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Evaluation of HIV-1 inhibition by stereoisomers and analogues of the sesquiterpenoid hydroquinone peyssonol A

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ABSTRACT

Peyssonol A, a brominated natural product with documented anti-HIV-1 activity, was synthesized racemically along with 6 isomers and 15 truncated analogues and synthetic precursors. These compounds were screened in a cell-based assay against a recombinant HIV-1 strain to investigate structure–activity relationships. The results obtained suggest that both the aliphatic and aromatic domains of peyssonol A are responsible for its potency, while the stereochemical configuration of the substituents on the aliphatic domain, including their bromine atom, are largely irrelevant. Although none of the analogues tested were as potent as the parent natural product, several exhibited greater therapeutic indices due to reduced cytotoxicity, noting that nearly all compounds tested were measurably cytotoxic.

The sesquiterpenoid hydroquinone and quinone natural products arguably contain a 'privileged scaffold'[1] due to their ubiquity in nature and their incredible diversity of bioactivities.[2,3] Across that broad range of biochemical properties, however, it is their potential as inhibitors of HIV-1 that has garnered much attention over the course of the past quarter century.[4–6] Perhaps the most well-known compound with such activity is avarol; it, along with its oxidized variant, avarone (**1** and **2**, Fig. 1), has been shown to inhibit HIV-1 in cell culture at concentrations as low as 300 nM.[7] Mechanistic studies have implicated an intriguing pathway for their activity that involves inhibition of the function of a suppressor tRNA required for the readthrough of a UAG termination codon in the *gag-pol* junction of the mRNA sequence.[8] In the absence of this particular tRNA, translation is terminated prior to synthesis of the proteins encoded by the *pol* gene (reverse transcriptase, integrase, and protease). Intriguingly, naturally occurring analogues such as avarol F (**3**) and avarone E (**4**),[9] as well as related sesquiterpenoid (hydro)quinones ilimaquinone (**5**)[10] and peyssonol A (**6**),[11,12] have all been found to be allosteric inhibitors of reverse transcriptase. Thus, despite the structural similarity between these compounds and avarol, it appears that they have a complementary mechanistic pathway for HIV-1 inhibition. Nevertheless, these compounds typically suffer from high cytotoxicity, precluding their use in humans as pharmaceuticals.

In an effort to improve upon the potency and cytotoxicity of the sesquiterpenoid (hydro)quinones, a number of analogues of avarol[13,14] and ilimaquinone[10] have been synthesized via modification of the parent structures and subsequently evaluated for HIV-1 inhibition. To date, these modifications have focused exclusively on the hydroquinone/quinone rings, and the resultant compounds have generally proven to be less potent and/or significantly cytotoxic. To the best of our knowledge, no structure–activity relationship (SAR) studies have been undertaken to examine the effects of modifying the aliphatic decalin portion of the molecules; this situation is presumably due to the difficulty of functionalizing this relatively inert alkane-based portion of the framework. Herein, we describe efforts that systematically investigate the importance of the decalin framework of the sesquiterpenoid hydroquinone natural product peyssonol A (**6**) for both anti-HIV-1 activity and overall background cytotoxicity.

Our interest in this question derives from our recently published racemic total synthesis of peyssonol A (**6**),[15] the key step being a bromonium-induced polyene cyclization cascade initiated by a reagent pioneered by our group known as BDSB ($Et_2SBr{\cdot}SbBrCl_5$, bromodiethylsulfonium bromopentachloroantimonate).[16] The key finding of these efforts was that the originally assigned structure for peyssonol A (**16**, vide infra), one postulating a *cis*-decalin framework, was inaccurate. This structural correction required the preparation of four different diastereomers of the final structure, efforts which began, as shown in Scheme 1, by transforming each of the four different geometric isomers of

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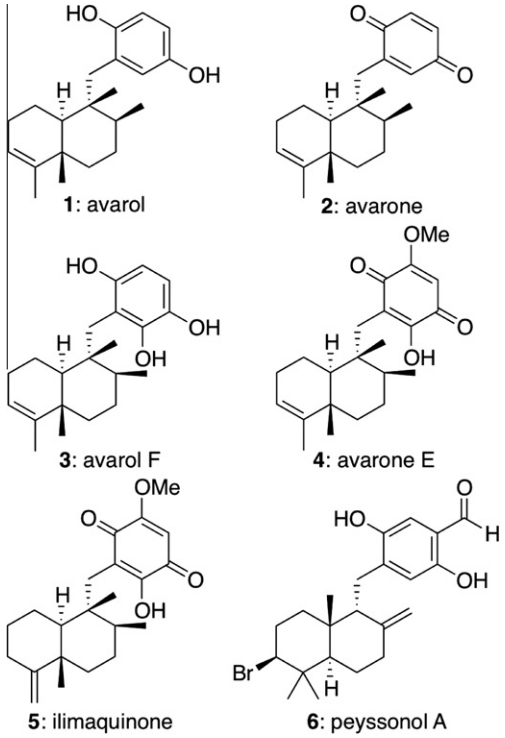
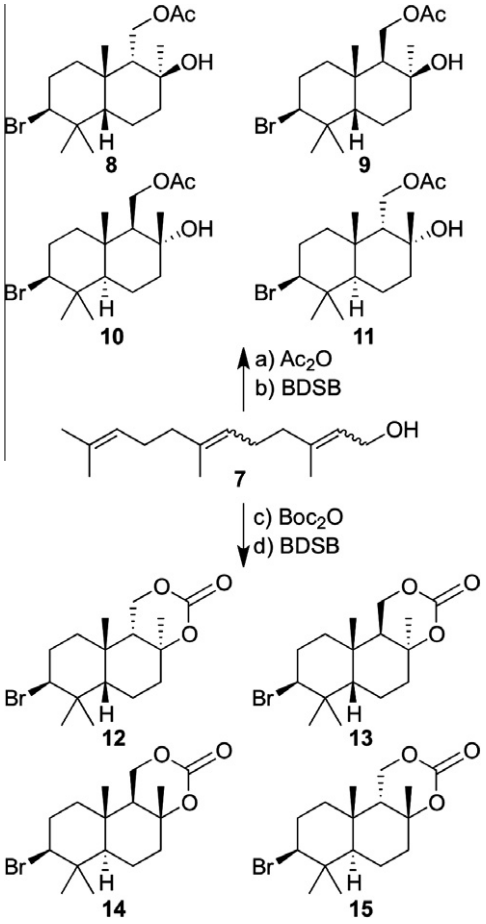


Figure 1. Sesquiterpenoid hydroquinone and quinone natural products which display anti-HIV-1 activity.

farnesol into a single stereoisomer of the requisite decalin framework, either with acetate/alcohol (i.e., **8**–**11**) or cyclic carbonate (i.e., **12**–**15**) functionality. From these synthetic precursors, we were able to produce four potential stereoisomers of the natural product (see Scheme 2); of these, *trans*-decalin **6** was spectroscopically identical to naturally occurring peyssonol A.

Given that these efforts provided access to a large array of racemic natural product analogues as well as their synthetic precursors, we sought to test their HIV-1 inhibition properties. However, in addition to those compounds depicted in Schemes 1 and 2, whose synthesis has been previously reported,[15] we also hoped to produce additional analogues to more thoroughly investigate the overall SAR effects of the aliphatic decalin portion of peyssonol A. Additionally, we recognized that the overall length of our total synthesis of peyssonol A (13 linear steps; 6% overall yield) could be a significant limitation in terms of material supply for more advanced assays; as such, we sought to identify bioactive analogues which could be prepared in far fewer steps.

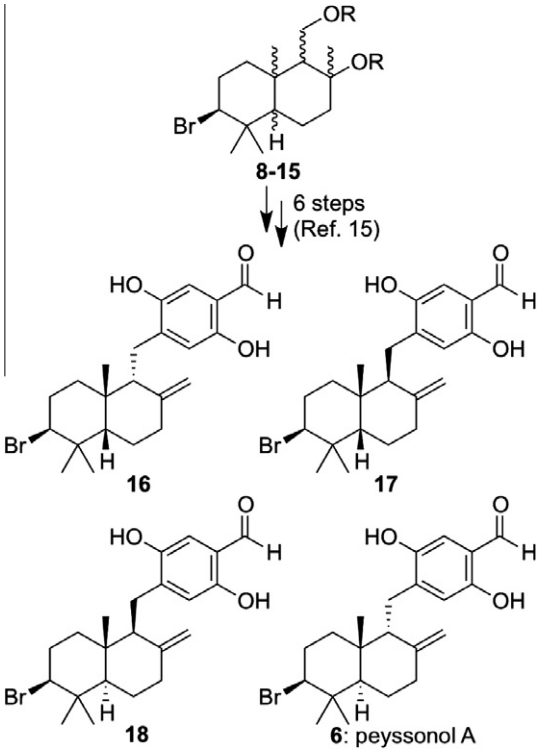
Our initial synthetic efforts along these lines were focused on the preparation of peyssonol A regioisomers (see Supplementary data for detailed Experimental procedures). These routes were inspired by our previously published total synthesis of a second natural product in the family, peyssonoic acid A (**20**),[17] and proved to be quite concise. As depicted in Scheme 3, (2*Z*,6*E*)-farnesyl bromide (**19**, accessible in five steps from commercial materials),[15] could be coupled with the aryllithium reagent derived from **21** (accessible in three steps) to produce polyene **22** in reasonably good yield. BDSB cyclization of **22** followed by in situ hydrolysis of the MOM ether and dioxolane groups resulted in tetracycle **23**. Finally, cleavage of the tertiary ether linkage with BCl_3 afforded a separable mixture of two additional peyssonol A regioisomers, **24** and **25**. As such, this modified route afforded access to three isomers of peyssonol A in fewer than 10 linear steps. It is worth noting that an even more facile synthesis, commencing with commercially available (2*E*,6*E*)-farnesol, could potentially access diastereomers of compounds **23**–**25** in only 5–6 steps, though these efforts were not undertaken in this study.



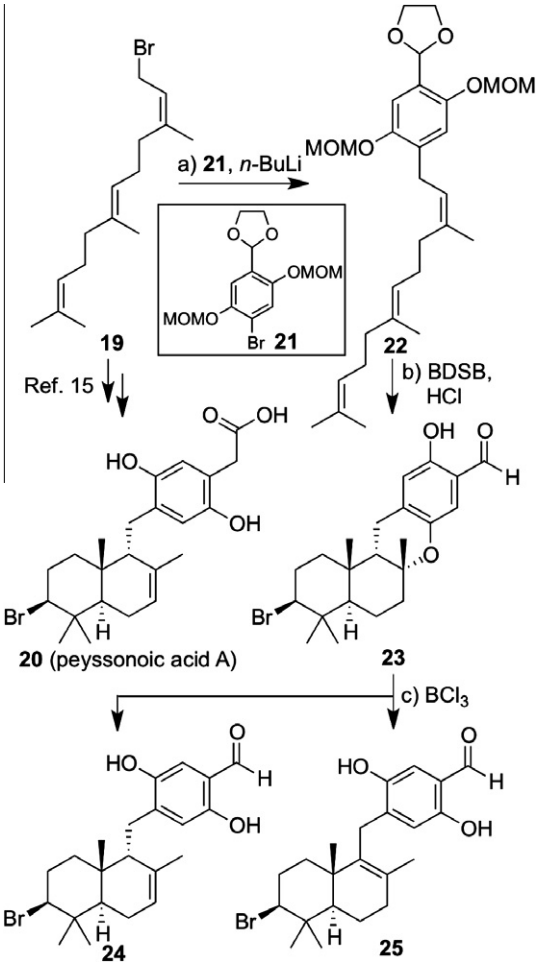
Scheme 1. Stereoselective syntheses of acetates **8**–**11** and carbonates **12**–**15**. Reagents and conditions: (a) Ac_2O, 4-DMAP, Et_3N, CH_2Cl_2, 30 min, 0 °C, 91–98%; (b) BDSB, $MeNO_2$, −25 °C, 5 min, 20–43%; (c) *n*-BuLi, Boc_2O, THF, 30 min, −78 °C to 25 °C, 91–93%; (d) BDSB, $MeNO_2$, −25 °C to 25 °C, 30 min, 26–56%.

Additional analogues were also synthesized to investigate the impact of the functional groups present on the decalin frameworks of **8**–**15**. Since carbonate **15** possesses the stereochemical configuration of the natural product (and could be prepared in higher yield than the corresponding acetate, **11**), derivatives of this compound were targeted in particular. Scheme 4 depicts the synthesis of four such compounds. Simple hydrolysis of cyclic carbonate **15** afforded **26** in high yield; this compound was targeted as a more polar variant of **11** and **15**. Meanwhile, radical debromination of **15** resulted in **27**, a compound integral to understanding whether or not the aliphatic bromide is relevant to the observed bioactivity. Likewise, the corresponding alkyl chloride, **29**, was produced via mercury-induced cyclization of (2*Z*,6*E*)-farnesol derivative **28**,[18] followed by stereoselective replacement of mercury with electrophilic chlorine.[19] Lastly, iodinated analogue **30** was synthesized by iodonium-induced cyclization initiated by IDSI, the iodine analogue of BDSB.[15]

The final compounds synthesized were intended to investigate the effects of partial or complete removal of the aliphatic portion of peyssonol A. As illustrated in Scheme 5, the phenols within commercially available 1,4-dibromo-2,5-dihydroquinone (**31**) could be protected with MOM ethers; subsequent methylation using lithium-halogen exchange followed by iodomethane addition provided **33**. A second lithium-halogen exchange facilitated a formylation with DMF, with a final acidic deprotection affording



Scheme 2. Synthesis of peyssonol A (**6**) and three diastereomers.



Scheme 3. Synthesis of peyssonoic acid A and isomers of peyssonol A (**23**, **24**, and **25**). Reagents and conditions: (a) **21**, *n*-BuLi, THF, −40 °C to 0 °C, 1 h, 67%; (b) BDSB, $MeNO_2$, −25 °C, 2 min, then H_2O, 25 °C, 1 h, 60%; (c) BCl_3, CH_2Cl_2, 0 °C, 45 min, 60% of **24**, 13% of **25**.

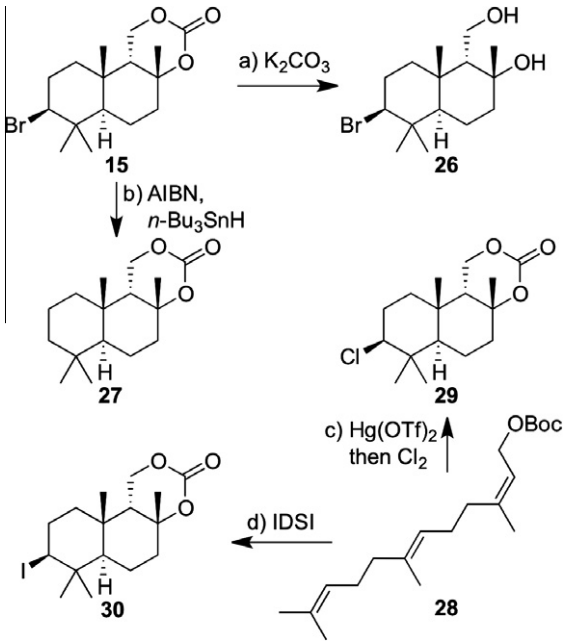
34 in good yield. Compound **36** was synthesized in a similar fashion, with its methylenecyclohexyl substituent incorporated to provide a group seemingly intermediate in size between the methyl group of **34** and the decalin system of peyssonol A and its isomers (**6**, **16**–**18**, **23**–**25**).

Bioactivity screening consisted of a cell-based assay measuring inhibition of a recombinant HIV-1 (Rep-Rluc Sac II, see Supplementary data) replicating in MT-2 human lymphocytes with cytotoxicity towards the host cell determined in a parallel experiment. To the best of our knowledge, this constitutes the first study of these natural products and analogs in cell culture for HIV-1 inhibition since previously published data[9–12] focus on RT inhibition; the results are depicted in Table 1. With an EC_{50} value of approximately 1 μM, peyssonol A (**6**) was indeed a fairly potent in vitro inhibitor of the recombinant HIV-1 although the therapeutic index (TI) was poor. Most surprisingly, however, the three diastereomers (**16**–**18**), two regioisomers (**24** and **25**) and one structural isomer (**23**) of peyssonol A exhibited bioactivity profiles nearly identical to the natural product itself with similar TIs. The consistency observed across diastereomers **16**–**18** argues that the three-dimensional shape of the aliphatic subunit is of little consequence for antiretroviral activity of peyssonol A. Meanwhile, the remarkably similar bioactivities observed for compounds **6**, **23**, **24**, and **25** suggest that the true biologically active molecule might in fact be **23**; this material could potentially be formed from the three others in a cellular environment, given that in a laboratory flask treatment of **6**, **24**, or **25** with acid affords **23**.

Interestingly enough, however, the aliphatic subunit does not strictly require the aromatic subunit to retain inhibitory activity. The single-digit micromolar EC_{50} values for nearly all the carbonates (**12**, **13**, **15**, **27**, and **30**) argue that, at least with a polar cyclic carbonate group (or diol, as in **26**) appended, the aliphatic subunit of peyssonol A is active enough to produce measurable inhibition of HIV-1. Again, the marked similarity of both potency and cytotoxicity values for compounds **15** and **26** may indicate that hydrolysis of the carbonate to the diol occurs in vivo. Most important, though, the collated results of compounds **8**–**15** serve to reinforce the notion that the stereochemical configuration of the decalin system is relatively unimportant with respect to potency. Additionally, the data from compounds **29**, **30**, and especially **27** seem to indicate that the aliphatic bromine atom is not playing a role in HIV-1 inhibition.

While the aforementioned data argue that the aliphatic subunit is sufficient for some level of HIV-1 inhibition, the results from compounds **34** and **36** make the same case for the aromatic subunit. Compound **34**, which is essentially just the aromatic ring of peyssonol A, is only a single order of magnitude less potent than the natural product with a similar TI. Meanwhile, compound **36** exhibits even better potency, as is to be expected with a larger aliphatic group appended. Additionally, the 50-fold drop in potency when comparing peyssonol A regioisomer **24** with peyssonoic acid A (**20**) indicates that the aryl aldehyde is an important contributor to the observed bioactivity.

Interestingly, peyssonol A (**6**) and its various fully elaborated regio-, stereo-, and structural isomers were the most potent of all compounds tested. However, their CC_{50} values reveal that they were also the most cytotoxic (as could be expected from their sesquiterpenoid hydroquinone structures and the presence of a potentially reactive aldehyde moiety). None of the seven peyssonol A isomers exhibited a TI >10. Meanwhile, although not quite as po-

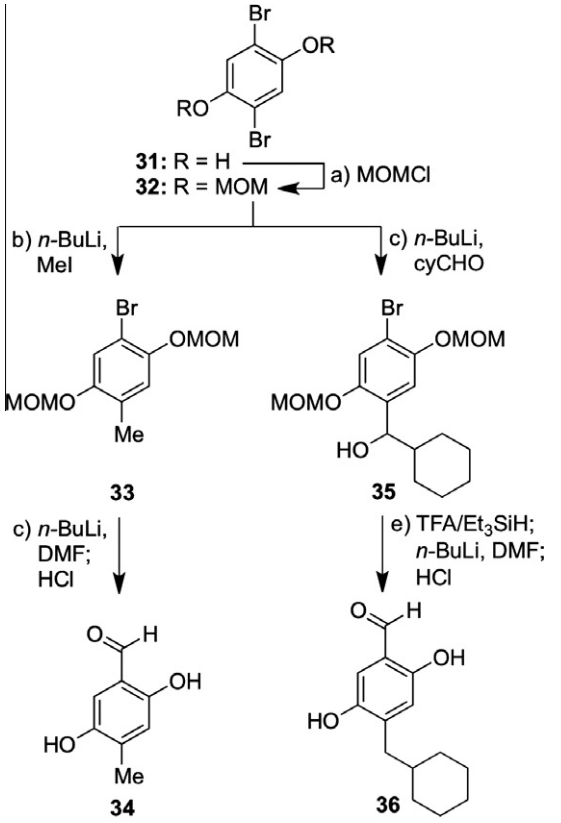


Scheme 4. Synthesis of analogues of **15**. Reagents and conditions: (a) K_2CO_3, MeOH, 40 °C, 3 h, 99%; (b) AIBN, n-Bu_3SnH, tol, 85 °C, 1 h, 90%; (c) $Hg(OTf)_2$, $MeCN/CH_2Cl_2$, 15 min, −20 °C; Cl_2, CH_2Cl_2/pyr, −40 °C, 30 min, 19% overall; (d) IDSI, $MeNO_2$, 0 °C to 25 °C, 20 min, 31%.

Table 1
Measured antiviral activities (EC_{50}) against HIV-1 NL-Rluc Virus, along with cytotoxicity values (CC_{50}) and therapeutic indexes (TI).

Compound	EC_{50}[a] (M)	CC_{50}[b] (M)	TI
6[c]	1×10^{-6}	4×10^{-6}	4
8	4×10^{-5}	$>1 \times 10^{-4}$	>2
9	2×10^{-5}	7×10^{-5}	3
10	1×10^{-5}	4×10^{-5}	3
11	1×10^{-5}	9×10^{-5}	8
12	9×10^{-6}	$>1 \times 10^{-4}$	11
13	7×10^{-6}	7×10^{-5}	9
14	1×10^{-5}	$>1 \times 10^{-4}$	>10
15	4×10^{-6}	9×10^{-5}	23
16	7×10^{-7}	5×10^{-6}	7
17	5×10^{-7}	4×10^{-6}	8
18	5×10^{-7}	2×10^{-6}	4
20[d]	5×10^{-5}	$>1 \times 10^{-4}$	>2
23	1×10^{-6}	5×10^{-6}	5
24	9×10^{-7}	4×10^{-6}	4
25	8×10^{-7}	4×10^{-6}	5
26	2×10^{-6}	9×10^{-5}	44
27	2×10^{-6}	3×10^{-5}	17
29	1×10^{-5}	4×10^{-5}	4
30	5×10^{-6}	3×10^{-5}	6
34	1×10^{-5}	3×10^{-5}	3
36	4×10^{-6}	$>1 \times 10^{-4}$	>25

[a] Virus was used to infect human lymphocyte cells (MT-2) in the presence of compounds; after 5 days of incubation, cells were processed and quantitated for virus growth by amount of expressed luciferase. Each value is an average of two to four experiments.
[b] Parallel cytotoxicity screen performed by exposing uninfected MT-2 cells to serially diluted compounds and measuring cell viability via XTT assay. Each value is an average derived from two to four experiments.
[c] Peyssonol A.
[d] Peyssonoic acid A.



Scheme 5. Synthesis of hydroquinones **34** and **36**. Reagents and conditions: (a) NaH, MOMCl, THF, 0 °C, 3 h, 95%; (b) n-BuLi, MeI, THF, −78 °C to 25 °C, 1 h, 74%; (c) n-BuLi, DMF, THF, −78 °C, 1 h, then 12 M HCl, 60 °C, 1 h, 64%; (d) n-BuLi, cyclohexane carboxaldehyde, THF, −78 °C to 25 °C, 20 min, 75%; (e) TFA, Et_3SiH, CH_2Cl_2, 0 °C, 8 h; n-BuLi, DMF, THF, −78 °C, 1 h; 12 M HCl, 60 °C, 1 h, 39% overall.

tent, nearly all the aliphatic carbonates (**12**–**15**, **27**, **29**, **30**) appeared to be less cytotoxic, such that TIs greater than ten were achieved. From this perspective, the most promising candidates for further elaboration are carbonate **15**, diol **26** and hydroquinone **36**, all three of which displayed single-digit micromolar EC_{50} values and TIs >20.[20] We do note, of course, that there could be differences in the mode of action given the significant changes in structure among these compounds.[21]

In conclusion, a brief SAR study of peyssonol A and several analogues revealed that all seven isomers of the natural product successfully inhibited HIV-1 at the micromolar or sub-micromolar range, seemingly without regard for the shape of the aliphatic portion of the molecule; unfortunately, all seven isomers also displayed high cytotoxicity. Simpler analogues composed of only the aliphatic or aromatic subunits of the natural product generally exhibited less potency. Accounting for both potency and cytotoxicity, and considering ease of synthesis, the greatest potential for future study and optimization likely lies with two very different compounds: aliphatic carbonate **14** (prepared in two steps from commercially available *E*,*E*-farnesol), and aromatic hydroquinone **36** (prepared in four steps from commercial materials).

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Supplementary data

Supplementary data (experimental procedures for synthesis of key compounds and raw HIV-1 screening data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.01.098.

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