint to the pharmacophore and was associated with a lower shift in potency in the presence of human serum albumin. In this manuscript, we describe efforts to improve the polymorphic coverage of the C-3 benzoic acid chemotype through modifications at the C-28 position of the triterpenoid core. The dimethylaminoethyl amides **17** and **23** delivered improved potency toward bevirimat-resistant viruses while increasing $C_{24}$ in rat oral PK studies.



Combination antiretroviral therapy (cART) has successfully slowed the spread of HIV-1 infection and made a life-threatening disease manageable through treatment with a combination of medicines from several drug classes.[1] Nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTIs), fusion inhibitors (FIs) and chemokine receptor antagonists (CCR5 antagonists) are all target classes that have yielded effective antiviral agents.[2] Several of these classes of compounds are often combined in current treatment regimens depending on a number of factors including, but not limited to, a patient's age, sex, history of illicit drug use, current medications, concurrent diseases and infections and prior experience to HIV-1 medications.[3] Although cART has been effective in extending the life of patients and reducing the spread of the disease, there is still a need for therapies with differentiated modes of action to address the emergence of resistance to existing therapies, issues with drug–drug interactions and allow for more diverse drug combination regimens to deal with the multitude of factors described above.[4]

We have recently discovered that the C-3 benzoic acid of **1** is a suitable replacement for the dimethyl succinate ester side chain of bevirimat (BVM, **2**)[5] as an HIV-1 maturation inhibitor (MI).[6] HIV-1 maturation inhibitors disrupt the late-stage protease-mediated cleavage of the capsid-spacer peptide 1 (CA-SP1) region of the Gag protein resulting in defective core condensation and the release of immature virions. Immature virions are non-infectious, as they lack the integral organization of the Gag proteins needed to form the functional core of the virion.[7,8]

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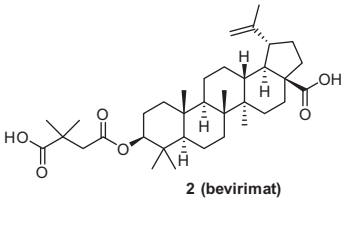


2 (bevirimat)

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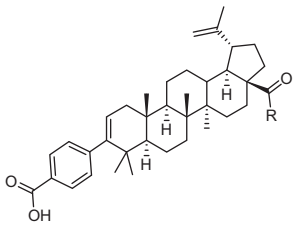
0960-894X/

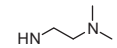
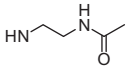
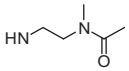
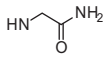
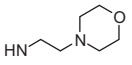
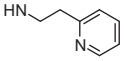
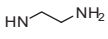
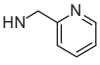
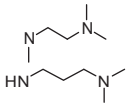
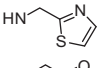
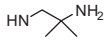
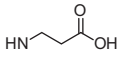
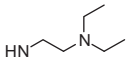
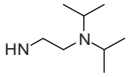
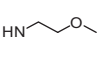
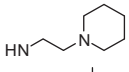
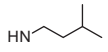
BVM (**2**) reached Phase II clinical trials before it was discontinued due, in part, to a poor response in patients with naturally occurring polymorphisms in the CA-SP1 region of the Gag polyprotein.[9–11] Polymorphic variations to the glutamine–valine–threonine (QVT) motif at residues 369–371 were not only associated with reduced sensitivity to BVM (**2**) in vivo and in vitro, but these polymorphisms were found in nearly half of subtype B patients and the majority of non-B subtype patients.[10,12,13] A 2nd generation MI needs to show substantial improvement in potency towards these polymorphism-containing viruses that are intrinsically resistant to BVM (**2**). Other issues that interfered with the advancement of BVM included high human serum binding and difficulties with formulation.[14]

In our recent Letter detailing the discovery of the C-3 benzoic acid, we began to address these issues.[5] The inhibitory activities of **1** and **2** toward wild-type (WT) and V370A viruses were comparable, while the potency of **1** against WT virus in the presence of 15 mg/mL of human serum albumin (HSA; an important component in human serum that shifts the potency of BVM) showed a considerable improvement when compared to **2** (Table 1). With this discovery in hand, and a clear goal for the profile of a next-generation HIV-1 maturation inhibitor that requires improved activity toward viruses containing Gag polymorphisms, the effect of modification of the C28 acid moiety was investigated. At the time that we initiated this effort, there was no indication from the prior structure–activity relationship (SAR) studies with BVM that structural modification at C-28 would lead to compounds with maturation inhibitory activity toward the polymorphic viruses. In addition to identifying compounds with improved virological properties, we sought molecules with a pharmacokinetic (PK) profile in preclinical species predictive of QD dosing, with a low dose projection preferred so that the compound could be combined in fixed dose combinations with other antiretroviral agents.

Two recombinant viruses containing Gag polymorphisms that are known to decrease potency to **2** were used as screening tools

**Table 1**
Antiviral activity toward wild type, V370A and ΔV370 viruses, cytotoxicity and serum shift of C-28 amide derivatives



| # | R | WT $EC_{50}$* (nM) | WT $EC_{50}$ w/HSA (nM) | V370A $EC_{50}$ (nM) | ΔV370 $EC_{50}$ (nM) | $CC_{50}$ (μM) | # | R | WT $EC_{50}$* (nM) | WT $EC_{50}$ w/HSA (nM) | V370A $EC_{50}$ (nM) | ΔV370 $EC_{50}$ (nM) | $CC_{50}$ (μM) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | OH | 16 | 150 | 233 | >6000 | 27 | **17** |  | 3 | 11 | 8 | 31 | 3 |
| **2** | BVM | 10 | 974 | 553 | >10,000 | 17 | **18** |  | 5 | 22 | 24 | 361 | 12 |
| **5** |  | 47 | 1516 | 282 | | 16 | **19** |  | 3 | | 2 | 224 | 9 |
| **6** |  | 59 | 166 | 67 | 1186 | 18 | **20** |  | 6 | | 32 | 2000 | 9 |
| **7** |  | 83 | 255 | 31 | 676 | 10 | **21** |  | 7 | 28 | 12 | 77 | 5 |
| **8** |  | 15 | | 19 | 415 | 8 | **22** |  | 3 | | 7 | 702 | 4 |
| **9** |  | 5 | 9 | 31 | | 1 | **23** |  | 6 | 17 | 38 | 25 | 3 |
| **10** |  | 15 | | 28 | 690 | 6 | **24** | | 2 | 3 | 9 | 50 | 0.5 |
| **11** |  | 106 | | >2000 | >2000 | >50 | **25** |  | 7 | | 21 | 800 | 20 |
| **12** |  | 37 | 449 | 427 | >4000 | 36 | **26** |  | 2 | | 7 | 39 | 33 |
| **13** |  | 7 | 39 | 41 | >2000 | 7 | **27** |  | 4 | | 13 | 69 | 3 |
| **14** |  | 17 | | 152 | | 14 | **28** |  | 7 | | 10 | 359 | 10 |
| **15** |  | 10 | 28 | 252 | | 7 | **29** |  | 47 | | 101 | 1613 | 11 |
| **16** |  | 369 | 1583 | | | 0.2 | | | | | | | |

* WT, WT w/15 mg/mL of HSA, V370A, ΔV370 and $CC_{50}$ and are the mean of at least two experiments.

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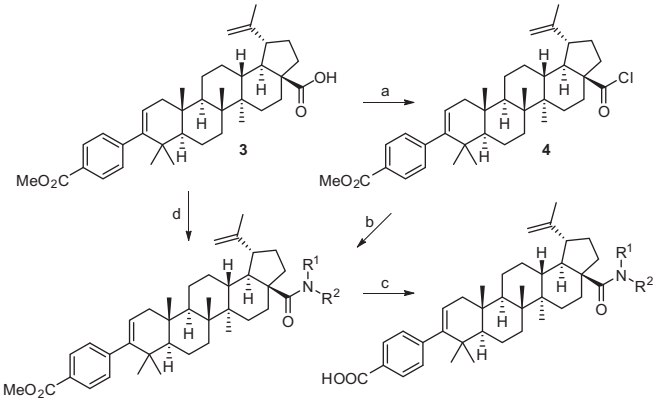
to discover compounds with improved virological profiles. V370A-containing virus was used since this is the most common polymorphic variation in the Gag protein in the SP1 region observed in HIV-1 subtype B-infected patients that leads to reduced susceptibility (50-fold) to **2**.[15] In addition, a ΔV370-contaning virus was used as it is a common polymorphic variation in non-subtype B viruses, although it is only present in a small percentage of the subtype B patient population (0.9%). Additionally, ΔV370 is one of the most BVM-resistant single polymorphic variations (>190-fold resistance), and improved potency against this virus concomitantly improved potency toward other clinically-relevant polymorphisms.[16] In vitro potency was measured utilizing a multiple cycle assay in MT-2 cells with an NL4-3-derived virus expressing the *Renilla* luciferase gene incorporated as a marker for virus growth (NLRepRlucP373S). V370A and ΔV370 viruses were identical to the WT virus except for a single amino acid substitution (V370A) or deletion (ΔV370) at the 370 position of the Gag protein.

Carboxylic acid **3**[6] was modified through one of two routes to give the C-28 amides and acyl sulfonamides (Scheme 1). The first route employed oxalyl chloride to form the acid chloride **4**, which was reacted with a series of amines to give the C-28 amides. The second route utilized HATU as the amide coupling agent with a reactant amine. Amide formation using both of these procedures was followed by hydrolysis of the C-3 benzoate ester using either NaOH or LiOH with heating at 50–85 °C in 1,4-dioxane and $H_2O$. Acidification and purification delivered the compounds compiled in Tables 1 and 4.[17]

The compounds compiled in Table 1 represent a selection of the C-28 amide and acyl sulfonamide side chains that were examined to screen for inhibition of WT and the two polymorphism-containing viruses. Initially, we screened C-28 acyl sulfonamides since this motif mimics the acidic nature of the carboxylic acid of **1**. Unfortunately, acyl sulfonamides **5**–**7** demonstrated reduced WT potency compared to **1** and insufficient potency against ΔV370 virus to be of further interest. The next avenue of exploration focused on heterocycles extending from the C-28 amide of the triterpenoid core as a means of improving potency and influencing ADME (absorption, distribution, metabolism and excretion) properties. While other labs have reported on the effects of modifying the C-28 amide in combination with the dimethyl succinate side chain of **2**,[18–21] at the time this work was conducted, there were no Letters suggesting that C28 amide derivatives would confer improved potency toward BVM-resistant viruses; however, several recent reports have suggested that BVM-resistant viruses can be inhibited through this kind of structural modification.[22,23] Entries **8**–**10** exemplify the initial alkyl-linked heterocyclic amide modifications examined and the results indicate that improved potency against BVM-resistant viruses can be achieved. Each heterocycle showed similar potency toward the WT virus as **1**, but with only a 1 to 6-fold decline in potency against the V370A virus. The potency shift in the presence of HSA was only evaluated for **9** because the series lacked significant inhibition of the ΔV370 virus; however, the serum shift for **9** was less than two-fold, preserving the improvement observed with **1** when compared to **2**. Unfortunately, **9** showed an increase in in vitro cytotoxicity, precluding it from further studies. Additional methyl-linked heterocycles were synthesized, but these failed to further improve the antiviral profile (data not shown). Extending the carboxylic acid moiety further from the core, as in **11** and **12**, was detrimental to potency against both WT and V370A viruses, although to a lesser extent with the ethyl-linked **12**. However, compounds **11** and **12** were associated with reduced in vitro cytotoxicity compared to heterocycles **8**–**10**. Replacing the carboxylic acid terminus with an alcohol, as exemplified by ethanolamide **13**, resulted in improved activity toward both WT and V370A virus, although this compound still failed to address the more challenging ΔV370 virus. Capping the alcohol as a methyl ether (**14**) resulted in a further decline in potency against both WT and V370A viruses. Small alkyl amides, including methyl amide **15**, were explored next. WT virus inhibitory activity was acceptable; however, the V370A-containing virus was more than 25-fold less susceptible to **15** than WT virus. The piperidinamide **16** exhibited greatly reduced activity toward both WT and V370A viruses while also showing increased cytotoxicity. However, an enhancement in potency toward both BVM-resistant test viruses was achieved when a basic dimethylamine functionality was incorporated into the amide moiety, as exemplified by the ethyl-linked compound **17**. In addition to improved WT potency, both polymorphic viruses were inhibited with $EC_{50}$ values below 35 nM, suggesting that polarity and/or basicity in the side chain was essential in this portion of the molecule in order to enhance antiviral coverage, especially in regard to the more challenging ΔV370 polymorph. In addition, potency in the presence of human serum albumin was also largely preserved, with a less than 4-fold serum shift for **17** compared to 9-fold for **1**. However, incorporating a basic amine into the side chain also led to an increase in the in vitro cytotoxicity (3 μM for **17** compared to 27 μM for **1**), perhaps not entirely surprising since basic amines have been associated with promiscuity.[24]

Since **17** exhibited the preferred virological profile, SARs of the amine-containing side chain were probed more deeply in order to identify compounds with ADME properties suitable for progressing into in vivo studies. Capping the amine as a terminal amide, exemplified by **18**–**20**, retained antiviral activity toward WT virus and showed only modest changes toward V370A virus compared to **17**; however, potency toward the ΔV370 virus declined by 7- to 65-fold compared to **17**. Less basic amines were also examined, including the morpholine-containing side chain in **21**; however, potency and in vitro cytotoxicity were not improved when compared to **17**. When both methyl groups of **17** were eliminated, as in the primary amine **22**, inhibitory potency against both WT and V370A viruses were retained, but inhibition of the ΔV370 virus was reduced by 23-fold compared to **17**. The tertiary amide **23** showed similar potency toward WT and ΔV370 virus but almost a 5-fold decline in potency toward the V370A virus when compared to progenitor **17**. Nevertheless, **23** offered an alternative to the free NH of the secondary amide of **17**, which may influence the permeability of the molecule. The three carbon side chain homolog **24** maintained potency but in vitro $CC_{50}$ values were 6-fold lower than for **17**. Installing a gem dimethyl group in the linker element α- to the amine gave the tertiary amine **25**, a




**Scheme 1.** Synthesis of C-28 amides and acyl sulfonamides derived from the C-3 benzoic acid-modified triterpenoid **3**. Reagents and conditions: (a) oxalyl chloride, DCM, rt, 2–5 h, 100%; (b) DIEA, DMAP, amine reactant, DCM, rt; (c) NaOH or LiOH monohydrate, 1,4-dioxane, $H_2O$, 50–85 °C; (d) HATU, THF, DIEA, amine reactant, rt.

structural change that improved in vitro cytotoxicity, but activity toward the ΔV370 polymorph suffered by over an order of magnitude while increasing the bulk at the amine terminus with the diethyl moieties in **26** or the diisopropyl groups in **27** maintained potency against all three viruses. Interestingly, **26** showed an 11-fold improvement in in vitro cytotoxicity compared to **17**. In analog **28**, the amine is enclosed in a piperidine ring, which resulted in decreased potency in the ΔV370 virus assay. Finally, replacing the amine in **17** with an aliphatic side chain that represents the same silhouette afforded the non-basic **29**, which showed decreased potency of more than 10-fold against all three viruses. In summary, exploration of C-28 amide modifications provided several compounds that achieved an excellent combination of WT, V370A and ΔV370 HIV-1 inhibition. In addition, the human serum albumin shift measured for these compounds was generally less than 5-fold, with the exception of when a carboxylic acid or a carboxylic acid isostere was part of the side chain.

Two of the more promising compounds, **8** and **17**, were selected for evaluation against a panel of BVM-resistant viruses (Table 2). The ethyl linked pyridine **8** exhibited improved inhibition of the BVM-resistant viruses tested; however it was still 27-fold less active toward ΔV370 than WT virus. The basic dimethyl amine **17**, on the other hand, showed a relatively flat virological profile with a less than a 3-fold variation in virus inhibitory activity against all BVM-resistant polymorphs other than ΔV370 which showed a 10-fold change.

The compounds that exhibited an acceptable potency profile were further evaluated in an in vitro metabolic stability assay utilizing human and rat liver microsomes to determine their respective half-lives. Compounds showing good in vitro metabolic stability in rat liver microsomes were evaluated in in vivo rat PK studies. Compounds were dosed at 1 mg/kg IV and 5 mg/kg PO using a mixture of poly(ethylene glycol) 300 (PEG 300), ethanol and tween 80 (TW80) (89:10:1 v/v) as the vehicle, unless otherwise noted. Several less potent compounds were also tested in vivo in order to provide data on structural modifications that could potentially inform for further refinements of the chemotype.

**Table 2**
Antiviral activity toward BVM-resistant viruses

| # | WT $EC_{50}$ (nM) | V370A $EC_{50}$ (nM) | ΔV370 $EC_{50}$ (nM) | V370M $EC_{50}$ (nM) | T371A $EC_{50}$ (nM) | ΔT371 $EC_{50}$ (nM) |
|---|---|---|---|---|---|---|
| BVM | 10 | 553 | >10,000 | 1810 | 40 | 77 |
| **8** | 15 | 19 | 415 | 61 | 19 | 38 |
| **17** | 3 | 8 | 31 | 10 | 3 | 3 |

In vivo studies were performed as either a rat PK screen, in which a snapshot 6 h experiment was used to expedite data turnaround, or as a full 24 h rat PK study. Data up to the 6 h time point were used to compare the 6 h rat screen compounds to the full 24 h rat PK data. Because of the long in vivo $t_{1/2}$ values for many of the test compounds, plasma concentration measured 24 h after dosing ($C_{24}$) and %*F* could not be determined in the 6 h rat screen because a significant amount of the data would need to be extrapolated (Table 3).

IV plasma clearance of the tested compounds ranged from 0.6 to 8.3 mL/min/kg, indicating generally low clearance (CL), while volume of distribution ($V_{ss}$) ranged from 0.1 to 2.7 L/kg. The oral $AUC_{total}$ (measured over 24 h) for the pyridine-containing analog **8** was 1232 nM * h, with a $C_{max}$ of 172 nM, an order of magnitude lower than for **2**. Although **8** had a promising $t_{1/2}$ of 5.7 h, the compound showed poor oral bioavailability (*F* = 3%), although the $C_{24}$ was, nevertheless, comparable to that measured for **2**. The carboxylic acid derivative **12** was selected for evaluation in vivo in order to assess the profile of a compound with the same overall charge as **2**. Interestingly, **12** exhibited rat oral exposure that was much higher than any other compound evaluated in this series. The $AUC_{total}$ for **12** was 5.8-fold higher than for **2** with a 20-fold improvement in the $C_{24}$ value. Because of its promising antiviral profile, the potent dimethylethylamine derivative **17** was examined in a full 24 h rat PK study. The $AUC_{total}$ was about half of that measured for **2**; however, with an extended $t_{1/2}$ and a similar %*F*, the $C_{24}$ was improved 4.5-fold compared to **2**. The tertiary C-28 amide **23** showed a 1.8-fold higher $AUC_{total}$ and a 4-fold improved $C_{24}$ compared to **17**, indicating that masking the amide NH of the prototype was beneficial. Unfortunately, attempts to further influence rat oral exposure by increasing lipophilicity in hopes of improving cell membrane permeability proved to be ineffective, as exemplified by the data associated with compounds **24**–**29** (Table 3). Surprisingly, elongating the side chain of **17** by a single $CH_2$ (**24**) resulted in a substantially decreased $AUC_{0-6h}$ while the more lipophilic amines **26** and **27** and the simple *iso*-pentyl amide **29** did not exhibit improved $AUC_{0-6h}$.

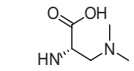
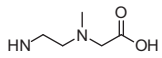
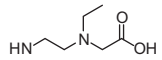
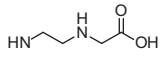
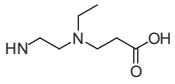
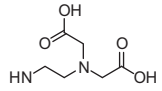
After analysis of the data from the in vivo rat PK studies, a series of compounds was synthesized that sought to combine the potency-enhancing basic amine portion of the side chain in **17** with the PK-enhancing carboxylic acid moiety present in **12**. Table 4 outlines the results from the evaluation of these combinations. Compound **30** directly combined the diethylaminoethyl amide side chain of **17** with a carboxylic acid substituent. Although potency was better than the prototype carboxylic acid-containing analogs **11** and **12**, compound **30** was still more than 15-fold less active toward WT virus and even less active against the

**Table 3**
In vivo pharmacokinetic properties of selected compounds in rats[a]

| # | Met. Stab., $t_{1/2}$ (min) human/rat | *F* (%) | $AUC_{0-6h}$[b] (nM·h) | $AUC_{total}$[b] (nM·h) | $C_{max}$[b] (nM) | $C_{24}$[b] (nM) | CL, IV (mL/min/kg) | $V_{ss}$, IV (L/kg) |
|---|---|---|---|---|---|---|---|---|
| **1** | – | 4 | 5724 | – | 1314 | – | 1.0 | 0.2 |
| **2** | – | 18 | 6390 | 13,763* | 2587 | 6 | 4.0 | 0.1 |
| **8** | 43/>120 | 3 | 721 | 1232 | 172 | 5 | 3.2 | 1.1 |
| **12** | 113/>120 | 71 | 43,159 | 79,949 | 9467 | 137 | 1.2 | 0.2 |
| **17**[c] | >120/>120 | 17 | 1623 | 3814 | 396 | 27 | 6.1 | 2.3 |
| **23** | >120/85 | 11 | 1420 | 6865 | 386 | 108 | 2.2 | 1.8 |
| **24**[d] | >120/>120 | – | 116 | – | 36 | – | 8.3 | 2.7 |
| **26**[e] | – | – | 809 | – | 229 | – | 3.3 | 1.7 |
| **27** | >120/>120 | – | 1315 | – | 302 | – | 4.3 | 1.6 |
| **29** | – | – | 1582 | – | 731 | – | 0.6 | 0.1 |

[a] Vehicle: Poly(ethylene glycol) 300 (PEG 300), ethanol, tween 80 (TW80) (89:10:1 v/v). Dose, 1 mg/kg IV; 5 mg/kg po.
[b] Oral $C_{max}$, AUC and $C_{24}$ level dosed at 5 mg/kg.
[c] Vehicle: Poly(ethylene glycol) 300 (PEG 300), ethanol, 0.1 N NaOH, TPGS (78:10:10:2 v/v).
[d] Vehicle: Poly(ethylene glycol) 300 (PEG 300), ethanol, 0.1 N HCl, tween 80 (TW80) (84.5:10:5:0.5 v/v).
[e] Vehicle: Poly(ethylene glycol) 300 (PEG 300), ethanol, 0.1 N HCl, tween 80 (TW80) (85:9.7:4.8:0.5 v/v).
* $AUC_{total}$ was calculated from a full 24 rat PK run in addition to a 6 h rat p*K* screen.

**Table 4**
Antiviral activity of wild type, V370A and ΔV370, cytotoxicity and serum shift of HIV-1 maturation inhibitors incorporating combinations basic and acidic moieties in the C-28 amide chain

| # | R | WT $EC_{50}$* (nM) | V370A $EC_{50}$ (nM) | ΔV370 $EC_{50}$ (nM) | $CC_{50}$ (μM) |
|---|---|---|---|---|---|
| **30** |  | 46 | 772 | >160 | 25 |
| **31** |  | 10 | 51 | 160 | 0.2 |
| **32** |  | 3 | 9 | 64 | 17 |
| **33** |  | 42 | 15 | 61 | >2.0 |
| **34** |  | 2 | 6 | 119 | >17 |
| **35** |  | >2000 | >2000 | >2000 | >20 |

* WT, V370A, ΔV370 and $CC_{50}$ are the mean of at least two experiments.

polymorphism-containing viruses. Substituting one of the methyl groups of **17** with an acetic acid moiety afforded **31**, which exhibited comparable potency to **30** toward WT and V370A viruses but poorer activity than targeted toward virus with the ΔV370 polymorphism. In addition to a decrease in potency, cytotoxicity appeared to be an issue with this modification, since the in vitro $CC_{50}$ value was 0.2 μM. However, compound **32**, the ethyl homolog of **31**, exhibited a modestly improved antiviral profile that was similar to **17**. Notably, the in vitro cytotoxicity value for **32** was improved by more than 85-fold, a beneficial effect that is also reflected in the replacement of the dimethyl amine substituents of **17** with the diethyl amine homolog in **26** ($CC_{50}$ was 33 μM). Interestingly, the substituted glycine **33** resulted in decreased WT virus inhibition without a significant decrease in inhibition of the V370A and ΔV370 viruses. Homologating the carboxylic acid moiety of **32** by a single $CH_2$ gave **34**, which showed a similar profile to the progenitor, with only a 2-fold loss in activity toward the ΔV370 virus. Finally, the iminodiacetic acid derivative **35** resulted in poor activity toward all three viruses in the testing panel. Although the combination of a basic amine and a carboxylic acid in the C-28 amide side chain provided several potent compounds, inhibition of the ΔV370 polymorphic virus was generally lower for this series. The most potent compound from this brief survey was **32** which showed excellent in vitro metabolic stability ($t_{1/2}$ > 120 min in both human and rat liver microsomes) and was, therefore, progressed into an in vivo rat PK study. IV plasma clearance and the volume of distribution of **32** were 26.7 mL/min/kg and 0.5 L/kg, respectively. However, the oral $AUC_{0-6h}$ was low at only 220 nM * h, precluding further work in the combined side chain series.

New treatment options for patients infected with HIV-1 virus continue to be needed because of the diverse patient population taking antiretroviral drugs today. With patients having different medical needs based on a number of factors, new drugs with differentiated modes of action will be paramount to future therapies. We have detailed our work towards identifying a second generation HIV-1 maturation inhibitor through combinations of the previously-described C-3 benzoic acid modification and careful SAR analysis of the C-28 position of this modified betulinic acid core. The compounds described herein were some of the first compounds in our survey of the MI chemotype to demonstrate improved in vitro coverage of polymorphisms identified in clinical trials to be associated with reduced response to BVM. They offer improvement over other C-28 modifications reported to date which exhibit a less balanced WT and V370A inhibitory profile while antiviral activity toward the more challenging ΔV370 virus has not been described. In addition to improved polymorphic virus inhibition, compounds **17** and **23** show increased $C_{24}$ values compared to **2** following oral administration to rats, a key improvement necessary for a QD dosing formulation. Inclusion of basic polar groups extending from a C-28 amide improved potency toward the polymorphic viruses, while a terminal carboxylic acid extending from the amide improved the overall rat PK; however, the combination did not prove additive. Although compounds from this series did not possess a combination of potency and oral exposure to allow consideration into progressing into more advanced preclinical studies, these results provided a basic understanding of the C-28 SARs from the perspective of both antiviral profile and PK properties that helped to define the path toward the identification of BMS-995176, a second generation HIV-1 maturation inhibitor currently in Phase II clinical trials.[16,25]

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## Supplementary data

Supplementary data (experimental details of the biological assays and compounds synthesized and characterizations of new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.03.019.

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17. Several of the compounds in Tables 1 and 4 required additional synthetic steps to assemble the side chain. The experimental details can be found in

Supporting information. In addition, additional C-28 amides were explored and their synthesis is reported in the following patent: Regueiro-Ren, A.; Liu, Z.; Swidorski, J.; Meanwell, N. A.; Sit, S. -Y.; Chen, J.; Chen, Y.; Sin, N. U.S. Patent 8,802,661, 2014.

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