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Synthesis and Biological Properties of Amino Acid Amide Ligand-Based Pyridinioalkanoyl Thioesters as Anti-HIV Agents

Yongsheng Song,[a,†] Atul Goel,[b,†] Venkatesha Basrur,[b] Paula E.A. Roberts,[c] Judy A. Mikovits,[c] John K. Inman,[d] Jim A. Turpin,[e] William G. Rice[a] and Ettore Appella[b,*]

[a]*Achillion Pharmaceuticals, Inc., 300 George Street, New Haven, CT O6511, USA*
[b]*Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA*
[c]*Laboratory of Antiviral Drug Mechanisms, SAIC Frederick, Frederick, MD 21702, USA*
[d]*Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892, USA*
[e]*Infectious Disease Research Department, Southern Research Institute, 431 Aviation Way, Frederick, MD 21702, USA*

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Abstract—Hyper-mutable retroviruses such as HIV can become rapidly resistant to drugs used to treat infection. Strategies for coping with drug-resistant strains of virus include combination therapies, using viral protease and reverse transcriptase inhibitors. Another approach is the development of antiviral agents that attack mutationally nonpermissive targets that have functions essential for viral replication. Thus, the highly conserved nucleocapsid protein, NCp7, was chosen as a prime target in our search for novel anti-HIV agents that can overcome the problem of viral drug resistance. Recently, we reported (*J. Med. Chem.* **1999**, *42*, 67) a novel chemotype, the pyridinioalkanoyl thioesters (PATEs), based on 2-mercaptobenzamides as the thiol component and having its amide nitrogen substituted with various phenylsulfonyl moieties. These compounds were identified as relatively nontoxic anti-HIV agents in the XTT cytoprotection assay. In this study, we wish to report a separate genre of active PATEs wherein the thiol component consists of an *N*-2-mercaptobenzoyl-amino acid derivative. Active derivatives (EC_{50} < 10 μM) reported herein were confined to amino acid primary amides or methyl amides having side chains no larger than isobutyl. Amino acids terminating in free carboxyl or carboxylic acid ester groups were mostly inactive. Selected compounds were shown to be active on chronically infected CEM/SK-1, TNFα-induced U1, ACH-2 cells and virucidal on cell-free virus, latently infected U1 cells and acutely infected primary peripheral blood mononuclear cells (PBMCs).

Introduction

The HIV/AIDS epidemic continues to take its toll worldwide, despite major advances in understanding the pathogenesis and treatment of HIV infection. One of the principal obstacles that has weakened the prospects for development of innovative therapeutic regimens is the tendency of HIV-1 to mutate to genotypes that are resistant to prevailing antiviral therapies.[1–4] One approach to overcome this problem has been the use of multidrug cocktails[5,6] that seek to cripple the virus on multiple fronts, thus slowing progression to resistance. An alternative approach is the development of antiviral agents that target novel or mutationally intolerant targets that are essential for virus replication.

The nucleocapsid p7 protein (NCp7) of HIV-1 has recently been proposed and developed as a novel antiretroviral target[7,8] for antiviral therapy based on both its biological mechanism of action and chemical properties. The biological importance of this target relates to functions of the two retroviral zinc finger domains of NCp7 that provide essential functions during early and late phases of HIV replication cycle. The Cys-Xaa_2-Cys-Xaa_4-His-Xaa_4-Cys (CCHC) motifs[9,10] are present in both zinc fingers of HIV-1 NCp7. The zinc chelating residues (three Cys, one His), as well as other specific amino acid residues in these structures, are highly conserved and mutationally intolerant.[11] Site-directed mutational studies have revealed that changes in any of the zinc chelating and a number of non-chelating residues in the zinc finger loops and adjacent sequences yields virions with defective RNA encapsidation and NCp7 function during reverse transcription that renders them noninfectious.[12–14]

*Corresponding author. Fax: +1-301-496-7220; e-mail: appellae@pop.nci.nih.gov
†These authors have made an equal contribution.

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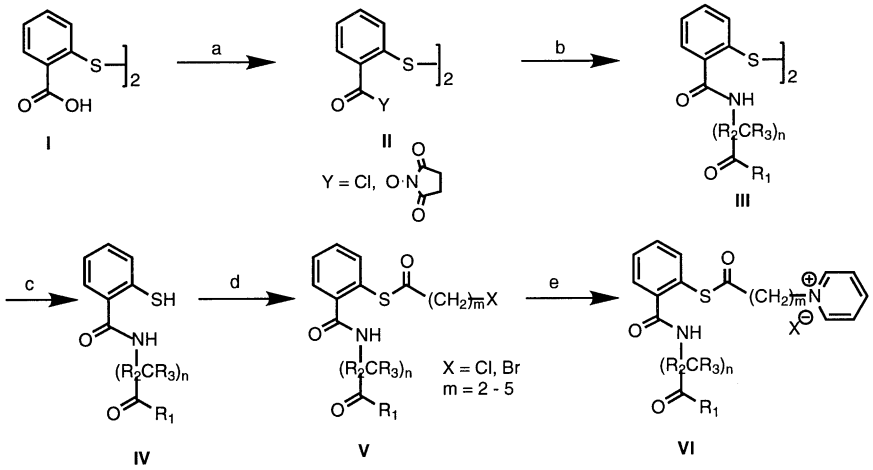


Scheme 1. Reagents and conditions: (a) $SOCl_2$, 80 °C or *N*-hydroxysuccinimide, DIC, THF-2-PrOH, 25 °C; (b) $H_2N(CR_2R_3)_nCOR_1{\cdot}HCl$, 4-methylmorpholine, DMA, 0 °C or $H_2N(CR_2R_3)_nCOR_1{\cdot}HCl$, Et_3N, DMF, 25 °C; (c) TCEP·HCl, Et_3N, DMF/H_2O (9:1), 25 °C; (d) $ClCO(CH_2)_mX$, DMA, 25 °C; (e) pyridine, under N_2, 25 °C.

Important chemical characteristics of these motifs are their spatial proximity and almost identically folded conformations around the metal ion. In each finger loop, the zinc is tetrahedrally coordinated to the sulfur atoms of three cysteine residues and the imidazole group of one histidine residue. This conformation maintains the cysteine sulfur atoms in an ionic state that is receptive to reaction with electrophiles.[15,16] This propensity for chemical modification through electrophilic attack has opened new avenues for antiretroviral drug development.

Selective covalent modification of HIV-1 zinc fingers was first demonstrated with the agent, 3-nitrosobenzamide (NOBA).[17] This initial discovery was expanded to include a number of disulfide-based molecules,[7,18–20] benzisothiazolones[18,21,22] and azodicarbonamides[23] possessing good antiviral activity and low cellular toxicity. Among them, some compounds showed significant in vitro antiviral activity ($EC_{50} = 0.5$–10 μM) with relatively low toxicity ($IC_{50} > 100$ μM). Zn finger reactivity and antiviral activity was abrogated by glutathione reduction. Thus, further development of Zn finger reactive compounds was needed to incorporate Zn finger reactivity, antiviral activity and lack of cellular cytotoxicity in the presence of cellular reducing agents.

Recently we reported[24] a novel chemotype, the pyridinioalkanoyl thioesters (PATEs), as anti-HIV-1 agents that selectively target the viral nucleocapsid protein zinc fingers without apparently affecting other viral or host zinc fingers while maintaining activity in the presence of reduced glutathione. A number of PATEs with phenylsulfonyl-based ligand structures were synthesized and tested for antiviral activity. However, efforts to generate compounds with substantially improved in vitro antiviral potencies over glutathione sensitive inhibitors was not successful. In this report, we describe the discovery of an additional class of ligand structures based on simple, aliphatic amino acid amides. PATEs with amino acid primary amide ligands offer several advantages over previously reported PATEs, such as increased antiviral potency, increased flexibility to position themselves on the NCp7 protein, low molecular weight (450–480), and enhanced water solubility without increased cytotoxicity. Several compounds with amino acid-based ligands bearing different terminal groups (primary and secondary amides, free carboxylate and carboxylate esters) were synthesized and evaluated for inhibition of HIV-1 replication and virus infectivity.

Chemistry

Various 2,2′-dithiobis (benzamides)(**III**), used as precursor molecules, were synthesized via coupling of 2,2′-dithiobis(benzoyl chloride) or *N,N*′-disuccinimidyl-2,2′-dithiosalicylate (**II**, prepared from the commercially available 2,2′-dithiosalicylic acid (**I**), *N*-hydroxysuccinimide and 1,3-diisopropylcarbodiimide) with the desired amino acid or amino acid derivative as shown in Scheme 1. These disulfide benzamides were then reduced to 2-mercaptobenzamides (**IV**) using tris(2-carboxyethyl)phosphine hydrochloride (TCEP.HCl) which, on further reaction with ω-bromo/chloroalkanoyl chlorides, afforded 2-(ω-bromo/chloro alkanoylthio)-benzamides (**V**). These compounds next were dissolved in pyridine. All bromo derivatives were easily converted to the corresponding pyridinium salt (**VI**) by stirring this solution at room temperature under nitrogen for 8–48 h; however, the chloroalkanoyl compounds required heating to 40–50 °C to effect conversion to the pyridinium salt. All synthesized compounds were characterized by spectroscopic analyses (NMR, MS), and data for representative compounds are given in the Experimental section.

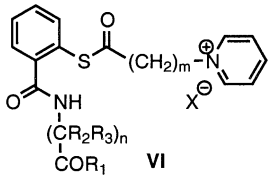
Results and Discussion

Table 1 summarizes the antiviral activity (EC_{50}), cellular toxicity (IC_{50}), and structure activity relationships of compounds having short-chain amino acid derivatives

as ligands linked to the benzoyl carbonyl group. In contrast to previously published[24] PATEs ($M_r > 500$) the present amino acid amide PATEs were designed to possess molecular weights ($M_r < 500$) that have been associated with favorable pharmacological parameters. Compounds with primary amide functionality at the terminus of the ligand ($R_1 = NH_2$) demonstrated significant anti-HIV activity as evident from the activity profiles of **2**–**10** and **15**–**19**, but not **1** and **11**–**14**. In contrast, compounds having free carboxylic acid ($R_1 = OH$) or ester ($R_1 = OMe,OEt$) functionalities were either inactive (**21** and **23**–**26**) or highly toxic (**22**). These findings may be explained by the hydrogen bond donor ability of the amide; this could potentially result in a greater binding affinity to binding sites on NCp7. Carboxylic acids and their esters are evidently poor hydrogen bonding partners with NCp7 consequently, leading to complexes that are either too weak or unfavorably oriented for effective transfer of the pyridinioalkanoyl group to the target zinc finger cysteine side chain (see ref 25a for discussion of this mechanism).

Based on these observations, several compounds terminating in primary amides, but differing in carbon chain length, were prepared and evaluated for in vitro anti-HIV activity. It was interesting to note that all the compounds of this type (**2**–**8**, **15**–**19** except **1** and **9**–**14**) showed similar EC_{50} values (0.9–2.3 μM), but exhibited a wide range of cellular toxicity (IC_{50}) values (5.0–200 μM). Short carbon chain ($n = 1$) homologue (**6**) demonstrated better therapeutic indices ($TI = IC_{50}/EC_{50}$) in comparison to higher homologues ($n > 2$), for example, **18** and **19**, where extension to three and four carbon molecules clearly resulted in greater compound cytotoxicity. Again, the increased activity for lower homologues may result from a conformational arrangement of the atoms that allow a more favorable positioning in the interaction cleft of NCp7. Among the straight chain (**6**, **16**, and **18**–**19**) and branched chain (**4**, **5**, **7**–**15**, and **17**) compounds, ones with a methylene spacer ($n = 1$; **6** and **16**), or those having a small side chain (R_2, $R_3 = H/CH_3$, CH_2CH_3; **4**, **7**, **8**, **15**, and **17**), exhibited potent antiviral activity ($EC_{50} < 2.3$ μM) and relatively low cytotoxicity ($IC_{50} > 100$ μM; except **4** and **15**). Compounds with larger branched chains (**5** and **9**–**11**), in general, were less efficacious; either lacking antiviral activity or possessing greater cytotoxicity. Changing from a small side chain ($R_3 = CH_3$ as in **7** and keeping other parameters the same) to more complex and bulky groups (R_3 = benzyl or carbamylalkyl as in **12**–**14**) resulted in significantly reduced antiviral activity.

Table 1. Antiviral activity of novel pyridinioalkanoyl thioesters (**VI**)



VI

Entry	R_1	R_2	R_3	n	m	X	Antiviral activity[a] EC_{50} (μM)	Cellular toxicity IC_{50} (μM)	TI
1	NH_2	H	H	1	2	Cl	I	6.8	—
2	NH_2	H	H	1	3	Cl	1.6	5.4	3.4
3	NH_2	H	H	1	3	Br	2.2	43	20
4	NH_2	H	CH_3	1	3	Br	1.5	63	43
5	NH_2	H	$CH(CH_3)(C_2H_5)$	1	3	Cl	0.92	10	11
6	NH_2	H	H	1	4	Br	1.8	>200	>109
7	NH_2	H	CH_3	1	4	Br	0.94	101	107
8	NH_2	H	CH_2CH_3	1	4	Br	1.9	200	103
9	NH_2	H	$CH(CH_3)_2$	1	4	Br	5.8	9.9	1.7
10	NH_2	H	$CH_2CH(CH_3)_2$	1	4	Br	3.9	5.2	1.3
11	NH_2	H	$CH(CH_3)(C_2H_5)$	1	4	Br	I	20.1	—
12	NH_2	H	$CH_2C_6H_5$	1	4	Br	I	4.5	—
13	NH_2	H	CH_2CONH_2	1	4	Br	31	>316	>10
14	NH_2	H	$CH_2CH_2CONH_2$	1	4	Br	71	>316	>4
15	NH_2	CH_3	CH_3	1	4	Br	0.87	23	26
16	NH_2	H	H	1	5	Br	2.3	110	48
17	NH_2	H	CH_3	1	5	Br	1.4	132	94
18	NH_2	H	H	3	4	Br	0.80	12	15
19	NH_2	H	H	4	4	Br	1.4	9	6.4
20	$NHCH_3$	H	H	1	4	Br	6.6	21	3.2
21	OH	H	H	1	4	Br	230	>316	>1.4
22	OH	H	H	2	4	Br	2.3	10	4.3
23	OC_2H_5	H	H	1	4	Br	I	>316	—
24	OC_2H_5	H	H	2	4	Br	I	1.4	—
25	OC_2H_5	H	H	3	4	Br	I	0.9	—
26	OCH_3	H	CH_3	1	4	Br	I	5.9	—

[a]Antiviral activity was measured by the XTT cytoprotection assay as described in the Experimental. I, no antiviral activity was observed at or below the concentration of compound that caused cellular toxicity. All determinations were performed in duplicate with triplicate determinations for each compound concentration. The variation of individual triplicates was 10% or less.

These results were consistent with mechanism of action studies suggesting that large, bulky side chains may alter the mode of binding to NCp7.

Previous mechanism of action studies[25a] demonstrated that the pyridinioalkanoyl moiety of some PATEs bind covalently to the cysteine sulfurs of NCp7 resulting in complete loss of nucleocapsid function as evidenced by loss of virus infectivity and inhibition of virus replication. Studies[24] have also suggested that the antiviral activity of this chemotype was influenced by the length of the alkyl spacer between the carbonyl group and the pyridinium nitrogen atom. These results prompted us to re-evaluate this parameter in amino acid amides. Accordingly, compounds **1**–**7** and **15**–**17** were synthesized with the alkyl spacer varying from $m = 2$–5. With regard to the positioning of the pyridinium cation, the spacing, $-(CH_2)_m$, where $m = 3$–5, appeared to have only a minor effect on anti-HIV potency (EC_{50} values in the range of 0.9–2.3 μM). An alkyl chain of four CH_2 units gave rise to the least toxic and most potent analogues when coupled to the most favorable ligand structures (**6**: $EC_{50} = 1.8$ μM, TI > 109; **7**: $EC_{50} = 0.94$ μM, TI = 107). In contrast, the shortest spacing tested (**1**, $m = 2$) gave rise to an inactive thioester, in a manner similar to previous observations.[24]

The discovery of the susceptibility of the NCp7 nucleocapsid protein to electrophilic agents has resulted in the identification of a panel of biochemical and cell-based methodologies to verify target selectivity. Table 2 summarizes the assessment of compounds **3**, **6**, **7**, **16**, and **17** for their ability to eject Zn^{2+} ions from NCp7, inactivate cell-free virus and inhibit virus replication in acute, chronic and post-integrative (latent) models of HIV-1 replication. In general, compared to the previously published PATEs, the lead amino acid amide PATEs demonstrate a 2- to 20-fold increase in antiviral potency in U1 cells, a 2-fold potency for virucidal activity (Table 9 in ref 24), and a 4- to 8-fold increase in potency for inhibition of acute HIV-1 infection.

All five lead amino acid amide-conjugated PATEs demonstrated ejection of Zn^{2+} ions from purified recombinant NCp7 in the presence of Ag^+. We have found in our previous studies[24,25a] that PATEs appear to be weakly reactive toward the target NCp7 cysteine side chains. However, this property may confer considerable advantage in target selectivity. Yet, in order to visualize the process of zinc ejection within the timeframe of our experimental protocol, it was necessary to accelerate the transacylation reaction by 'activation' with Ag^+ ions.[25a–c] It was shown that $AgNO_3$ alone did not cause detectable release of Zn^{2+} in this assay. We have clear evidence by NMR that in the absence of Ag^+, these types of molecules can modify the target cysteine sulfurs by a transacylation (thioester exchange) reaction (unpublished results). Furthermore, we found by mass spectral analysis that the only observed mass increments to the target NCp7 were due to the addition of one or two pyridiniovaleryl moieties (Δ 162 mass unit each). Thus, we could rule out an alternative reaction mechanism, such as 1,2- or 1,4-additions of a target cysteine thiol to the pyridinio group. The potential of these compound to mediate their antiviral activity via interaction with the NCp7 protein was further demonstrated by their ability to inactivate cell-free virions.[23,24,26] All 5 compounds reduced virus infectivity with 50% inhibitory concentrations in the 5–6 μM range, following a 4 h incubation at 37 °C and removal of residual compound by centrifugation (18,000*g*, 2 h, 4 °C). The control compounds AZT and dextran sulfate failed to reduce virus infectivity under the same conditions (data not shown). Thus the lead compounds maintain a functional phenotype that is consistent with antiviral activity mediated by ejection of zinc from NCp7.

We next assessed the five lead compounds for activity in acute, chronic and latently infected cells. The proven abilities of NCp7 inhibitors to inactivate cell-free virus,[23,24,26] disrupt reverse transcription[27] and inhibit post-integrative events during HIV replication provides potent inhibition of virus replication in a variety of in vitro HIV-1 replication models. All five compounds were potent inhibitors of acute infection in peripheral blood mononuclear cells (PBMCs), with compounds **7** and **16** exhibiting submicromolar EC_{50} values of 0.8 and

Table 2. Range of antiviral action for selected pyridinioalkanoyl thioesters[a]

Compd	Reactivity with NCp7[b]	Chronically infected cells[c] (EC_{50} μM)	U1 cells[d] (EC_{50} μM)	PBMC[e] (EC_{50} μM)	Virucidal[f] (IC_{50} μM)
3	+	15.4	5.0	2.8	5.5
6	+	18.2	4.9	9.8	6.3
7	+	6.8	2.0	0.8	6.8
16	+	6.4	4.2	0.9	6.6
17	+	16.4	5.0	1.9	6.4

In all cases, except compound **17**, the concentration resulting in 50% loss of cell viability (IC_{50}) was greater than 90 μM. For compound **17** the IC_{50} was greater than 70 μM.

[a]All determinations were preformed in triplicate with the intra-triplicate variation of the mean less than 10%.

[b]Ejection of Zn^{2+} from purified NCp7 protein was performed in the presence of Newport Green and $AgNO_3$ as described previously.[24]

[c]CEM-SS cells chronically infected with the SK-1 strain of HIV-1 were assessed for supernatant RT following 6 days of treatment.

[d]U1 cells were TNFα induced and expression of supernatant RT determined at day 3 post-treatment.

[e]Expression of supernatant RT was determined 6 days post-infection with HIV-1 RoJo.

[f]Reduction in infectivity of HIV-1 IIIB following a 4 h incubation with PATEs. Residual infectivity was determined in HeLa CD4 LTR β-gal cells by chemiluminescence.

0.9 μM, respectively. Compound **6** was the least potent with an EC_{50} of 9.8 μM. As shown in Table 1, compounds **6** and **16** vary by four versus five methylene spacer groups, respectively between the thioester and pyridinium group and **6** and **7** vary by the presence of a methyl side-chain group in the ligand portion of the PATE, further supporting the observations above of an optimal fit for the amino acid amide PATEs with the NCp7 molecule for successful inhibition of virus replication. Interestingly, the ligand with a methyl group at R_3 (**17**) resulted in a 2-fold loss on potency versus **16** (EC_{50} 0.91 vs 1.9 μM, respectively) in the five methylene spacer configuration. Finally, all five compounds were nontoxic at a high test concentration of 100 μM in PBMCs.

The lead compounds were next assessed for activity against CEM-SS cells chronically infected with the SK-1 isolate of HIV-1. Although, the compounds were less potent at inhibiting virus replication in chronically infected CEM-SS cells than PBMCs, all five compounds prevented virus replication with EC_{50}'s ranging from 6 to 18 μM (Table 2). Interestingly, the observed differences between compounds **6**, **7**, **16**, and **17** were maintained, although the magnitude of difference in the EC_{50}'s decreased from approximately 10- to 3-fold. As observed with the PBMCs, the five lead compounds were also non-cytotoxic to chronically infected CEM-SS cells ($IC_{50} > 90$ μM). Similar effects were seen in using H9 cells that were chronically infected with HIV IIIB (data not shown).

Previously,[24] we have shown that the PATEs are potent inhibitors of HIV-1 replication in post-integrative latent models of HIV-1 infection. Interaction of TNFα-induced U1 cells with the PATEs and other zinc finger inhibitors, are characterized by extensive modification and cross-linkage of HIV-1 Gag precursor polyproteins containing the CCHC zinc finger motif.[28] The five lead amino acid amide PATEs were micromolar inhibitors (EC_{50} 2–5 μM) of HIV-1 replication in TNFα-induced monocytic U1 (Table 2) and T-lymphocytic ACH-2 cells (data not shown). In contrast to inhibition of virus replication in acutely and chronically infected cells, antiviral activity does not appear to be as sensitive to the structural differences between **6** and **7** or **16** and **17**. However in contrast to the acute and chronic infection models, **17** possessed limited cytotoxicity in both U1 (IC_{50} 67 μM) and ACH-2 (IC_{50} 79.6 μM) cells, suggesting loss of specificity for the NCp7 zinc finger and increased reactivity with cellular Zn fingers.[15] In previous studies,[24] we have shown that the effects of PATEs on U1 cells was associated with extensive cross-linking of NCp7 and retardation of gag precursor polyprotein processing in both cells and viruses released by the U1 cells. Western blotting revealed the expected patterns of 2-mercaptoethanol reversible disulfide cross-linking of NCp7 and inhibition of $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ polyprotein processing in U1 cells (data not shown). Therefore the amino acid amide-linked PATEs represent a new class of Zn finger inhibitors with increased antiviral potency.

Conclusions

The involvement of HIV-1 NCp7 zinc fingers in multiple phases of the HIV-1 replication cycle and their mutationally nonpermissive nature has provided incentives for developing molecules to successfully interact with these structures as targets for antiretroviral therapy. Our previous efforts yielded pyridinioalkanoyl thioester (PATE) derivatives that overcame the cellular toxicity and sensitivity to reductive cleavage associated with disulfide benzamides; yet these compounds had not been optimized with regard to antiviral potency or molecular size. The efforts described here were designed to further optimize the in vitro therapeutic indices of the PATEs and potentially identify new lead pharmacophores within this class of compounds. By optimizing the length of the alkanoyl chain and the substituent group to the benzamide nitrogen, we have identified a subclass of PATEs that show improved antiviral potency. Previous PATEs incorporated phenylsulfonyl-based benzamide substituents, whereas the corresponding structures reported here were derived from amino acid primary amides. This new subclass of amino acid-based PATEs demonstrated activity in latently infected U1 and ACH-2 cells, peripheral blood mononuclear cells and chronically infected cells, and mechanistically mediated zinc ejection from purified NCp7 protein, were virucidal to cell-free virus and altered Gag polyprotein protein processing, thus showing that their mechanism(s) of action are compatible with NCp7 inhibition. The new PATE chemotypes reported here also demonstrated increased antiviral activity with the EC_{50}'s of selected amino acid amide-liganded PATEs in the submicromolar range, while maintaining low or no cellular toxicity. In several cases, the PATEs with amino acid amide-based ligands showed substantial improvement of antiviral activity over previously published PATEs.[24] Finally, this new generation of PATEs acts both intracellularly and extracellularly to render virus incapable of replicating, functions that were segregated to specific PATE congers in previous studies. Thus, we have significantly improved antiviral potency and constructed a new PATE pharmacophore that incorporates the ability to disrupt HIV-1 NCp7 retroviral zinc finger function during late phases of virus assembly and release, as well as in cell-free virions.

The observed characteristics of this new family of PATEs are extremely interesting and in light of recent observations of persistent, chronically infected reservoirs in patients undergoing standard chemotherapies for HIV-1 disease.[29–31] Thus, they provide the potential for chemotherapeutic elimination of these reservoirs. Multiple studies have shown that reservoir persistence is due to continual low levels of re-infection or re-seeding of specific T cell populations, even during suppression of virus replication below current detection methodologies. The use of low molecular weight amino acid amide PATEs in conjunction with current combination chemotherapies and HAART could be an effective strategy to circumvent re-seeding by reducing the infectivity of both cell-free and cell-associated virus, thus potentially resulting in the reduction or sterilization of virus reservoirs by disruption of the re-seeding process.

Table 3. 2,2′-Dithiobisbenzamides (**III**)

Entry	Starting material (RH)	Method	Yield (%)	^{1}H NMR (DMSO-d_6)
a	Glycinamide	A	65	8.9 (t, 2H), 7.85 (d, 2H), 7.7 (d, 2H), 7.53 (m, 4H), 7.38 (t, 2H), 7.13 (s, 2H), 3.9 (d, 4H)
b	L-Alaninamide	D	92	8.66 (d, 2H), 7.84 (d, 2H), 7.7 (d, 2H), 7.55–7.4 (m, 4H), 7.35 (t, 2H), 7.1 (brs, 2H), 4.45 (q, 2H), 1.4 (d, 6H)
c	Isoleucinamide	D	95	8.5 (d, 2H), 7.72 (t, 2H), 7.56–7.44 (m, 6H), 7.35 (t, 2H), 7.16 (brs, 2H), 4.36 (t, 2H), 2.05–1.85 (m, 2H), 1.7–1.45 (m, 2H), 1.35–1.15 (m, 2H), 1.05–0.8 (m, 12H)
d	L-2-Aminobutyric acid[a]	C, E	84	8.6 (d, 2H), 7.82 (d, 2H), 7.7 (d, 2H), 7.5 (m, 4H), 7.35 (t, 2H), 7.13 (s, 2H), 4.37 (q, 2H), 1.8 (m, 4H), 1.0 (t, 6H).
e	L-Valinamide	B	84	8.47 (d, 2H), 7.73 (q, 4H), 7.5 (t, 4H), 7.35 (t, 2H), 7.17 (s, 2H), 4.35 (t, 2H), 2.17 (m, 2H), 1.0 (d, 12H).
f	L-Leucinamide	B	96	8.65 (d, 2H), 7.8 (d, 2H), 7.7 (d, 2H), 7.5 (m, 4H), 7.35 (t, 2H), 7.07 (s, 2H), 4.5 (m, 2H), 1.85–1.5 (m, 6H), 0.95 (d, 12H).
g	L-Phenylalaninamide	B	93	8.75 (d, 2H), 7.7–7.15 (m, 22H), 4.7 (m, 2H), 3.25–2.9 (m, 4H).
h	L-Asparaginamide	B	78	8.75 (d, 2H), 7.8 (d, 2H), 7.7 (d, 2H), 7.5 (t, 2H), 7.38 (m, 6H), 7.15 (s, 2H), 6.98 (s, 2H), 4.75 (q, 2H), 2.65 (d, 4H).
i	L-Glutaminamide	B	72	8.75 (d, 2H), 7.85 (d, 2H), 7.7 (d, 2H), 7.6–7.3 (m, 8H), 7.14 (s, 2H), 6.85 (s, 2H), 4.38 (m, 2H), 2.25 (m, 4H), 2.0 (m, 4H).
j	2-Aminoisobutyric acid[a]	C, E	43	8.48 (s, 2H), 7.84 (d, 2H), 7.7 (d, 2H), 7.5 (t, 2H), 7.35 (t, 2H), 7.22 (s, 2H), 6.98 (s, 2H), 1.5 (s, 12H).
k	4-Aminobutyramide	A	40	8.7 (t, 2H), 7.68 (d, 4H), 7.5 (t, 2H), 7.35 (t, 2H), 6.83 (s, 4H), 3.32 (q, 4H), 2.2 (t, 4H), 1.82 (quintet, 4H).
l	5-Aminovaleramide[b]	F, C	89	8.65 (t, 2H), 7.66 (d, 4H), 7.48 (t, 2H), 7.3 (m, 4H), 6.76 (s, 2H), 3.38 (q, 4H), 2.15 (t,4H), 1.6 (m, 8H).
m	*N*-Methylglycinamide	D	90	8.94 (t, 2H), 7.94 (brs, 2H), 7.05 (d, 2H), 7.68 (d, 2H), 7.54 (t, 2H), 7.36 (t, 2H), 3.9 (d, 4H), 2.66 (d, 6H).
n	Glycine-*t*-butyl ester	B	81	9.05 (t, 2H), 7.73 (q, 4H), 7.52 (t, 2H), 7.37 (t, 2H), 3.97 (d, 4H), 1.48 (s, 18H).
o	D-Alanine-*t*-butyl ester	B	91	8.75 (t, 2H), 7.65 (q, 4H), 7.5 (t, 2H), 7.35 (t, 2H), 3.5 (q, 4H), 2.55 (t, 4H), 1.45 (s, 18H).
p	Glycine ethyl ester	B	91	9.15 (t, 2H), 7.8 (d, 2H), 7.72 (d, 2H), 7.53 (t, 2H), 7.38 (t, 2H), 4.2 (q, 4H), 4.08 (d, 4H), 1.26 (t, 6H).
q	D-Alanine ethyl ester	B	83	8.78 (t, 2H), 7.65 (m, 4H), 7.5 (t, 2H), 7.35 (t, 2H), 4.13 (q, 4H), 3.55 (q, 4H), 2.65 (t, 4H), 1.25 (t, 6H).
r	Ethyl 4-aminobutyrate	B	90	8.68 (t, 2H), 7.8 (d,4H), 7.5 (t, 2H), 7.35 (t, 2H), 4.1 (q, 4H), 3.35 (m, 4H), 2.45 (t, 4H), 1.77 (quintet, 4H), 1.23 (t, 6H).

[a]Disulfide benzamide having a free terminal carboxyl groups are first prepared by method C and then converted to amide by method E.
[b]5-Aminovaleramide was synthesized from 5-(*tert*-butoxy carbonylamino)valeric acid according to method F.

Experimental

Antiviral methods

XTT cytoprotection assay. Antiviral activity of compounds was performed with CEM-SS cells and HIV-1_{RF} (MOI = 0.01) using the XTT (2,3-bis[2-methoxy-4-nitro-5 - sulfophenyl] - 5 - [(phenylamino)carbonyl] - 2*H* - tetrazolium hydroxide) cytoprotection assay as previously described.[17,26] Effective antiviral concentrations providing 50% cytoprotection (EC_{50}), and cellular growth inhibitory concentrations causing 50% cytotoxicity (IC_{50}) were calculated. Efficacy of the compounds was further evaluated by calculation of the in vitro therapeutic index (IC_{50}/EC_{50}) which represents a relative measurement of antiviral success. 3′-Azido-2′-3′-dideoxythymidine (AZT) and dextran sulfate were utilized as reference compounds for antiviral activity.

Cell-free virus inactivation. Cell-free virus inactivation (virucidal assay) assays were performed as previously described.[27] Reverse transcription of human immunodeficiency virus type 1 is blocked by retroviral zinc finger inhibitors. Briefly, a known titer of the IIIB strain of HIV-1 was preincubated for 4 h at 37 °C with test materials and excess compound removed by centrifugation at 18,000*g* for 2 h at 4 °C. The virus pellets were suspended in RPMI 1640 supplemented with 10% heat inactivated fetal calf serum, 2 mM L-glutamate, 100 U/mL penicillin and 200 μg/mL streptomycin and placed on HeLa CD4 LTR β-gal cells for 48 h. At 48 h, the media was removed and β-galactosidase enzyme expression determined by chemiluminescence per manufacturers instructions (Gal-screen™, Tropix, Bedford, MA, USA). Compound toxicity was monitored on a sister plate using CellTiter96® reagent (Promega Corp. Madison, WI, USA), a single solution formazan-based dye reduction system similar to XTT for measuring cell survival and viability.

NCp7 reactivity. The methods to produce NCp7 protein and monitor ejection of Zn^{2+} from the protein have been described in detail elsewhere.[25a] Briefly, Zn ejection was measured following addition of compounds to NCp7 (1 μM) in zinc ejection assay buffer containing 10 μM Newport Green (Molecular Probes, Eugene, OR, USA) and 20 μM $AgNO_3$ in 20 mM phosphate buffer. Changes in fluorescence of the Newport Green following chelation of Zn were monitored at 540 nm (λ_{ex} 490 nm).

Antiviral assays. Acute infection assays were performed on PBMCs isolated from hepatitis and HIV sero-negative donors by ficoll hypaque gradient centrifugation as previously described.[32] Antiviral assays were accomplished with 3–day–old phytohemagglutinin/IL-2 stimulated PBMC. All antiviral evaluations were performed

in triplicate in RPMI 1640 supplemented with 10% fetal bovine serum, L-glutamate (2 mM), penicillin (100 U/mL) and streptomycin (100 μg/mL). HIV replication in PBMC cultures was determined by measurement of supernatant reverse transcriptase (RT) activity.[33] Cell viability was determined using the CellTiter® reagent. All determinations were performed in triplicate and the standard deviation within triplicates was less than 10%. AZT was used as a positive control for all assays.

Chronic infections were preformed with CEM-SS cells chronically infected with the SK-1 strain of HIV (CEM/SK-1). CEM/SK-1 cells were cultured in RPMI 1640 supplemented as described above. Fifty thousand (5.0×10^4) exponentially growing, CEM/SK-1 cells were incubated for 6 days (37 °C, 5% CO_2) in a final volume of 200 μL with or without test compound. Virus replication and compound cytotoxicity were measured by RT expression in cell-free supernatants and CellTiter96® dye reduction, respectively.

The effect of compounds on virus expression from TNFα-induced, latently infected cells was performed using U1 and ACH-2 cells obtained from the AIDS Research and Reference Reagent Program (Bethesda, MD, USA). The cells were maintained in RPMI 1640 supplemented as described above. U1 cells are derived from the histocytic leukemia cell line U937, and contain a single integrated (HIV IIIB)cytokine inducible provirus.[34] ACH-2 cells are derived from the A3.01 T lymphoblastic cell line, and contain two copies of the HIV IIIB provirus.[35] Fifty thousand U1 cells were induced with 10 ng/mL of TNFα (R& D Systems, Minneapolis, MN, USA) in the presence of the test compounds. Cultures were incubated for 3 days, and virus expression measured by supernatant RT activity, and cytotoxicity determined by CellTiter96® dye reduction.

Organic synthesis. All organic reagents and intermediates were obtained from Sigma and Aldrich (St Louis, MO, USA). Solvents and others chemicals were reagents grade. Melting points were determined in open capillary tubes on a Buchi 510 melting point apparatus and are uncorrected. Proton magnetic resonance (NMR) spectra were recorded on a 250 MHz Perkin–Elmer spectrometer. Chemical shifts are reported in δ values relative to tetramethylsilane. Electron-impact ionization (EI) and fast-atom bombardment (FAB) mass spectra were recorded on VG-70E and Jeol JMS-SX102 spectrometer, respectively. TLC was carried out with precoated silica gel on glass (EM Science Silica Gel 60 F_{254}) TLC plates. Column chromatography was used for purification of final products with a Silica gel (70–270 mesh, 60 Å, from Aldrich) using methanol/acetic acid (9:1) or ethyl acetate/methanol (7:3) as eluent. The synthesis and spectral data of all the intermediate compounds were not given, but one example of each intermediate is reported in this section.

General procedures for the preparation of parent disulfide benzamides (III). All the disulfide benzamides were synthesized according to general procedures A–E, and data are shown in Table 3.

Method A. To a solution of amide hydrochloride (25 mmol) in *N*,*N*-dimethylacetamide (DMA, 30 mL) and water (5 mL) was added 4-methylmorpholine (NMM, 5 mL, 45 mmol) and 2,2′-dithiobisbenzoyl chloride (3.43 g, 10 mmol) at 0 °C. The solution was stirred at 0 °C for 1 h and at room temperature for 1–3 days. Reaction was monitored by TLC. The reaction mixture was poured into 0.5 M HCl (300 mL). The precipitate thus obtained was collected, washed with water and dried under vacuum.

Method B. This was the same as described in method A but in the absence of water (5 mL).

Method C. To a solution of potassium *t*-butoxide (2.8 g, 25 mmol) in 2-propanol (25 mL) was added amine hydrochloride (10.8 mmol) at 0 °C. To the clear solution was then added 2,2′-dithiobisbenzoyl chloride (1.7 g, 5 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h and at room temperature over night. The mixture was added dropwise into 0.1 M HCl (200 mL) with constant stirring. The precipitate was collected, washed with water and dried.

Method D. To a solution of 2,2′-dithiosalicylic acid (2 mmol) in a mixture of THF (14 mL) and 2-propanol (6 mL) was added *N*-hydroxysuccinimide (4.4 mmol) and 1,3-diisopropylcarbodiimide (4.1 mmol). The solution was stirred at room temperature for 4 h. The white precipitate thus obtained was filtered, washed with 2-propanol (20 mL) in portions, yielding *N,N*′-disuccinimidyl-2,2′-dithiosalicylate (89%). A solution of this compound (1.0 mmol) in DMF (5 mL) was added amino acid amide hydrochloride (2.2 mmol) and triethylamine (0.28 mL). The solution was stirred at room temperature for 4–6 h. After completion of the reaction, the solvent was removed under vacuum, and the remaining residue was treated with 20% hot ethanol (40 mL). The white precipitate was collected and washed with hot water yielding the corresponding 2,2′-dithiobisbenzamides (90–95%).

Method E. To a clear solution of *N*-hydroxysuccinimide (2.84 g, 24.8 mmol) in 2-propanol (30 mL) and DMA (15 mL) was added disulfide benzamide bearing terminal carboxyl groups (10.6 mmol) at room temperature. The clear solution was cooled to 0 °C and 1,3-diisopropylcarbodiimide (3.51 mL, 24.8 mmol) was added. The reaction mixture was stirred at 0 °C for 0.5 h and at room temperature for 3 h. To the mixture was added gradually 2 M NH_3 in 2-propanol (10.8 mL, 21.6 mmol) at room temperature. The mixture was stirred at room temperature overnight and added into ethyl ether (400 mL). The white precipitate was collected by filtration and dissolved in DMF (40 mL). The solution was then added dropwise to 1 M HCl (400 mL) with stirring. The white precipitate was collected, washed with water and dried under vacuum to yield an amide ligand-based disulfide benzamide.

Method F. The starting material of 5-aminovalerylamide was first prepared using 5-(*tert*-butoxycarbonylamino)valeric acid. To a solution of 5-(*tert*-

butoxycarbonylamino)valeric acid (8.7 g, 40 mmol) in 2-propanol (150 mL) was added *N*-hydroxysuccinimide (5.1 g, 44 mmol) at room temperature. The mixture was cooled to 0 °C and 1,3-dicyclohexylcarbodiimide (8.7 g, 42 mmol) was added dropwise. The yellow mixture was stirred at 0 °C for 0.5 h and at room temperature for 3 h. It was cooled again to 0 °C, and 2 M ammonia in 2-propanol (40 mL, 80 mmol) was added dropwise. The mixture was stirred at 0 °C for 0.5 h and at room temperature overnight. The reaction mixture was flash-evaporated at 60 °C to dryness. To the residue was added water (140 mL). The mixture was shaken strongly. The white precipitate (by product, dicyclohexylurea) was removed by filtration and washed with water (2×70 mL). To the combined filtrate (280 mL) was added NaCl (32.7 g) to form a precipitate suspended over the solution. The mixture was extracted with a solution (3×70 mL) of dichloromethane (178 mL) and 2-propanol (32 mL). The organic layer was flash-evaporated. It was dried in vacuum to give 10.1 g of raw product that was dissolved in ethyl acetate (50 mL) at reflux temperature (77 °C). The hot solution stood at 4 °C overnight. The white precipitate was collected, washed with ethyl ether and dried to give 3.5 g of 5-(*tert*-butoxycarbonylamino)valerylamide. The protecting group was removed by dissolving the 5-(*tert*-butoxycarbonylamino)valerylamide (3.5 g) in a solution of formic acid (2 mL) and trifluoroacetic acid (TFA, 10 mL). The clear solution was stirred for 3 h and then added to ethyl ether (200 mL). The resulting crystals were collected, washed with ethyl ether and dried to yield 3.7 g of 5-aminovalerylamide trifluoroacetate (total yield 40%).

***N,N′*-(2,2′-Dithiobisbenzoyl)-bis(L-alaninamide) (IIIb).** This compound was prepared according to the general procedure as described in method D using *N,N′*-disuccinimidyl-2,2′-dithiosalicylate and L-alaninamide hydrochloride. ^{1}H NMR data is shown in Table 3. MS (EI) *m*/*z* 446 [M$^+$].

***N*-(2-Mercaptobenzoyl)-L-alaninamide (IV, $n=1$, $R_1=NH_2$, $R_2=H$, $R_3=CH_3$).** To the mixture of *N,N′*-(2,2′-dithiobisbenzoyl)-bis(L-alaninamide) (**IIIb**, 2.0 g, 4.5 mmol) in DMF (18 mL) and water (2 mL) was added tris(carboxyethyl)phosphine hydrochloride (TCEP.HCl) (1.5 g, 5.2 mmol) and triethylamine (0.5 mL, 4.6 mmol). The mixture was stirred at room temp for 1 h and then added to ethyl ether (200 mL). The precipitate was collected, washed with water (3×20 mL) and dried under vacuum to yield 1.56 g (77%) of the title compound. ^{1}H NMR (DMSO-d_6) δ 8.52 (d, 1H), 7.66 (d, 1H), 7.40–7.56 (m, 2H), 7.36 (t, 1H), 7.24 (t, 1H), 7.10 (brs, 1H), 5.42 (brs, 1H), 4.40 (quintet, 1H), 1.34 (d, 3H). MS (EI) *m*/*z* 224 [M$^+$].

***N*-[2-(5-Bromovaleroylthio)benzoyl]-L-alaninamide (V, $n=1$, $m=4$, X = Br, $R_1=NH_2$, $R_2=H$, $R_3=CH_3$).** To a solution of *N*-(2-mercaptobenzoyl)-L-alaninamide (0.22 g, 1 mmol) in DMA (5 mL) was added 5-bromovaleryl chloride (0.30 g, 1.5 mmol) under nitrogen at room temperature. It was stirred for 3 h and then the solvent was removed under vacuum. The remaining residue was vigorously stirred with ethyl ether (10 mL). The precipitate, thus obtained, was collected and washed with water to yield 70% of the title compound. ^{1}H NMR (DMSO-d_6) δ 9.15 (d, 2H), 8.66 (t, 1H), 8.40 (d, 1H), 8.22 (t, 2H), 7.48–7.70 (m, 4H), 7.34 (brs, 1H), 7.14 (brs, 1H), 4.68 (t, 2H), 4.36 (quintet, 1H), 2.78 (t, 2H), 2.02 (quintet, 2H), 1.62 (quintet, 2H), 1.30 (d, 3H). MS (EI) *m*/*z* 386 [M$^+$], 343, 326.

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-L-alaninamide bromide (VI, $n=1$, $m=4$, X = Br, $R_1=NH_2$, $R_2=H$, $R_3=CH_3$) (7).** A solution of *N*-[2-(5-bromovaleroylthio)benzoyl]-L-alaninamide (0.2 g, 0.5 mmol) in pyridine (4 mL) was stirred at room temperature under nitrogen overnight. The solvent was evaporated to dryness and the product was purified by column chromatography using ethyl acetate/methanol (7:3) as eluent to yield colorless oil (65%). ^{1}H NMR (DMSO-d_6) δ 9.15 (d, 2H), 8.66 (t, 1H), 8.40 (d, 1H), 8.22 (t, 2H), 7.70–7.48 (m, 4H), 7.34 (brs, 1H), 7.14 (brs, 1H), 4.68 (t, 2H), 4.36 (q, 1H), 2.78 (t, 2H), 2.02 (quintet, 2H), 1.62 (quintet, 2H), 1.30 (d, 3H). MS (EI) *m*/*z* 386 [M$^+$], 343, 326.

***N*-[2-(3-Pyridiniopropanoylthio)benzoyl]glycinamide chloride (1).** Yellowish oil; isolated yield: 66%; ^{1}H NMR (DMSO-d_6) δ 9.18 (d, 2H), 8.65 (t, 1H), 8.50 (t, 1H), 8.22 (t, 2H), 7.65–7.45 (m, 4H), 7.32 (brs, 1H), 7.12 (brs, 1H), 4.90 (t, 2H), 3.82 (d, 2H), 3.30 (t, 2H). MS (EI) *m*/*z* 344 [M$^+$], 301, 286.

***N*-[2-(4-Pyridiniobutanoylthio)benzoyl]glycinamide chloride (2).** Yellowish oil; isolated yield: 59%; ^{1}H NMR (DMSO-d_6) δ 9.18 (d, 2H), 8.68 (t, 1H), 8.58 (t, 1H), 8.24 (t, 2H), 7.70–7.50 (m, 4H), 7.35 (brs, 1H), 7.15 (brs, 1H), 4.70 (t, 2H), 3.88 (d, 2H), 2.88 (t, 2H), 2.26 (quintet, 2H). MS (EI) *m*/*z* 358 [M$^+$], 341, 313.

***N*-[2-(4-Pyridiniobutanoylthio)benzoyl]glycinamide bromide (3).** Colorless oil; isolated yield: 65%; ^{1}H NMR (DMSO-d_6) δ 9.16 (d, 2H), 8.66 (t, 1H), 8.58 (t, 1H), 8.22 (t, 2H), 7.72–7.50 (m, 4H), 7.35 (brs, 1H), 7.15 (brs, 1H), 4.68 (t, 2H), 3.82 (d, 2H), 2.86 (t, 2H), 2.28 (quintet, 2H). MS (EI) *m*/*z* 358 [M$^+$], 341, 313.

***N*-[2-(4-Pyridiniobutanoylthio)benzoyl]-L-alaninamide bromide (4).** Colorless oil; isolated yield: 53%; ^{1}H NMR (DMSO-d_6) δ 9.15 (d, 2H), 8.66 (t, 1H), 8.45 (d, 1H), 8.22 (t, 2H), 7.70–7.50 (m, 4H), 7.38 (brs, 1H), 7.12 (brs, 1H), 4.70 (t, 2H), 4.36 (q, 1H), 2.85 (t, 2H), 2.26 (quintet, 2H), 1.32 (d, 3H). MS (EI) *m*/*z* 372 [M$^+$], 327, 281.

***N*-[2-(4-Pyridiniobutanoylthio)benzoyl]-L-isoleucinamide chloride (5).** Yellowish oil; isolated yield: 48%; ^{1}H NMR (DMSO-d_6) δ 9.16 (d, 2H), 8.66 (t, 1H), 8.30 (d, 1H), 8.26 (t, 2H), 7.70–7.50 (m, 4H), 7.48 (brs, 1H), 7.15 (brs, 1H), 4.70 (t, 2H), 4.25 (t, 1H), 2.82 (t, 2H), 2.26 (quintet, 2H), 1.85 (m, 1H), 1.20 (m, 2H), 0.98–0.82 (m, 6H). MS (FAB) *m*/*z* 415 [M$^+$ + 1].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]glycinamide bromide (6).** Colorless oil; isolated yield: 90%; ^{1}H NMR (DMSO-d_6) δ 9.2 (d, 2H), 8.67 (t, 1H), 8.56 (t, 1H), 8.25

(t, 2H), 7.7–7.45 (m, 4H), 7.36 (s, 1H), 7.2 (s, 1H), 4.72 (t, 2H), 3.83 (d, 2H), 2.82 (t, 2H), 2.03 (quintet, 2H), 1.65 (quintet, 2H). MS (FAB) m/z 373 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-(DL-2-aminobutyramide) bromide (8).** Colorless oil; isolated yield: 61%; ^{1}H NMR (DMSO-d_6) δ 9.16 (d, 2H), 8.66 (t, 1H), 8.32 (d, 1H), 8.23 (t, 2H), 7.55 (m, 4H), 7.35 (s, 1H), 7.15 (s,1H), 4.66 (t, 2H), 4.26 (m, 1H), 2.78 (t, 2H), 2.0 (quintet, 2H), 1.8–1.5 (m, 4H), 0.95 (t, 3H). MS (FAB) m/z 401 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-L-valinamide bromide (9).** Colorless oil; isolated yield: 58%; ^{1}H NMR (DMSO-d_6) δ 9.15 (d, 2H), 8.65 (t, 1H), 8.25–8.1 (m, 3H), 7.55 (m, 4H), 7.36 (s, 1H), 7.15 (s, 1H), 4.65 (t, 2H), 4.25 (t, 1H), 2.75 (t, 2H), 2.15–1.85 (m, 3H), 1.55 (quintet, 2H), 1.95 (m, 6H). MS (FAB) m/z 415 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-L-leucinamide bromide (10).** Colorless oil; isolated yield: 71%; ^{1}H NMR (DMSO-d_6) δ 9.15 (d, 2H), 8.66 (t, 1H), 8.4 (d, 1H), 8.23 (t, 2H), 7.57 (m, 4H), 7.3 (s, 1H), 7.1 (s, 1H), 4.68 (t, 2H), 4.36 (m, 1H), 2.75 (t, 2H), 2.0 (quintet, 2H), 1.85–1.45 (m, 5H), 0.92 (d, 6H). MS (FAB) m/z 429 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-L-isoleucinamide bromide (11).** Yellowish oil; isolated yield: 51%; ^{1}H NMR (DMSO-d_6) δ 9.16 (d, 2H), 8.66 (t, 1H), 8.25–8.15 (m, 3H), 7.70–7.50 (m, 4H), 7.38 (brs, 1H), 7.15 (brs, 1H), 4.68 (t, 2H), 4.26 (t, 1H), 2.78 (t, 2H), 2.0 (quintet, 2H), 1.85 (m, 1H), 1.60 (quintet, 2H), 1.20 (m, 2H), 0.98–0.82 (m, 6H). MS (EI) m/z 428 [M^+], 401, 356.

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-L-phenylalaninamide bromide (12).** Colorless oil; isolated yield: 54%; ^{1}H NMR (DMSO-d_6) δ 9.15 (d, 2H), 8.66 (t, 1H), 8.53 (d, 1H), 8.23 (t, 2H), 7.6–7.1 (m, 11H), 4.6 (m, 3H), 3.15 (d, 1H), 2.9 (t, 1H), 2.7 (t, 2H), 1.96 (quintet, 2H), 1.56 (quintet, 2H). MS (FAB) m/z 463 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-L-asparaginamide bromide (13).** Colorless oil; isolated yield: 86%; ^{1}H NMR (DMSO-d_6) δ 9.15 (d, 2H), 8.67 (t, 1H), 8.48 (d, 1H), 8.24 (t, 2H), 7.7–7.5 (m, 4H), 7.43 (s, 1H), 7.22 (s, 2H), 6.97 (s, 1H), 4.67 (m, 3H), 2.8 (t, 2H), 2.55 (m, 2H), 2.0 (quintet, 2H), 1.6 (quintet, 2H). MS (FAB) m/z 430 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-L-glutaminamide bromide (14).** Colorless oil; isolated yield: 44%; ^{1}H NMR (DMSO-d_6) δ 9.16 (d, 2H), 8.67 (t, 1H), 8.38 (d, 1H), 8.22 (t, 2H), 7.7–7.5 (m, 4H), 7.38 (s, 1H), 7.35 (s, 1H), 7.17 (s, 1H), 6.83 (s, 1H), 4.68 (t, 2H), 4.33 (q, 1H), 2.78 (t, 2H), 2.22 (t, 2H), 2.1–1.8 (m, 4H), 1.62 (quintet, 2H). MS (FAB) m/z 444 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-(2-aminoisobutyramide)bromide (15).** Colorless oil; Isolated yield: 62%; ^{1}H NMR (DMSO-d_6) δ 9.16 (d, 2H), 8.66 (t, 1H), 8.3 (s, 1H), 8.23 (t, 2H), 7.75–7.45 (m, 4H), 7.16 (s, 1H), 7.05 (s, 1H), 4.67 (t, 2H), 2.77 (t, 2H), 2.0 (quintet, 2H), 1.63 (quintet, 2H), 1.45 (t, 6H). MS (FAB) m/z 401 [$M^+ + 1$].

***N*-[2-(6-Pyridiniohexanoylthio)benzoyl]glycinamide bromide (16).** Colorless oil; isolated yield: 59%; ^{1}H NMR (DMSO-d_6) δ 9.15 (d, 2H), 8.65 (t, 1H), 8.50 (t, 1H), 1.36 (quintet, 2H), 8.20 (t, 2H), 7.72–7.48 (m, 4H), 7.30 (brs, 7.15 (brs, 1H), 4.65 (t, 2H), 3.78 (d, 2H), 2.72 (t, 2H), 1.98 (quintet, 2H), 1.66 (quintet, 2H). MS (EI) m/z 386 [M^+], 341, 313.

***N*-[2-(6-Pyridiniohexanoylthio)benzoyl]-L-alaninamide bromide (17).** Colorless oil; isolated yield: 58%; ^{1}H NMR (DMSO-d_6) δ 9.18 (d, 2H), 8.66 (t, 1H), 8.38 (d, 1H), 8.22 (t, 2H), 7.70–7.46 (m, 4H), 7.34 (brs, 1H), 7.16 (brs, 1H), 4.66 (t, 2H), 4.36 (q, 1H), 2.72 (t, 2H), 1.96 (quintet, 2H), 1.65 (quintet, 2H), 1.36 (quintet, 2H), 1.30 (d, 3H). MS (EI) m/z 400 [M^+], 365, 355, 329.

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-4-aminobutyramide bromide (18).** Colorless oil; isolated yield: 95%; ^{1}H NMR (DMSO-d_6) δ 9.16 (d, 2H), 8.67 (t, 1H), 8.4 (t, 1H), 8.25 (t, 2H), 7.55 (s, 4H), 7.38 (s, 1H), 6.85 (s, 1H), 4.68 (t, 2H), 3.2 (quartet, 2H), 2.8 (t, 2H), 2.12 (t, 2H), 2.03 (quintet, 2H), 1.8–1.5 (m, 4H). MS (FAB) m/z 401 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-5-aminovalerylamide bromide (19).** Colorless oil; isolated yield: 82%; ^{1}H NMR (DMSO-d_6) δ 9.16 (d, 2H), 8.67 (t, 1H), 8.35 (t, 1H), 8.25 (t, 2H), 7.6–7.45 (m, 4H), 7.33 (s, 1H), 6.7 (s, 1H), 4.68 (t, 2H), 3.2 (quartet, 2H), 2.8 (t, 2H), 2.12 (t, 2H), 2.05 (quintet, 2H), 1.7–1.4 (m, 6H). MS (FAB) m/z 415 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-*N'*-methylglycinamide bromide (20).** Yellowish oil; isolated yield: 57%; ^{1}H NMR (DMSO-d_6) δ 9.12 (d, 2H), 8.64 (t, 1H), 8.52 (t, 1H), 8.18 (t, 2H), 7.80 (quartet, 1H), 7.66–7.45 (m, 4H), 4.68 (t, 2H), 3.78 (d, 2H), 2.66 (d, 3H), 2.28 (t, 2H), 1.98 (quintet, 2H), 1.52 (quintet, 2H). MS (EI) m/z 386 [M^+], 339, 325.

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]glycine bromide (21).** A solution of *N*-[2-(5-bromovalerylthio)benzoyl]glycine-*t*-butyl ester (0.9 g, 2.1 mmol) in pyridine (3 mL) was stirred under nitrogen overnight. Ethyl ether (45 mL) was added and the resulting residue was collected, washed with ethyl ether and dissolved in a solution of formic acid (3 g) and TFA (10 mL). The mixture was stirred overnight at room temperature. The reaction solution was evaporated to 2 mL under nitrogen and treated with ethyl ether (45 mL) with stirring. The resulting residue was washed 2–3 times with ethyl ether and dried. Isolated yield: 0.3 g (32%); ^{1}H NMR (DMSO-d_6) δ 9.13 (d, 2H), 8.67 (m, 2H), 8.2 (t, 2H), 7.57 (m, 4H), 4.67 (t, 2H), 3.9 (d, 2H), 2.78 (t, 2H), 2.0 (quintet, 2H), 1.63 (quintet, 2H). MS (FAB) m/z 374 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-β-alanine bromide (22).** Isolated yield: 53%; ^{1}H NMR (DMSO-d_6) δ 9.15 (d, 2H), 8.66 (t, 1H), 8.44 (t, 1H), 8.22 (t, 2H), 7.5 (m,

4H), 4.65 (t, 2H), 3.42 (quartet, 2H), 2.8 (t, 2H), 2.52 (t, 2H), 2.0 (quintet, 2H), 1.65 (quintet, 2H). MS (FAB) m/z 388 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]glycine ethyl ester bromide (23).** Colorless oil; isolated yield: 68%; ^{1}H NMR (DMSO-d_6) δ 9.14 (d, 2H), 8.8 (t, 1H), 8.66 (t, 1H), 8.23 (t, 2H), 7.57 (m, 4H), 4.65 (t, 2H), 4.15 (quartet, 2H), 3.96 (d, 2H), 2.78 (t, 2H), 2.0 (quintet, 2H), 1.65 (quintet, 2H), 1.25 (t, 3H). MS (FAB) m/z 402 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-β-alanine ethyl ester bromide (24).** Colorless oil; isolated yield: 67%; ^{1}H NMR (DMSO-d_6) δ 9.16 (d, 2H), 8.66 (t, 1H), 8.46 (t, 1H), 8.23 (t, 2H), 7.53 (m, 4H), 4.66 (t, 2H), 4.12 (q, 2H), 3.45 (q, 2H), 2.78 (t, 2H), 2.55 (t, 2H), 2.02 (quintet, 2H), 1.63 (quintet, 2H), 1.22 (t, 3H). MS (FAB) m/z 416 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-4-aminobutyrate ethyl ester bromide (25).** Colorless oil; isolated yield: 82%; ^{1}H NMR (DMSO-d_6) δ 9.15 (d, 2H), 8.66 (t, 1H), 8.4 (t, 1H), 8.23 (t, 2H), 7.53 (m, 4H), 4.66 (t, 2H), 4.12 (q, 2H), 3.23 (q, 2H), 2.8 (t, 2H), 2.4 (t, 2H), 2.02 (quintet, 2H), 1.76 (quintet, 2H), 1.63 (quintet, 2H), 1.22 (t, 3H). MS (FAB) m/z 430 [$M^+ + 1$].

***N*-[2-(5-Pyridiniovalerylthio)benzoyl]-L-alanine methyl ester bromide (26).** Colorless oil; Isolated yield: 43%; ^{1}H NMR (DMSO-d_6) δ 9.14 (d, 2H), 8.82 (d, 1H), 8.66 (t, 1H), 8.22 (t, 2H), 7.64–7.50 (m, 4H), 4.66 (t, 2H), 4.42 (q, 1H), 3.70 (s, 3H), 2.78 (t, 2H), 2.00 (quintet, 2H), 1.62 (quintet, 2H), 1.35 (d, 3H). MS (EI) m/z 401 [M^+], 370, 342.

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