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Triterpene based compounds with potent anti-maturation activity against HIV-1

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ABSTRACT

Efforts towards developing orally bioavailable HIV-1 maturation inhibitors starting from betulinic acid **1** are described. SAR resulted in improved potency, physicochemical properties, and enhanced oral absorption in rats.

Acquired immunodeficiency syndrome (AIDS), and its cause, HIV-1 infection, remain a life-threatening health problem that has taken the lives of more than 25 million people worldwide during the 27 years since its initial outbreak and characterization in 1981.[1] In the absence of anti-retroviral treatment, HIV-1+ individuals will suffer depressed CD4 T lymphocyte counts resulting in increased susceptibility to opportunistic pathogens and ultimately death. Today's clinician has an array of chemotherapeutics available for control of viremia, including inhibitors of HIV-1 fusion/entry, reverse transcriptase, integrase, and protease. While current therapies are effective, severe adverse effects and viral resistance necessitate continued efforts in the discovery of new agents with both improved tolerability and with novel mechanisms of action.

There has been a resurgent interest in naturally derived and semi-synthetic triterpenes as promising platforms for discovery of novel anti-HIV agents.[2,3] Triterpenes effect their anti-viral activity by intervening with one or more[4] of the critical steps in the virus life cycle including fusion/entry,[5] GAG processing,[6] reverse transcription,[7] maturation,[8] as well as undefined mechanisms.[9]

Several laboratories have recognized the utility of betulinic acid (**1**) (itself only weakly anti-viral) as a starting point for creating potent anti-viral agents. Indeed, the anti-maturation derivative 3-*O*-(3',3'-dimethylsuccinyl)-betulinic acid (**DSB**, **2**)[10] is currently in phase-IIB clinical trials for treatment of HIV viremia. Additionally, isomeric C-28 amides RPR103611 (**3**)[5d,e] and IC9564 (**4**)[5b] are potent fusion (syncytium formation) inhibitors but apparently never entered development (see Fig. 1).

We too recognized the opportunity to discover potent, anti-maturation derivatives by amide formation at C-28.[11] To this end, synthesis was relatively straight-forward and proceeded by either of two routes (Scheme 1). In the first approach, the C-3 hydroxyl group of **1** was masked as an acetate group followed by

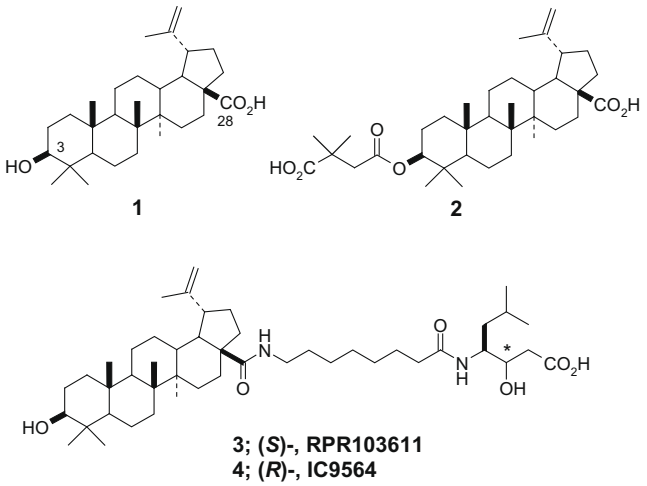

Figure 1. Structure of betulinic acid and anti-HIV derivatives.

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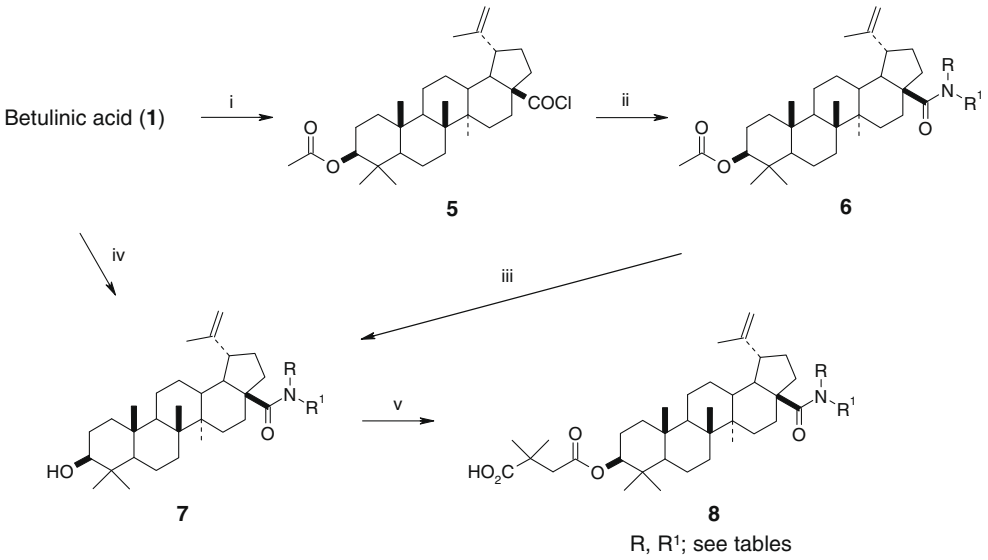


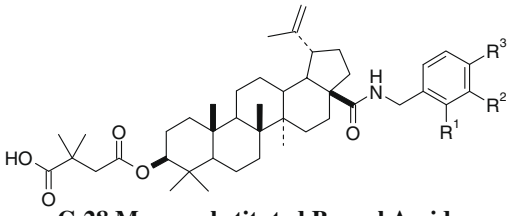
Scheme 1. Reagents and conditions: (i) Ac_2O, pyr., 4-DMAP, then $SOCl_2$, cat. DMF, reflux or $(COCl)_2$, cat. DMF, CH_2Cl_2, rt; (ii) $RNHR^1$, *i*-Pr_2NEt, 4-DMAP, CH_2Cl_2, rt then 4 N NaOH, THF, MeOH; (iv) EDCI, HOAt, NMM, $RNHR^1$, DMF, rt; (v) 3,3-dimethylsuccinic anhydride, 4-DMAP, pyr., 105 °C.

conversion to acid chloride **5** with either oxalyl chloride or thionyl chloride. Without purification, a solution of **5** in CH_2Cl_2 was allowed to react with an appropriate amine and stoichiometric 4-DMAP. Deprotection (4 N NaOH, THF, MeOH) provided C-28 amides **7**. Alternatively, **1** could be used directly by activation with water soluble carbodiimide (EDCI) in combination with HOAt and tertiary amine base such as NMM. Regardless of coupling method, intermediate amides **7** were converted in good yields to the target C-3 ester/C-28 amides **8** by esterification with 3,3-dimethylsuccinic anhydride in pyridine with 2 equivalents of 4-DMAP followed by purification (silica gel flash chromatography or preparative HPLC). Interestingly, catalytic or even stoichiometric amounts of the DMAP failed to provide significant improvements in coupling efficiencies (see Scheme 1).

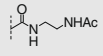
In vitro anti-viral activity was assessed with two different anti-viral assays.[12] Compounds were first assessed for efficacy against the NL4-3 strain in the MT-4 cytoprotection assay. Compounds with significant activity in this assay were further profiled for activity against the IIIB strain in an anti-viral assay with human PBMCs. The data are presented in the following tables. Since we discovered early on that anilides had little to no anti-viral activity (data not shown), we focused our efforts solely on exploring the SAR of secondary and tertiary aryl alkyl amides. In general, the SAR was tolerant of a wide variety of substituted benzyl and phenethyl amides. Noteworthy compounds in class are *o*-methoxy derivative **9**, *o*-carboxyl acid **15**, *p*-chloro **23** and methylene dioxyphenyl **26** (Table 1). Each derivative had low sub-micromolar potency in both the cytoprotection and PBMC anti-viral assays and a therapeutic index (TI, TC_{50}/EC_{50} or TC_{50}/IC_{50}) >400.

We also explored the effects on potency of higher order aryl alkyl amides (Table 2). Similarly, methoxy groups were tolerated with phenethyl derivative **28** showing the best potency profile. Interestingly, the phenethyl *o*-carboxamide derivative **32** was found to be exceptionally potent with the best TI (>1000) overall. In general, heteroaromatic derivatives (Table 3) demonstrated good potency in the MT-4 cytoprotection assay. Particularly noteworthy are hydroxyquinoline and benzimidazole derivatives **37** and **38**, respectively, which also exhibited good activity in the PBMC assay. Unfortunately, microsomal instability of many of the more potent analogs in Table 3 precluded further pharmacokinetics evaluation *in vivo*. Finally, tertiary and branched alkyl aryl amides (Table 4) were assessed. While amino acid derivatives **44** and **45** demonstrated good potency in the cytoprotection assay,

Table 1
SAR of C28 benzyl amide derivatives.

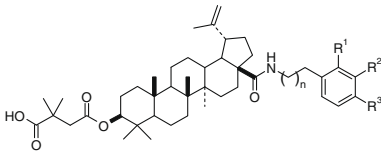


C-28 Mono-substituted Benzyl Amides

	R^1	R^2	R^3	MT-4 cytoprotection NL4-3 virus		PBMC IIIB virus	
				EC_{50} (μM)	TC_{50} (μM)	IC_{50} (μM)	TC_{50} (μM)
8	H	H	H	0.023[a]	>10	0.011[a]	>10
9	OMe			0.024[a]	>10	0.007[a]	>10
10		OMe		0.019	>10	0.014[a]	>10
11			OMe	0.026	>10	0.0235	>10
12	Me			0.085	>10		
13	Ph			0.290[a]	>10		
14			Ph	0.267	>10		
15	CO_2H			0.006	4.5	0.016	9.7
16		CO_2H		0.480[a]	7.6		
17			CO_2H	0.165	8.9		
18	CO_2H		F	0.052	3.2	0.004	1.5
19				0.005[a]	9.3		
20				0.010[a]	10		
21				0.030[a]	10		
22			NMe_2	0.030[a]	1		
23			Cl	0.0179	>10	0.027	>10
24		OCF_3		0.110[a]	5.2	NA	NA
25			OCF_3	0.190[a]	1	NA	NA
26		$-OCH_2O-$		0.023[a]	>10	0.009[a]	>10

[a] a = *n* of 1.

Table 2
SAR of C28 phenethyl and higher order amide derivatives.



C-28 Phenethyl and Higher Amides

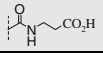
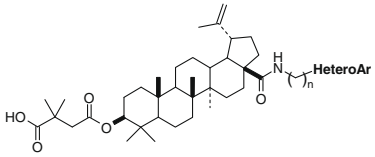
	n	R^1	R^2	R^3	MT-4 cytoprotection NL4-3 virus		PBMC IIIB virus	
					EC_{50} (μM)	TC_{50} (μM)	IC_{50} (μM)	TC_{50} (μM)
27	1		OMe		0.040	>10		
28	1			OMe	0.012	>10	0.060	1
29	2			OMe	0.080	10		
30	2		OMe	OMe	0.020	10		
31	1			CO_2H	1	1		
32	1				0.0031	9.6	0.0094	10.98

Table 3
SAR of C28 heteroaromatic amide derivatives.



C-28 Heteroaromatic Amides

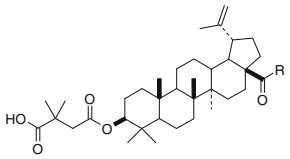
	n	Hetero-Ar	MT-4 cytoprotection NL4-3 virus		PBMC IIIB virus	
			EC_{50} (μM)	TC_{50} (μM)	IC_{50} (μM)	TC_{50} (μM)
33	1		0.129	>10		
34	1		1.70[a]	>10		
35	1		0.024	>10	0.030	>10
36	1		0.026	>10		
37	1		0.003	>10	0.004	>10
38	1		0.005	8.6	0.001[a]	>10
39	1		0.033	6.8	0.003	>10
40	1		0.007	>10	0.007[a]	>10

[a] a = *n* of 1.

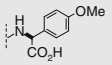
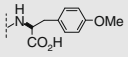
we opted not to pursue them further due to the presence of a stereogenic carbon and problematic syntheses.

The small-molecule HIV-1 maturation inhibitor DSB (**2**) prevents HIV-1 replication by delaying cleavage of the CA-SP1 junction during Gag processing. The incomplete Gag processing leads to impaired maturation of the viral core and release of noninfectious particles from infected cells.[13] In agreement with the mechanism of action of maturation inhibitors, virions released from virus-producing cells treated with compound **15** display abnormal Gag processing with a dose-dependent inhibition of CA-SP1 cleavage to the mature CA protein as compared to control cells. Subsequent infection of cells with culture supernatants containing these defective virions was impaired as well.

Table 4
SAR of C28 tertiary and alpha-branched amide derivatives.



C-28 Tertiary and α-Branched Amides

	R	MT-4 cytoprotection NL4-3 virus	
		EC_{50} (μM)	TC_{50} (μM)
41		0.070[a]	>10
42		0.200[a]	>10
43		0.180[a]	>10
44		0.040	6.8
45		0.009[a]	5.6

[a] a = *n* of 1.

Table 5
Pharmacokinetics and oral bioavailability of selected derivatives following oral administration in rats. iv data (not shown) was obtained at 2.5 mg/kg.

Compound	PK parameters [12] (PO 5 mg/kg, *n* = 3–5) male Sprague–Dawley rats					
	Dose (mg/kg)	T_{max} (h)	$t_{1/2}$ (h)	C_{max} (μM)	AUC(0-inf) (h ng/mL)	$\%F_{po}$
9	5	2.00	2.00	0.04	114	ND
15	5	3.20	2.00	0.58	1695	56.4
23	5	2.00	8.00	0.27	1342	3.91
28	5	2.00	4.8	0.04	245	1.84
32	7.5	1.00	4.54	0.86	1652	15.53
37	10	4.00	7.8	0.07	1259	1.6

With potent compounds in hand we focused our attentions on assessing their ADME properties in vitro and in vivo (Table 5). Compounds were first assessed for stability in pooled liver microsomes (rat and/or human). Compounds **15** and **23** had >60% remaining after 40 min, **32** and **37** had >40% remaining while the other compounds in Table 1 were relatively unstable with 10% to 20% remaining after 40 min. Plasma exposure was determined for each compound after oral administration in rats. Compounds **9**, **28** and **37** were poorly absorbed as judged by their relatively low C_{max} values and AUC. This is most likely due to the overall poor aqueous solubility of these derivatives.

Fortunately, bis-carboxylic acid derivatives **15** and **32** were well absorbed and demonstrated the highest C_{max} and plasma $T_{1/2}$ values. Indeed, **15** was superior in all regards with 56% oral bioavailability.

In summary, we have found that amides of 3-*O*-(3′,3′-dimethylsuccinyl)-betulinic acid are potent inhibitors of HIV-1 maturation. The majority of the benzyl, phenethyl and heteroaromatic derivatives studied posses nanomolar potencies in both MT-4 cytoprotection and PBMC assays. Importantly, the SAR is sufficiently tolerant to a variety of substitutions which in effect allows for fine-tuning of physical properties (aqueous solubility, metabolic stability, etc.) and thus in vivo ADME properties.

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12. *MT-4 cytoprotection assay*. The HTLV-1 transformed T cell line, MT-4, is highly susceptible to HIV-1 infection. Compounds were evaluated in this target cell line by protection from HIV-induced cytopathic effects in a spreading infection. Viability of both HIV-1 and mock-infected cells were assessed to determine cytoprotection. Briefly, exponentially growing MT-4 cells were mock-infected or batch-infected with the HIV-1 laboratory strain, NL4-3, at a multiplicity of infection of 0.0005. Following a two hour infection, the cells were washed to remove unbound virus and plated in the presence of increasing concentrations of compound. After four days incubation, cytoprotection in the infected cells and compound toxicity in mock-infected cells were analyzed with a cell viability assay (ATP Lite).*PBMC drug susceptibility assay*. Human peripheral blood mononuclear cells (PBMCs) were used to test compound anti-viral activity as an indicator for clinical efficacy. PBMCs were isolated from two donors using a Ficoll-Hypaque density gradient, pooled and stimulated with PHA-L for 3 days. After stimulation, the cells were washed and maintained in culture medium containing IL-2. The stimulated cells were then mock-infected or batch-infected with the strain $HIV\text{-}1_{IIIB}$ at MOI 0.01 for 1 h. Cells (unwashed) were then plated in the presence of increasing concentrations of compound and incubated for 7 days. Viral replication in these cultures was monitored by either determining the concentration of HIV-1 p24 in the supernatant or by measuring reverse transcriptase activity. Compound toxicity in mock-infected cells was determined using a cell viability assay.
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