Discovery and Biological Activity of Orally Active Peptidyl Trifluoromethyl Ketone Inhibitors of Human Neutrophil Elastase

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Previously we had shown that tripeptidyl trifluoromethyl ketones (TFMKs) possessing an N-terminal diarylacylsulfonamide, such as ICI 200,880 and ICI 200,355, displayed unparalleled protection against the lung damage induced by human neutrophil elastase (HNE) when the inhibitors were administered intratracheally. Since the diarylacylsulfonamides were designed specifically to afford a long residence time in the lung, it was not unexpected that inhibitors from this class of TFMKs were not active when administered orally. Upon evaluating a large number of peptidyl TFMKs possessing a variety of N-terminal groups, several compounds were identified which demonstrated oral activity. Compounds were evaluated for their oral activity by measuring their ability to inhibit the increase in lung weight relative to body weight (Lw/Bw), the increase in red blood cells, and the increase in white blood cells induced by intratracheally administered HNE (100 μg/hamster). A number of tripeptidyl trifluoromethyl ketones containing neutral N-terminal groups displayed good oral activity, while those containing basic, acidic, or polar groups did not. Compound **50**, possessing an N-terminal 4-$(CH_3O)C_6H_4CO$ group, was particularly effective, reducing Lw/Bw by 77%, red cells by 89%, and white cells by 91% when dosed at 37.5 mg/kg orally. Thus, by modifying the N-terminal group of tripeptidyl TFMKs, inhibitors can be designed which are effective *in vivo* when administered either orally or intratracheally.

Introduction

For more than a decade, we have been developing inhibitors of the serine protease human neutrophil elastase (HNE, EC 3.4.21.37).[1] HNE has been implicated in initiating or exacerbating a number of pathological conditions such as pulmonary emphysema,[2] cystic fibrosis,[3] and chronic bronchitis.[4] These pulmonary diseases could effectively be treated with drugs administered via an aerosol. Therefore, a major part of our research has focused on the development of HNE inhibitors which had a long duration of action following intratracheal administration. This effort has resulted in the identification of three series of peptidyl electrophilic ketones that displayed excellent *in vivo* activity in animal models following intratracheal administration: trifluoromethyl ketones,[5,6] α,α-difluoro-β-keto amides[7], and α-ketobenzoxazoles.[8]

However, there are a number of elastase-mediated diseases for which intratracheal administration is either less than optimal or inappropriate. Included among this group of conditions are acute respiratory distress syndrome,[9] myocardial reperfusion injury,[10,11] and arthritis.[12] Thus, having successfully developed a number of peptide-based elastase inhibitors suitable for aerosol administration, we focused our efforts on the identification of compounds which could be administered orally. Frequently, peptides have only limited oral bioavailability as a result of poor absorption, extensive metabolism, and/or rapid elimination. Thus, we mounted a large effort to identify nonpeptidic compounds that might have a superior *in vivo* profile to that of peptidic inhibitors following oral administration. This work led to the discovery of several series of nonpeptidic trifluoromethyl ketones including the pyridones,[13−17] pyrimidinones,[18] carbolines,[19] and pyridopyrimidines.[20] Compounds from some of these series demonstrated excellent oral activity in an animal model of elastase-induced lung injury.[16,18]

Several classes of orally active, nonpeptidic inhibitors of HNE have been described including benzisothiazolones[21] and β-lactams.[22−24] Although peptides generally suffer from poor oral bioavailability, there are examples of the successful development of orally active peptides, for example renin inhibitors.[25] Thus, while we pursued the design of orally active nonpeptidic HNE inhibitors, we also investigated the identification of peptidic inhibitors which would possess the desired level of *in vivo* activity following oral administration. Peptidyl pentafluoroethyl ketones (PFEKs) have been described which inhibit elastase-induced lung injury following oral administration.[26−28] In this report we describe the discovery of several peptidyl trifluoromethyl ketones which possess excellent oral activity. Recently it was reported that peptidyl TFMKs were not orally active while the corresponding PFEKs were active.[27] Not only have we identified orally active peptidyl TFMKs, but have identified ones which have superior activity to that reported for the pentafluoroethyl ketones. These results

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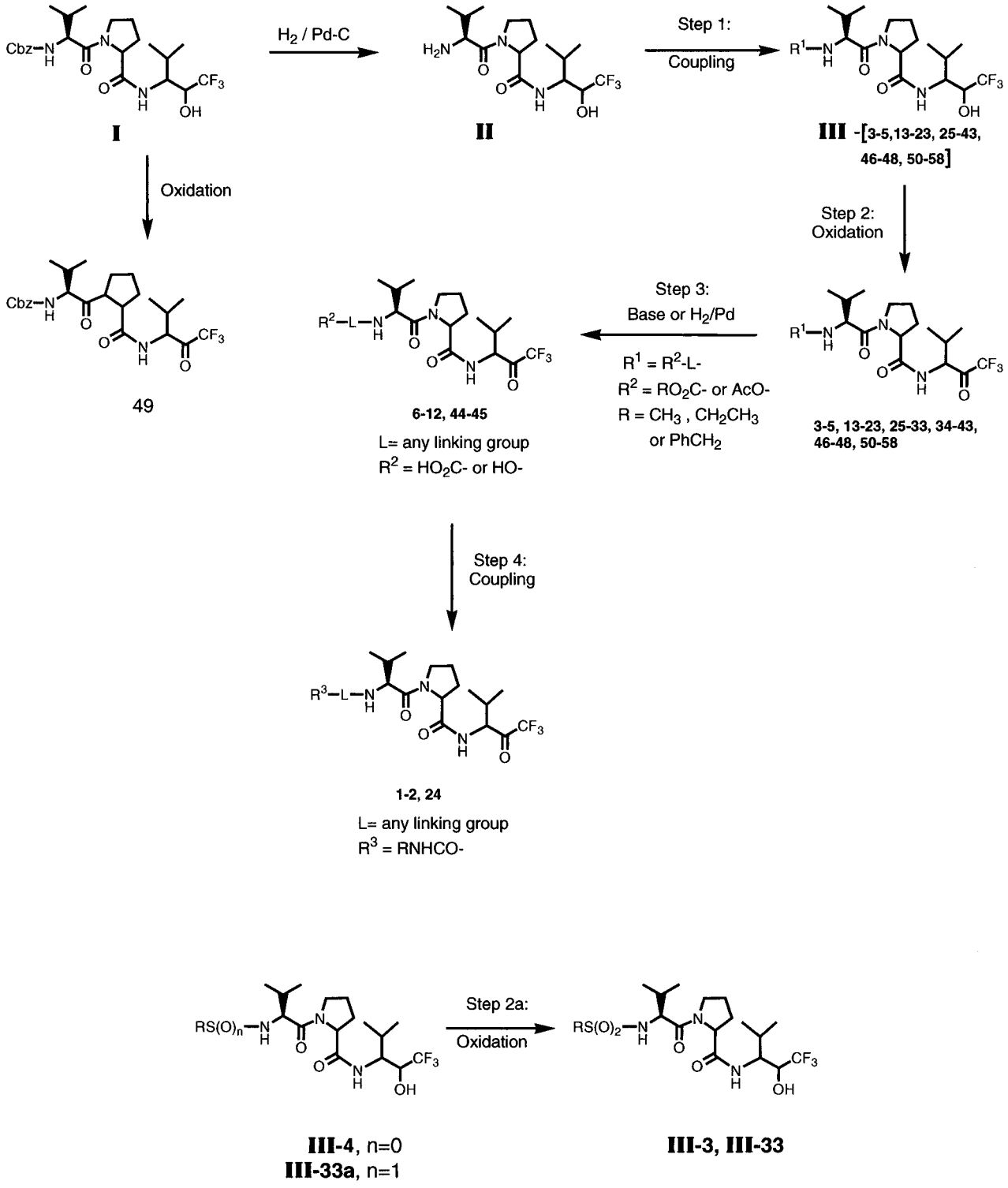
Scheme 1. Synthesis of Peptidyl Trifluoromethyl Ketones **1**−**58**

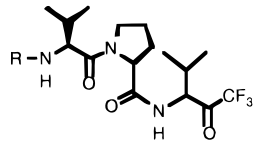


highlight a finding we have previously observed with peptidyl electrophilic ketones: interchanging mechanistic pharmacophores does not lead to predictable biological activity since the observed *in vivo* activity is a result of the physiochemical properties of the entire molecule.

Chemistry

The tripeptidyl TFMKs in this study were prepared starting from the previously described H-Val-Pro-Val-trifluoromethyl alcohol **II**[29] (Scheme 1). Amino alcohol **II** was coupled with a variety of electrophiles to yield the trifluoromethyl alcohols **III.** (See Table 1 for reagents.) Oxidation of alcohols **III** gave the corresponding trifluoromethyl ketones. Peptidyl TFMKs containing N-terminal substituents incorporating esters or phenolic acetates were converted to the corresponding acids and phenols via either hydrogenation or base hydrolysis. Further modification of the keto acids was achieved by coupling with various nucleophilic nitrogen compounds. TFMKs with an N-terminal sulfone (**III-3** and **III-33**) were prepared by oxidation of the corresponding sulfide (**III-4**) or sulfoxide (**III-33a**) to the sulfone and subsequent oxidation of the trifluoromethyl

Table 1. Method of Preparation and Physicochemical Properties of Peptidyl TFMKs **1**−**58**



compd	R	step in Scheme 1					yield (%)	MS[d] (M + 1)	formula[e]
		1	2a	2	3	4			
1	1-Npth-$SO_2NHCOCH_2CH_2CO$					12;C	45	ND	$C_{30}H_{37}F_3N_4O_7S\cdot0.5H_2O$
2	4-(CH_3SO_2NHCO)C_6H_4CO					10;C	60	ND	$C_{25}H_{34}F_3N_5O_7\cdot0.5H_2O$
3	4-(CH_3SO_2)C_6H_4CO		4;L	N			86	548	$C_{24}H_{32}O_6N_3F_3S\cdot1.0H_2O$
4	4-(CH_3S)C_6H_4CO	A		N			84	516	$C_{24}H_{32}O_4N_3F_3S$[f]
5	4-(CH_3CONH)$C_6H_4SO_2$)	H		P			84	ND	$C_{24}H_{33}F_3N_4O_6S\cdot1.0H_2O$
6	4-(HO_2C)C_6H_4CO				52;S		93	ND	$C_{24}H_{30}N_3F_3O_6\cdot1.0H_2O$
7	*trans*-4-(HO_2C)C_6H_4CH=$CHCO$				17;S		73	ND	$C_{26}H_{32}F_3N_3O_6\cdot0.35H_2O$
8	3,5-(HO_2C)$_2C_6H_4CO$				18;S		91	501	$C_{25}H_{30}N_3O_8\cdot0.8H_2O$
9	HO_2CCO				14;S		72	438	$C_{18}H_{26}F_3N_3O_6\cdot0.75H_2O$
10	4-(HO_2C)C_6H_4NHCO				15;S		92	ND	$C_{24}H_{31}F_3N_4O_6\cdot0.5H_2O$
11	3-(HO_2C)-2-Npth-CO				16;U		61	ND	$C_{28}H_{32}F_3N_3O_6$
12	$HO_2CCH_2CH_2CO$				13;S		87	ND	intermediate
13	$CH_3O_2CCH_2CH_2CO$	K		Q			74	ND	intermediate
14	CH_3O_2CCO	H		N			57	452	$C_{19}H_{28}F_3N_3O_6\cdot0.65H_2O$
15	4-($C_2H_5O_2C$)C_6H_4NHCO	I		P			78	ND	$C_{26}H_{35}F_3N_4O_6$
16	3-($PhCH_2O_2C$)-2-Npth-CO	B		O			84	ND	$C_{35}H_{38}F_3N_3O_6\cdot0.75H_2O$
17	*trans*-4-(EtO_2C)C_6H_4CH=$CHCO$	E		R			65	ND	$C_{28}H_{36}F_3N_3O_6$
18	3,5-(CH_3O_2C)$_2C_6H_4CO$	A		O			94	586	$C_{27}H_{34}F_3N_3O_8$
19	3-pyridyl-CO	A		N			32	471	$C_{22}H_{29}F_3N_4O_4\cdot1.1H_2O$
20	4-pyridyl-CO	A		N			24	471	$C_{22}H_{29}F_3N_4O_4\cdot0.65H_2O$
21	2-pyridyl-CO	A		N			38	471	$C_{22}H_{29}F_3N_4O_4\cdot0.33H_2O$
22	4-imidazole-CO	A		N			21	460	$C_{20}H_{28}F_3N_5O_4\cdot1.0H_2O$
23	4-(Me_2N)C_6H_4CO	A		N			93	513	$C_{25}H_{35}F_3N_4O_4\cdot0.6H_2O$
24	4-($Me_2NCH_2CH_2NHCO$)C_6H_4CO					6;B	46	584	$C_{28}H_{40}F_3N_5O_5\cdot0.5H_2O$
25	($PhCH_2CH_2CH_2$)$_2$	J		P			70	650	$C_{34}H_{46}F_3N_3O_6\cdot1.0HCl\cdot1.0H_2O$
26	CH_3CO	F		N			11	408	$C_{18}H_{28}F_3N_3O_4$
27	$CH_3CON(nPr)CH_2CO$	A		N			26	507	$C_{23}H_{37}F_3N_4O_5\cdot0.9H_2O$
28	$CH_3CON(Bn)CH_2CO$	A		N			55	555	$C_{27}H_{37}N_4O_5F_3\cdot0.9H_2O$
29	4-(NO_2)C_6H_4CO	A		N			79	515	$C_{23}H_{29}F_3N_4O_6\cdot0.3H_2O$
30	PhNHCO	I		P			76	ND	$C_{23}H_{31}F_3N_4O_4\cdot0.75H_2O$[g]
31	4-F-C_6H_4NHCO	I		P			42	ND	$C_{23}H_{30}F_4N_4O_4$
32	CF_3SO_2	H		N			67	498	$C_{17}H_{25}F_6N_3O_5S\cdot0.25H_2O$
33	1-Adamantyl-SO_2	H	M	Q			40	ND	$C_{26}H_{40}N_3O_5F_3S\cdot0.4H_2O$
34	$PhSO_2$	H		N			61	506	$C_{22}H_{30}F_3N_3O_5S\cdot0.25H_2O$
35	4-Cl-$C_6H_4SO_2$	H		N			41	540	$C_{22}H_{29}ClF_3N_3O_5S$
36	4-(NO_2)$C_6H_4SO_2$	H		N			37	551	$C_{22}H_{29}F_3N_4O_7S$
37	4-(CH_3O)$C_6H_4SO_2$	H		N			68	536	$C_{23}H_{32}F_3N_3O_6S\cdot0.25H_2O$
38	pentafluorophenyl-SO_2	H		Q			74	596	$C_{22}H_{25}F_8N_3O_5S\cdot0.5H_2O$
39	2,4,6-(^iPr)$_3C_6H_4SO_2$	H		N			66	632	$C_{31}H_{48}F_3N_3O_5S\cdot0.20H_2O$
40	2-Npth-SO_2	H		O			47	ND	$C_{26}H_{32}F_3N_3O_5S\cdot0.5H_2O$
41	4-(CH_3CO_2)C_6H_4CO	B		O			91	ND	$C_{25}H_{32}N_3O_6F_3\cdot0.6H_2O$
42	3,4-(CH_3CO_2)$_2C_6H_4CO$	D		O				ND	not isolated
43	3,5-(CH_3CO_2)$_2C_6H_4CO$	B		O			61	ND	$C_{27}H_{34}F_3N_3O_8\cdot0.25H_2O$
44	4-(HO)C_6H_4CO				41;T		62	ND	$C_{23}H_{30}N_3O_5F_3\cdot0.55H_2O$
45	3,4-(HO)$_2C_6H_4CO$				42;T		32	502	$C_{23}H_{30}F_3N_3O_6\cdot1H_2O$[h]
46	2,6-(CH_3O)$_2C_6H_4CO$	B		N			60	530	$C_{25}H_{34}N_3O_6F_3$
47	4-F-C_6H_4CO	A		N			44	488	$C_{23}H_{29}F_4N_3O_4\cdot0.2H_2O$
48	4-F-$C_6H_4OC(O)$	H		N			67	504	$C_{23}H_{29}F_4N_3O_5\cdot0.25H_2O$
49	Cbz			*I*;P			45	ND	$C_{24}H_{32}F_3N_3O_5\cdot0.5H_2O$
50	4-(CH_3O)C_6H_4CO	H		N			87	500	$C_{24}H_{32}F_3N_3O_5\cdot0.33H_2O$
51	PhOC(O)	H		N			80	486	$C_{23}H_{30}F_3N_3O_5\cdot0.4H_2O$
52	4-(CH_3O_2C)C_6H_4CO	C		Q			55	ND	$C_{25}H_{32}F_3N_3O_6\cdot0.5H_2O$
53	2-Npth-CO	A		O			71	520	$C_{27}H_{32}F_3N_3O_4\cdot0.75H_2O$
54	3,4-(CH_3)$_2C_6H_4CO$	B		N			65	498	$C_{25}H_{34}F_3N_3O_4$
55	PhCO	A		N			62	470	$C_{23}H_{30}F_3N_3O_4\cdot0.25H_2O$
56	3,4-(CH_3O)$_2C_6H_4CO$	B		N			32	530	$C_{25}H_{34}N_3O_6F_3$
57	CF_3CO	G		N			62	462	$C_{18}H_{25}F_6N_3O_4\cdot0.2H_2O$
58	4-(PhO)C_6H_4CO	A		N			41	562	$C_{29}H_{34}F_3N_3O_5\cdot0.5H_2O$

[a] A, RCO_2H, EDAC, with or without DMAP; B, RCO_2H (or RNH_2 for **24**), EDAC, HOBt; C, RNH_2 (or RCO_2H for **52**), DCC, HOBT or DMAP; D, RCO_2H, $^iBuOCOCl$, NMM; E, RCO_2H, CDI, THF; F, Ac_2O, TEA; G, CF_3CO_2Et, TEA; H, active chloride (ROCOCl, RSO_2Cl, RSOCl, or RCOCl), TEA or K_2O_3; I, RNCO, TEA; J, ROH, Tf_2O, Proton Sponge; K, RCOCl, NaOH; L, MCPBA; M, $KMnO_4$; N, EDAC, Cl_2CHCO_2H, DMSO, toluene; O, DMP; P, $(COCl)_2$, DMSO; Q, Ac_2O, DMSO; R, CrO_3, pyridine; S, NaOH; T, K_2CO_3; U, H_2, Pd/C. [d] ND = not determined. [e] All elemental analyses for C, H, and N agree within ±0.4% of calculated values unless otherwise noted. [f] N: calculated, 7.42; found, 6.31. [g] N: calculated, 11.25; found, 10.72. [h] N: calculated, 8.08; found, 7.44

alcohol to the ketone. All compounds in this report were roughly 1:1 mixtures of epimers at the stereogenic center α to the ketone carbonyl group and thus are mixtures of diastereomers.

Pharmacological Evaluation

The oral activity of the TFMK inhibitors was assessed in two *in vivo* models. When HNE is administered intratracheally to hamsters, a hemorrhagic lesion de-

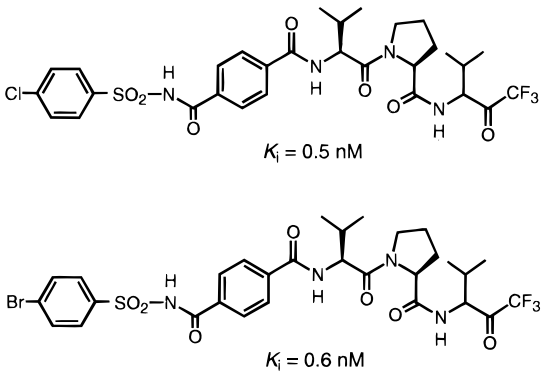


Figure 1. ICI 200,880 and ICI 200,355.

velops which is characterized by an increase in lung weight relative to body weight (Lw/Bw) as a result of edema, an increase in white blood cells, and an increase in red blood cells. In the acute lung injury model (ALIM), all three parameters are measured.[5] In the acute hemorrhagic assay (AHA), only the degree of hemorrhage is determined as measured by the amount of hemoglobin produced.[30] In both assays, the effect of the test compound is compared to HNE and saline controls. Compounds showing 100% inhibition are identical to saline, and those showing 0% are identical to HNE control. Compounds with a negative value for per cent inhibition exacerbate the effects of HNE and produce a greater degree of lung damage than HNE alone.

Results and Discussion

The first phase of our elastase program was directed at the development of elastase inhibitors which would have the physical properties suitable for administration as an aerosol. Our primary goal was the identification of compounds which had a long duration of action in the ALIM following it administration. Within the series of trifluoromethyl ketones, ICI 200,880 and ICI 200,355 (Figure 1) emerged as leading compounds with a superior duration of action. From a structural standpoint, this is the result of the diarylacylsulfonamide N-terminal group or N-cap. In physical terms, this long duration of action is the result of the compound's relative inability to cross the lung epithelial membrane, pass through the interstitial space, and finally cross the endothelium and enter the blood stream.

Thus, it was no surprise that peptides with acylsulfonamide N-caps (ICI 200,355, **1**, and **2**, Table 2) were not active in the ALIM following oral administration. The same properties of the peptidyl acylsulfonamides which contribute to their long duration of action following it administration also retard their absorption from the gastrointestinal tract into, and migration out of, the blood stream. Pharmacokinetic studies indicated that the acylsulfonamides were not absorbed into the systemic circulation from the intestinal tract. One possible explanation for the lack of oral absorption of these peptides might be that they are ionized at the pH of the intestinal tract. However, related compounds in which the charge of the acylsulfonamide is removed (**3**−**5**) were also inactive.

It was clear, therefore, that a different SAR would have to be developed for an orally active elastase inhibitor than that for an inhibitor delivered intratracheally. As part of our aerosol program, we had prepared a large number of compounds with a diverse set of physicochemical properties before we identified the unique properties of the acylsulfonamides. Thus we had a large stable of compounds which could be tested orally. Table 2 lists representative examples of the compounds which were tested orally in the ALIM. Consistent with our findings with the peptidyl acylsulfonamides, other acidic compounds were inactive (**7**−**11**). However, the corresponding esters were also inactive (**17**−**18**). A number of basic compounds were found to be inactive in our oral model as well (**19**−**25**).

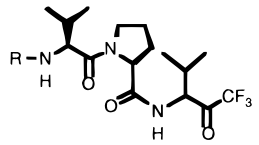
Finally, a large collection of neutral compounds were evaluated. These inhibitors contained N-caps possessing a broad range of lipophilicities and functional groups including amides (**26**−**29**), ureas (**30** and **31**), alkyl and aryl sulfonamides (**32**−**40**), and a number of phenols (**44** and **45**), phenolic esters (**43**), and phenolic ethers (**46**). While the classification used to group the peptidic inhibitors above is somewhat ill-defined and overlapping, it serves to highlight the general strategy we employed: modification of the charge, hydrogen bond donor/acceptor ability, hydrophobicity/lipophilicity, and solubility. The lack of activity for compounds **1**−**5**, **7**−**11**, **17**−**40**, and **43**−**47** indicates that obtaining the correct physical properties of a compound to promote oral absorption/activity within this series of peptidyl TFMKs is not simply a function of the presence of a particular class of N-terminal group.

One possible explanation for the lack of oral activity observed for the peptidyl TFMKs described above is that the physical or chemical properties of the trifluoromethyl group itself renders the attached peptide inactive following oral administration. Indeed, it was recently reported that peptidyl TFMKs that lacked oral activity could be converted into compounds with good activity in the AHA following oral dosing by replacing the trifluoromethyl group with a pentafluoroethyl group.[27] Thus, the TFMKs **60** and **62** (Table 3) were inactive at a dose of 50 mg/kg when administered 30 min prior to a 50 μg it dose of HNE, while the pentafluoroethyl analogs **59** and **61** significantly reduced the lesion at the same dose and displayed excellent reduction in the HNE (10 μg/animal) induced hemorrhage when dosed at 25 mg/kg. Thus it would appear from these results that the pentafluoroethyl group possesses special properties which render an attached peptide orally active.

The reported difference in oral activity between the TFMKs and the PFEKs may reside in the relative degree of ketone hydration. A number of studies have shown that the TFMKs are heavily, if not completely, hydrated in aqueous solution while other electrophilic peptidyl ketones such as α-keto esters, α,α-difluoro β-keto amides, and α-keto hetereocycles are not significantly hydrated.[31] Pentafluoroethyl ketones have been shown to be much less hydrated than TFMKs, being only about 80% hydrated. Thus, it has been speculated that the hydrated form of the TFMKs is either not well absorbed or very rapidly eliminated.[27]

However, we have found several peptidyl trifluoromethyl ketones that possess excellent oral activity, exceeding the reported activity of pentafluoroethyl ketones. Compounds **48**−**58** (Table 2) are part of a subset of peptidyl TFMKs we have found that demonstrate oral activity in the ALIM. For the most part, these compounds are all structurally similar, possessing a relatively small aromatic ring attached to the peptide backbone through an amide or carbamate linkage, and

Table 2. Potency and Oral Activity in ALIM Assay of Peptidyl TFMKs **1**−**58**



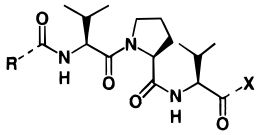
compd	R	$K_{i\,(nM)}$[a]	Lw/Bw[b,c]	red cells[b,c]	white cells[b,c]
ICI 200,355	4-[4-Br-PhSO$_2$NHCO]PhCO	0.62 ± 0.1	−0.5 (10)	20 (9)	30 (9)
1	1-Npth-SO$_2$NHCOCH$_2$CH$_2$CO	6.1 ± 1.1	−80* (10)	2 (9)	−212* (8)
2	4-(CH$_3$SO$_2$NHCO)C$_6$H$_4$CO	4.2 ± 0.7	−9 (9)	40* (9)	43 (9)
3	4-(CH$_3$SO$_2$)C$_6$H$_4$CO	2.0 ± 0.6	−16 (10)	2 (10)	58 (10)
4	4-(CH$_3$S)C$_6$H$_4$CO	2.3 ± 0.7	20 (9)	73* (8)	36 (9)
5	4-(CH$_3$CONH)C$_6$H$_4$SO$_2$	55 ± 6.5	2 (9)	0.3 (8)	32 (9)
7	*trans*-4-(HO$_2$C)C$_6$H$_4$CH=CHCO	1.8 ± 0.7	13 (10)	−11 (10)	68 (10)
8	3,5-(HO$_2$C)$_2$C$_6$H$_4$CO	43 ± 8.7	−45 (8)	−12 (7)	−105 (7)
9	HO$_2$CCO	810 ± 170	2 (7)	13 (7)	38 (6)
10	4-(HO$_2$C)C$_6$H$_4$NHCO	10 ± 2.0	−1 (8)	17 (8)	48 (7)
11	3-(HO$_2$C)-2-Npth-CO	1.7 ± 0.6	23 (8)	29 (8)	53 (8)
17	*trans*-4-(EtO$_2$C)C$_6$H$_4$CH=CHCO	0.43 ± 0.13	−122* (9)	17 (8)	−48 (7)
18	3,5-(CH$_3$O$_2$C)$_2$C$_6$H$_4$CO	11 ± 4.0	6 (9)	25 (8)	6 (9)
19	3-pyridyl-CO	7.8 ± 1.7	−6 (9)	1 (6)	23 (6)
20	4-pyridyl-CO	3.8 ± 1.0	−15 (9)	9 (9)	−45 (9)
21	2-pyridyl-CO	20 ± 4.0	8 (*)	64* (8)	44 (8)
22	4-imidazole-CO	63 ± 8.0	−42 (9)	−27 (8)	−267* (8)
23	4-(Me$_2$N)C$_6$H$_4$CO	2.5 ± 0.9	36 (10)	39* (10)	0 (10)
24	4-(Me$_2$NCH$_2$CH$_2$NHCO)C$_6$H$_4$CO	5.4 ± 4.2	20 (9)	5 (7)	71 (7)
25	(PhCH$_2$CH$_2$CH$_2$)$_2$	1.7 ± 0.4	−23 (10)	6 (9)	−39 (9)
26	CH$_3$CO	45 ± 12	8 (10)	51* (8)	−120 (9)
27	CH$_3$CON(^{n}Pr)CH$_2$CO	2.0 ± 0.7	24 (10	53* (9)	78 (10)
28	CH$_3$CON(Bn)CH$_2$CO	0.96 ± 0.5	20 (10)	16 (9)	36 (10)
29	4-(NO$_2$)C$_6$H$_4$CO	1.2 ± 0.4	54 (10)	70* (10)	4 (10)
30	PhNHCO	2.6 ± 0.7	−2 (8)	−8 (8)	−184* (8)
31	4-F-C$_6$H$_4$NHCO	11 ± 2.1	−3 (10)	33 (10)	8 (10)
32	CF$_3$SO$_2$	150 ± 40	20 (8)	38 (8)	17 (8)
33	1-adamantyl-SO$_2$	1.2 ± 0.34	−36 (9)	−70 (9)	61 (9)
34	PhSO$_2$	14 ± 6.0	29 (10)	17 (8)	−48 (9)
35	4-Cl-C$_6$H$_{42}$	3.3 ± 0.5	−41 (9)	41 (10)	7 (8)
36	4-(NO$_2$)C$_6$H$_4$SO$_2$	5.2 ± 1.6	−36 (9)	28 (9)	−118* (9)
37	4-(CH$_3$O)C$_6$H$_4$SO$_2$	8.0 ± 2.7	29 (10)	62 (*10)	−42 (10)
38	pentafluorophenyl-SO$_2$	1.6 ± 0.2	−24 (10)	28 (10)	27 (10)
39	2,4,6-(^{i}Pr)$_3$C$_6$H$_4$SO$_2$	3.2 ± 1.0	−17 (10)	−16 (7)	−186* (7)
40	2-Npth-SO$_2$	2.7 ± 0.7	−26 (10)	−69 (8)	20 (9)
43	3,5-(CH$_3$CO$_2$)$_2$C$_6$H$_4$CO	60 ± 30	−38 (8)	−21 (7)	−12 (8)
44	4-(HO)C$_6$H$_4$CO	3.9 ± 0.4	35 (8)	21 (7)	28 (7)
45	3,4-(HO)$_2$C$_6$H$_4$CO	16 ± 4.0	−73* (10)	−44 (9)	−41 (10)
46	2,6-(CH$_3$O)$_2$C$_6$H$_4$CO	15 ± 5.0	20 (8)	7 (6)	−177* (6)
47	4-F-C$_6$H$_4$CO	2.9 ± 0.9	44 (10)	23 (9)	22 (10)
48	4-F-C$_6$H$_4$OC(O)	0.63 ± 0.2	63* (10)	99* (9)	−67 (10)
49	Cbz	1.6 ± 0.3	53* (10)	78* (9)	38 (9)
50	4-(CH$_3$O)C$_6$H$_4$CO	1.9 ± 0.8	77* (9)	89* (9)	91* (8)
51	PhOC(O)	1.3 ± 0.4	62* (10)	97* (9)	77 (9)
52	4-(CH$_3$O$_2$C)C$_6$H$_4$CO	1.8 ± 0.2	49* (9)	50* (8)	56* (8)
53	2-Npth-CO	0.87 ± 0.13	43* (10)	50* (10)	73* (10)
54	3,4-(CH$_3$)$_2$C$_6$H$_4$CO	1.0 ± 0.4	75* (10)	91* (8)	55* (8)
55	PhCO	6.8 ± 1.1	62* (9)	62 (*9)	−7 (9)
56	3,4-(CH$_3$O)$_2$C$_6$H$_4$CO	1.1 ± 0.3	66* (10)	91* (9)	46 (10)
57	CF$_3$CO	15 ± 3.0	67 (7)	77* (6)	91* (6)
58	4-(PhO)C$_6$H$_4$CO	0.41 ± 0.2	31 (10)	61* (9)	78* (10)

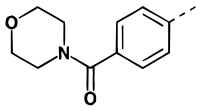
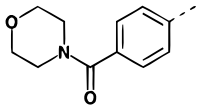
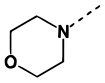
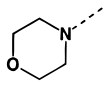
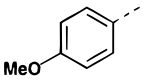
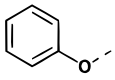
[a] Inhibition of HNE-catalyzed hydrolysis of the synthetic substrate MeO-Suc-Ala-Ala-Pro-Val-pNa. [b] Values of wet lung weight (Lw) relative to body weight (Bw), red cells, and white blood cells are the percent reduction relative to HNE and saline controls: 100% indicates identical with saline control; 0% indicates identical with HNE control; a negative value indicates parameter was worse than HNE control. An asterisk (*) indicates value is statistically significant relative to control ($p = 0.05$). Number in parentheses is number of animals used in experiment. Dose of HNE was 100 mg/animal; dose of inhibitor was 37.5 mg/kg po administered 30 min prior to HNE administration. [c] NT = not tested in the particular assay.

incorporating small, relatively nonpolar substituents on the aromatic ring. Particularly interesting is a comparison of the active compounds **48** and **56** with the inactive analogs **47** and **46**, respectively, which illustrates the subtle balance of properties necessary for obtaining oral activity.

We designed the ALIM[5] as an *in vivo* screen to assist in the development of compounds which would be suitable for administration by aerosol. When evaluating compounds intratracheally, we used an enzyme dose of 400 μg/animal. This dose of enzyme causes an extensive hemorrhagic lesion in the lung. Since the inhibitors were administered directly into the lung in our it program, compounds such as ICI 200,880 and ICI 200,355 were able to block the HNE-induced lung damage when predosed up to 36 h prior to administration of the enzyme. However, this dose of enzyme presents a very severe challenge for a compound administered orally since a significantly larger dose of

Table 3. Comparative Oral Activity of Peptidyl Trifluoromethyl and Pentafluoroethyl Ketones in AHA



Compd[a]	R	X	K_i (nM)	HNE Dose (μg/hamster)	Compd Dose (mg/kg)[d]	% Inhibition
59		---CF_2CF_3	25[b,c]	10	25	74
60		---CF_3	12[b]	50	50	inactive
61		---CF_2CF_3	170[b,c]	10	25	69
62		---CF_3	195[b]	50	50	inactive
50		---CF_3	1.9	50	10	84
51		---CF_3	1.3	50	10	91

[a]All compounds are 1:1 mixtures of epimers at the stereogenic center α to the ketone carbonyl, except **50** which is the *S* epimer. [b] All data for compounds **59-62** from reference 27. [c] These compounds were also shwon to significantly reduce the lesion induced by a 50 μg/animal dose of HNE. [d] Compounds were dosed orally 30 min prior to adminisration of an intratrachael dose of HNE.

compound must be administered orally to obtain an equivalent concentration in the lung. Thus we chose a dose of 100 μg/animal of enzyme when evaluating our compounds orally.

Even a dose of 100 μg/animal represents a fairly severe challenge for an orally administered compound. However, it is difficult to obtain a consistent hemorrhagic response in all three parameters measured in the ALIM if a dose much less than 100 μg of HNE is used. On the other hand, lower doses of HNE do generate a reproducible response in the degree of hemorrhage. This is the basis of the acute hemorrhagic assay (AHA), a model that is similar to the ALIM (see Pharmacological Evaluation section) except that only the degree of hemorrhage is determined and quantified by spectrophotometrically measuring the amount of hemoglobin in lavage rather than the number of red cells.[30]

Because of the ease of analysis and the ability to generate reproducible responses using low doses of enzyme, the AHA has been widely used for evaluating the activity of elastase inhibitors following oral administration. Table 3 lists the activity of several peptidyl PFEKs and TFMKs in the AHA. It can be seen that while the TFMKs **60** and **62** were inactive in this model, the TFMKs **50** and **51**, even at low doses of inhibitor (10 mg/kg), can significantly block the hemorrhage induced by a 50 μg/animal insult of HNE. Significantly, **50** and **51** demonstrated a greater degree of protection with a more severe enzyme challenge (50 μg/animal) and smaller dose of inhibitor (10 mg/kg) than the PFEKs **59** and **61** at a lower dose of enzyme (10 μg/animal) and larger dose of inhibitor (25 mg/kg).

Thus, it is not the properties of the electrophilic ketone activating group *per se* which are responsible for the differences in the oral activity between the TFMKs and PFEKs. Rather, it is the overall physicochemical properties of the molecules which render them active or inactive following oral administration. Therefore, if the extensive hydration of the TFMKs is a factor which decreases oral activity, this can be compensated for by the appropriate choice of N-terminal group. Clearly, hydration of the ketone carbonyl does not preclude oral activity. On the basis of the results from the current study, the difference between the TFMKs and PFEKs may have as much to do with the different lipophilicities of the pentafluoroethyl and trifluoromethyl groups as

it does with ketone hydration. As was observed with the development of TFMKs having a long duration of action following it administration, the *in vivo* activity of peptidyl electrophilic ketones following oral administration is dependent upon the overall properties of the entire molecule, and not upon a particular functionality.

Conclusion

Several tripeptidyl TFMKs have been identified which display excellent *in vivo* activity following oral administration in two different models of HNE-induced lung injury. Within the series of TFMKs, the *in vivo* profile can be controlled by modifications of the N-terminal group. Thus, compounds containing a diarylacylsulfonamide, such as ICI 200,880, that displayed excellent activity following it administration were not active orally. By changing the N-terminal group to a small, noncharged substituent, compounds with excellent orally activity can be obtained. These results contradict a previous report which concluded that TFMKs as a class lacked oral activity as a result of extensive hydration of the ketone carbonyl. Our results demonstrate that, with the proper N-terminal group, peptidyl TFMKs possess excellent oral activity. It should be possible to extrapolate this finding to other peptidyl electrophilic ketones. With the proper balance of physicochemical properties between the ketone activating group and the N-terminal substituents, oral activity should be obtainable in many different series of peptidyl ketones.

Experimental Section

General. Analytical samples were homogeneous by TLC and afforded spectroscopic results consistent with the assigned structures. Proton NMR spectra were obtained using either a Bruker WM-250 or AM-300 spectrometer. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane as internal standard. Mass spectra (MS) were recorded on a Kratos MS-80 instrument or a Finnigan MAT-60 operating in the chemical ionization (DCI) mode (only peaks ≥10% of the base peak are reported). Elemental analyses for carbon, hydrogen, and nitrogen were determined by the Analytical Section, ZENECA Pharmaceuticals, on a Perkin-Elmer 241 elemental analyzer and are within ±0.4% of theory for the formulas given. Analytical thin-layer chromatography (TLC) was conducted on prelayered silica gel GHLF plates (Analtech, Newark, DE). Visualization of the plates was accomplished using either UV light or phosphomolybdic acid−ethanol or iodoplatinate charring. Flash chromatography was conducted on Kieselgel 60, 230−400 mesh (E. Merck, Darmstadt, West Germany). Solvents were either reagent or HPLC grade. Reactions were run at ambient temperature and under a nitrogen atmosphere unless otherwise noted. Solvent mixtures are expressed as volume:volume ratios. Solutions were evaporated under reduced pressure on a rotary evaporator. All starting materials were commercially available unless otherwise indicated.

General Procedures for the Preparation of Trifluoromethyl Alcohols III. Method A: (2*RS*,3*SR*)-[[4-(Methylthio)phenyl]carbonyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-4). A solution of amino alcohol **II** (1.00 g, 2.72 mmol), 4-(methylthio)benzoic acid (520 mg, 3.09 mmol), and EDAC (650 mg, 3.39 mmol) in DMF (15 mL) was treated with DMAP (754 mg, 6.17 mmol) and stirred at room temperature for 18 h. The resulting solution was diluted with ethyl acetate, washed with 1 N HCl, saturated $NaHCO_3$, H_2O, and brine, dried ($MgSO_4$), and evaporated. The crude material was purified by flash chromatography eluting with chloroform/methanol (95:5) to afford trifluoromethyl alcohol **III-4** (1.16 g, 78%) as a white foam which was used without further purification: TLC $R_f = 0.70$, chloroform/methanol (9:1).

Method B: (2*RS*,3*SR*)-(4-Acetoxybenzoyl)-L-valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-41). A 0 °C solution of amino alcohol **II** (1.00 g, 2.72 mmol) in THF (15 mL) was treated with 4-acetoxybenzoic acid (738 mg, 4.08 mmol), HOBt (1.10 g, 8.16 mmol), and EDAC (783 mg, 4.08 mmol), warmed to room temperature, stirred for 18 h, and evaporated. The residue was taken up in ethyl acetate, washed with 1 N HCl, saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography eluting with diethyl ether afforded trifluoromethyl alcohol **III-41** (1.2 g, 86%) as a white solid: TLC $R_f = 0.38$, diethyl ether; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.86−1.01 (12H, m), 1.72−2.27 (6H, m), 2.29 (3H, s), 3.73 (1H, m), 3.81 (2H, m), 4.10 (1H, m), 4.45 (2H, m), 7.21 (2H, d, $J = 7.5$ Hz), 7.96 (2H, d, $J = 7.5$ Hz).

Method C: (2*RS*,3*SR*)-[[4-(Methoxycarbonyl)phenyl]-carbonyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-52). A 0 °C solution of amino alcohol **II** (4.5 g, 12.2 mmol), 4-carbomethoxybenzoic acid (2.2 g, 12.2 mmol), and HOBt (3.3 g, 24.5 mmol) in THF (250 mL) was treated with DCC (2.84 g, 13.8 mmol), warmed to room temperature, stirred for 18 h, and filtered and the filtrate evaporated. The residue was taken up in chloroform, washed with 20% aqueous citric acid and saturated $NaHCO_3$, dried ($MgSO_4$), and evaporated. Purification by flash chromatography eluting with chloroform/methanol (97:3) afforded trifluoromethyl alcohol **III-52** (5.13 g, 79%) as a pale yellow foam: TLC $R_f = 0.36$ and 0.40, chloroform/methanol (95:5); ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.83−1.20 (12H, m), 1.63−2.23 (6H, m), 3.63 (1H, m), 3.71−3.90 (5H, m), 4.13 (1H, m), 4.30−4.55 (2H, m), 6.46 (0.5H, d, $J = 8.2$ Hz), 6.56 (0.5H, d, $J = 8.2$ Hz), 7.24 (0.5H, d, $J = 8.2$ Hz), 7.67 (0.5H, d, $J = 8.2$ Hz), 8.02 (4H, m), 8.75 (1H, t, $J = 8.2$ Hz).

Method D: (2*RS*,3*SR*)-(3,4-Diacetoxybenzoyl)-L-valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-42). A −40 °C solution of 3,4-diacetoxybenzoic acid (640 mg, 3.00 mmol) and *N*-methylmorpholine (1.0 g, 0.33 mL, 3.00 mmol) in THF (10 mL) was treated dropwise with isobutyl chloroformate (0.411 g, 0.390 mL, 3.00 mmol). The resulting suspension was stirred at −40 °C for 35 min and was treated with a solution of amino alcohol **II** (1.00 g, 2.72 mmol) in CH_2Cl_2 (2 mL), and the mixture was allowed to warm to room temperature and stirred for 18 h. The reaction mixture was diluted with ethyl acetate, washed with 1 N HCl, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. The crude material was purified by flash chromatography, eluting with chloroform/methanol (95:5). The fractions containing the desired product were combined, dissolved in ethyl acetate, washed with saturated $NaHCO_3$, dried ($MgSO_4$), and evaporated to afford alcohol **III-42** (1.16 g, 76%) as a solid: HPLC $t_R = 2.51$ and 3.38, H_2O/CH_3CN (3:2), flow rate = 2 mL/min, Zorbax ODS analytical column (4.6 mm × 25 cm); ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.83 (6H m), 0.97 (6H, m), 1.72−2.30 (6H, m), 2.31 (6H, s), 3.55−4.1 (4H, m), 4.32−4.58 (2H, m), 7.37 (1H, d, $J = 8.0$ Hz), 7.90 (2H, m). Anal. ($C_{27}H_{36}F_3N_3O_8 \cdot 0.5H_2O$) C, H, N.

Method E: (2*RS*,3*SR*)-[(*E*)-3-[4-(Ethoxycarbonyl)phenyl]-1-oxoprop-2-enyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-17). A solution of 4-carbethoxy-(*E*)-cinnamic acid (6.82 g, 30.9 mmol) and CDI (5.02 g, 30.9 mmol) in THF (150 mL) was warmed to 40 °C until CO_2 evolution ceased. The resulting solution was cooled to room temperature, treated with amino alcohol **II** (735 mg, 2.00 mmol), stirred for 18 h, and evaporated. The residue was taken up in ethyl acetate, washed with saturated $NaHCO_3$, 1 N HCl, and brine, dried ($MgSO_4$), and evaporated to afford alcohol **III-17** (15.7 g, 89%). The crude material was used without further purification: TLC $R_f = 0.26$, chloroform/methanol (94:6); MS (DCI) $m/z = 570$ (M + 1, base).

Method F: (2*RS*,3*SR*)-Acetyl-L-valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-26). A solution of amino alcohol **II** (4.00 mg, 1.13 mmol) and TEA (445 mg, 0.613 mL, 4.40 mmol) in CH_2Cl_2 (5 mL) was treated with acetic anhydride (224 mg, 0.207 mL, 2.20 mmol), stirred at room temperature for 2 h, and evaporated. The residue was diluted with ether, filtered, and evaporated. The crude mate-

rial was purified by flash chromatography, eluting with chloroform/methanol (95:5) to afford alcohol **III-26** (483 mg, 100%) as a white foam: TLC $R_f = 0.4$, chloroform/methanol (9:1); MS (DCI) $m/z = 410$ (M + 1, base), 269; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (12H, m), 1.89 (1.5H, s), 1.92 (1.5H, s), 1.94 (6H, m), 3.55 (1H, m), 3.73 (2H, m), 4.09 (1H, m), 4.42 (2H, m), 7.20 (1H, d, $J = 10$ Hz), 7.63 (1H, d, $J = 10$ Hz).

Method G: (2*RS*,3*SR*)-(Trifluoroacetyl)-L-valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-57). To a solution of amino alcohol **II** (0.79 g, 2.15 mmol) and ethyl trifluoromethyl acetate (0.360 g, 0.301 mL, 2.50 mmol) in methanol (10 mL) was added TEA (0.500 g, 0.685 mL, 4.95 mmol), and the mixture was stirred at room temperature for 18 h and evaporated. The residue was taken up in ethyl acetate, washed with 1 N HCl, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated to afford alcohol **III-57** (0.870 g, 87%) as a white foam which was used without further purification: TLC $R_f = 0.41$ and 0.44, chloroform/methanol (95:5).

Method H: (2*RS*,3*SR*)-[(4-Methoxyphenyl)carbonyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-50). A 0 °C suspension of amino alcohol **II** (175 g, 476 mmol) and K_2CO_3 (126 g, 1.19 mol) in THF (2.5 L) was treated dropwise over 1 h with *p*-anisoyl chloride (81.3 g, 476 mmol), maintaining a reaction temperature of ≤3 °C. After being stirred at 0 °C for 2 h, the reaction mixture was filtered and the filter cake washed with THF (500 mL) and ethyl acetate (1 L). The combined filtrates were washed with 0.15 N HCl, brine, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. The crude material was recrystallized from ethyl acetate (1 L, two crops) to afford 174 g. The mother liquors were evaporated, and the residue was purified by chromatography, eluting with a gradient of dichloromethane/methanol (100:0, 99:1, 97.5:2.5). The chromatographed material was further purified by recrystallization from ethyl acetate to afford 34.5 g. The material from all crystallizations was combined to afford alcohol **III-50** (209 g, 87%) as a white solid: TLC $R_f = 0.33$, chloroform/methanol (95:5); ^{1}H NMR (300 MHz, DMSO-d_6/TFA) δ 0.94 (12H, m), 1.77−2.31 (6H, m), 3.63 (1H, m), 3.82 (3H, s), 3.86 (1H, m), 4.12 (1H, m), 4.49 (2H, m), 6.98 (2H, d, $J = 8.9$ Hz), 7.92 (2H, d, $J = 8.9$). Anal. ($C_{24}H_{34}F_3N_3O_5$) C, H, N.

Method I: (2*RS*,3*SR*)-[[[4-(Ethoxycarbonyl)phenyl]-amino]carbonyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-15). A solution of amino alcohol **II** (2.5 g, 6.80 mmol) in chloroform (25 mL) was treated with ethyl 4-isocyanatobenzoate (1.33 g, 6.80 mmol), stirred at room temperature for 4 h, and evaporated to afford alcohol **III-15** (4.49 g, >100%) as a gray foam which was used without further purification: TLC $R_f = 0.34$, methanol/chloroform (5:95); ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.9 (12H, m), 1.3 (3H, t, $J = 8$ Hz), 1.9 (6H, m), 3.7 (3H, m), 4.1 (1H, m), 4.3 (2H, q, $J = 8$ Hz), 4.4 (2H, m), 7.5 (2H, d, $J = 10$ Hz), 7.8 (2H, d, $J = 10$ Hz).

Method J: (2*RS*,3*SR*)-[Bis(4-phenylpropyl)]-L-valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-25). A 0 °C solution of 3-phenyl-1-propanol (370 mg, 2.72 mmol) and Proton Sponge ((Aldrich; 580 mg, 2.72 mmol) in CH_2Cl_2 (10 mL) was treated with $(CF_3SO_2)_2O$ (672 mg, 0.450 mL, 2.72 mmol) and stirred for 10 min. The reaction mixture was treated dropwise with a solution of amino alcohol **II** (1.00, 2.72 mmol) and Proton Sponge (580 mg, 2.72 mmol) in CH_2Cl_2 (10 mL), stirred at room temperature for 4 d, and evaporated. The residue was taken up in ethyl acetate, and filtered, and the filtrate washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography on silica gel eluting with hexanes/ethyl acetate (7:3) afforded trifluoromethyl alcohol **III-25** (300 mg, 18%) as a colorless oil: TLC $R_f = 0.43$, ethyl acetate/hexanes (30:70); MS (DCI) $m/z = 604$ (M + 1, base), 308; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.90 (12H, m), 1.73 (10H, m), 2.50 (8H, m), 3.08 (1H, m), 3.27 (1H, m), 3.57 (1H, m), 3.73 (1H, m), 4.09 (1H, m), 4.36 (1H, m), 6.44 (0.25H, d, $J = 7$ Hz), 6.57 (0.75H, d, $J = 7$ Hz), 7.18 (10H, m).

Method K: 2(*RS*,3*SR*)-[[2-(Methoxycarbonyl)ethyl]carbonyl]valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-13). A 0 °C solution of amino alcohol **II** (10.0 g, 27.2 mmol) and NaOH (27.2 mL, 1.00 N, 27.2 mmol) in dichloromethane (200 mL) was treated with a solution of 3-carbomethoxypropionyl chloride (4.10 g, 27.2 mmol) in CH_2Cl_2 (20 mL) and stirred at 0 °C for 0.5 h. The reaction mixture was acidified to pH 2 with 1 N HCl, the organic phase separated, and the aqueous phase extracted with two portions of CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and evaporated. Purification by flash chromatography eluting with acetone/hexanes (1:1) afforded trifluoromethyl alcohol **III-13** (11.1 g, 84%) as a white foam: TLC $R_f = 0.45$, chloroform/methanol (97:3); ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.85 (12H, m), 1.75−2.00 (6H, br m), 2.41 (4H, m), 3.56 (4H, s), 3.71 (2H, m), 3.85 (1H, m), 4.32 (2H, m), 6.35−8.08 (2H, multiple fractional peaks due to the NH resonances of the four diastereomers).

General Procedure for Oxidation of Sulfides III-4. Method L: (2*RS*,3*SR*)-[[4-(Methylsulfonyl)phenyl]carbonyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-3). A solution of sulfide **III-4** (590 mg, 1.14 mmol) and $NaHCO_3$ (925 mg, 1.14 mmol) in CH_2Cl_2 (10 mL) was treated with MCPBA (756 mg, 80−85%, 4.40 mmol) and stirred at room temperature for 1 h. The reaction mixture was treated with an excess of triphenylphosphine (>4.4 mmol), diluted with ethyl acetate, washed with 1 N NaOH, H_2O, and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography eluting with chloroform/methanol (95:5) afforded sulfone **III-3** (580 mg, 93%) as a white foam: TLC $R_f = 0.3$, dichloromethane/methanol (95:5); MS (DCI) $m/z = 546$ (M + 1), 267 (base), 99; ^{1}H NMR (300 MHz, DMSO-d_6/TFA) δ 0.91 (12H, m), 1.83 (6H, m), 3.26 (1.5H, s), 3.37 (1.5H, s), 3.65 (1H, m), 3.84 (2H, m), 4.47 (3H, m), 8.01 (2H, d, $J = 8$ Hz), 8.12 (2H, d, $J = 8$ Hz).

General Procedures for Oxidation of Sulfoxides III-33a. Method M: (2*RS*,3*SR*)-[(Tricyclo[3.3.1.1^{3,7}]dec-1-yl)-sulfonyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-2-hydroxy-4-methylpentyl)]-L-prolinamide (III-33). A refluxing solution of sulfoxide **III-33a** (860 mg, 1.56 mmol) in acetone (75 mL) was treated dropwise with a saturated solution of $KMnO_4$ in acetone (excess, ca. 150 mL). Following addition of $KMnO_4$, the reaction mixture was refluxed for an additional 15 min, cooled to room temperature, filtered through diatomaceous earth, and evaporated. Purification by flash chromatography eluting with hexanes/ethyl acetate (3:2) afforded sulfone **III-33** (637 mg, 72%) as a white solid: TLC $R_f = 0.46$ and 0.50, chloroform/methanol (95:5); ^{1}H NMR (300 MHz, DMSO-d_6/TFA) δ 0.76−1.00 (12H, m), 1.50−2.12 (21H, m), 3.58 (2H, m), 3.74 (2H, m), 4.02 (1H, m), 4.39 (1H, m). Anal. ($C_{26}H_{42}N_3O_5F_3S\cdot 2.5H_2O$) C, H, N.

General Procedures for the Oxidation of Trifluoromethyl Alcohols III. Method N: (3*RS*)-[[4-(Methylsulfonyl)phenyl]carbonyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-4-methyl-2-oxopentyl)]-L-prolinamide (3). A solution of trifluoromethyl alcohol **III-3** (650 mg, 1.18 mmol) and EDAC (2.30 g, 12.0 mmol) in DMSO/toluene (1:1, 0.8 mL) was treated with dichloroacetic acid (400 mg, 4.8 mmol) and stirred at room temperature for 18 h. The reaction mixture was diluted with ethyl acetate, washed with 1 N HCl, H_2O, and brine, dried ($MgSO_4$), and evaporated. Purification was by flash chromatography eluting with a gradient of hexanes/acetone (40:60, 60:40) to afford trifluoromethyl ketone **3** (560 mg, 86%) as a white foam: TLC $R_f = 0.3$, acetone/hexane (2:3); MS (DCI) $m/z = 548$ (M + 1), 282, 267 (base), 99; ^{1}H NMR (300 MHz, DMSO-d_6/TFA) δ 0.84 (3H, m), 0.91 (9H, m), 1.99 (4H, m), 2.19 (2H, m), 3.31 (3H, s), 3.41 (1H, m), 3.64 (2H, m), 4.45 (2H, m), 8.02 (2H, d, $J = 8$ Hz), 8.13 (2H, d, $J = 8$ Hz). Anal. ($C_{24}H_{32}O_6N_3F_3S\cdot 1.0\ H_2O$) C, H, N.

Method O: (3*RS*)-(4-Acetoxybenzoyl)-L-valyl-*N*-[3-(1,1,1-trifluoro-4-methyl-2-oxopentyl)]-L-prolinamide (41). A suspension of amino alcohol **III-41** (903 mg, 1.70 mmol) and DMP (2.16 g 5.1 mmol) in dichloromethane (6 mL) was treated with TFA (584 mg, 395 mL, 5.1 mmol) and stirred at room temperature for 18 h. The reaction mixture was partitioned between a solution of saturated $Na_2S_2O_3$/saturated $NaHCO_3$

(1:1) and ethyl acetate, the ethyl acetate layer was separated, washed with saturated $Na_2S_2O_3$/saturated $NaHCO_3$ (1:1) and brine, dried ($MgSO_4$), and evaporated to afford analytically pure trifluoromethyl ketone **41** (820 mg, 91%) as a white solid: TLC R_f = 0.32 and 0.38, diethyl ether; ^{1}H NMR (300 MHz, DMSO-d_6/TFA) δ 0.94 (12H, m), 1.71−2.33 (6H, m), 2.29 (3H, s), 3.60 (1H, m), 3.87 (1H, m), 4.03 (0.5 H, m, CH α to CF_3 carbonyl for hydrate), 4.48 (2.2H, m), 4.57 (0.3H, d, J = 6.7 Hz, CH α to CF_3 carbonyl for ketone), 7.21 (2H, d, J = 8.5 Hz), 7.98 (2H, d, J = 8.5 Hz). Anal. ($C_{25}H_{32}N_3O_6F_3 \cdot 0.6H_2O$) C, H, N.

Method P: (3*RS*)-[[[4-(Ethoxycarbonyl)phenyl]amino]carbonyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-4-methyl-2-oxopentyl)]-L-prolinamide (15). A −60 °C solution of DMSO (42.4 g, 38.7 mL, 544 mmol) and oxalyl chloride (34.4 g, 23.3 mL, 272 mmol) in dichloromethane (275 mL) was stirred for 15 min and treated with a solution of trifluoromethyl alcohol **III-15** (7.80 g, 13.6 mmol) in CH_2Cl_2 (50 mL). The mixture was stirred for 1 h, treated dropwise with diisopropyl ethylamine (70.3 g, 94.8 mL, 544 mmol), and allowed to warm to room temperature. The reaction mixture was washed with 1 N HCl and brine, dried (Na_2SO_4), and evaporated. The crude mixture was purified by flash chromatography eluting with a gradient of ether/hexanes (50:50, 70:30, 80:20) and then chloroform/methanol (95:5). The impure fractions were purified by a second chromatography eluting with chloroform/methanol (97:3). The material from both chromatographies was dissolved in dichloromethane and evaporated to afford trifluoromethyl ketone **15** (6.09 g, 80%) as an off-white solid: TLC R_f = 0.40, methanol/chloroform (5:95); ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.9 (12H, m), 1.3 (3H, t, J = 7 Hz), 2.0 (6H, m), 3.6 (1H, m), 3.7 (1H, m), 4.3 (2H, q, J = 7 Hz), 4.4 (1H, m), 4.5 (1H, m), 4.6 (1H, m), 6.6 (1H, m), 7.5 (2H, d, J = 9 Hz), 7.8 (2H, d, J = 9 Hz), 8.62 (1H, m), 9.10 (1H, s). Anal. ($C_{26}H_{35}N_4O_6F_3$) C, H, N.

Method Q: (3*RS*)-[[4-(Methoxycarbonyl)phenyl]carbonyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-4-methyl-2-oxopentyl)]-L-prolinamide (52). A solution of amino alcohol **III-52** (4.00 g, 7.56 mmol), acetic anhydride (30.3 g, 28 mL, 300 mmol), and DMSO (35.2 g 32 mL, 451 mmol) was stirred at room temperature for 18 h. The reaction mixture was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography eluting with chloroform/methanol (98:2) afforded trifluoromethyl ketone **52** (2.21 g, 55%) as a white foam: TLC R_f = 0.44 and 0.50, methanol/chloroform (5:95); ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.69−1.03 (12H, m), 1.75−2.26 (6H, m), 3.63 (1H, m), 3.84−4.0 (4H, m), 4.1 (0.6H, m), 4.50 (2H, m), 4.59 (0.4H, t, J = 8.2 Hz), 6.67−6.95 (0.4H, m), 7.35−7.59 (0.3H, m), 8.01 (4H, s), 8.59 (0.3H, m), 8.77 (1H, m). Anal. ($C_{25}H_{32}F_3N_3O_6 \cdot 0.5H_2O$) C, H, N.

Method R: (3*RS*)-[(*E*)-3-[4-(Ethoxycarbonyl)phenyl]-1-oxoprop-2-enyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-4-methyl-2-oxopentyl)]-L-prolinamide (17). A suspension of CrO_3 (5.00 g, 50.0 mmol) in CH_2Cl_2 (20 mL) was stirred for 15 min, treated with diatomaceous earth (5 g), stirred an additional 15 min, and treated with trifluoromethyl alcohol **III-17** (1.00 g, 1.75 mmol). The reaction mixture was stirred at room temperature for 2.5 h, filtered, diluted with ethyl acetate, washed with 1 N HCl, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography eluting with dichloromethane/methanol (98:2) afforded trifluoromethyl ketone **17** (650 mg, 65%) as a white foam: TLC R_f = 0.37, methanol/chloroform/acetic acid (3:97:1); ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.82−1.01 (12H, m), 1.35 (3H, t, J = 7.2 Hz), 1.70−2.42 (6H, m), 3.60 (1H, m), 3.86 (1H, m), 4.12 (0.6H, m), 4.33 (2H, q, J = 7.2 Hz), 4.52 (2H, m), 4.65 (0.4H, m), 6.95 (1H, d, J = 15.8 Hz), 7.50 (1H, J = 15.8 Hz), 7.70 (2H, d, J = 8.3 Hz), 8.02 (2H, d, J = 8.3 Hz). Anal. ($C_{28}H_{36}F_3N_3O_6$) C, H, N.

Method S: (3*RS*)-[[4-(Hydroxycarbonyl)phenyl]carbonyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-4-methyl-2-oxopentyl)]-L-prolinamide (6). A solution of ester **52** (700 mg, 1.32 mmol) in methanol/H_2O (1:1, 16 mL) was treated with NaOH (1 N, 2.99 mL, 2.99 mmol) and stirred at room temperature for 18 h. The reaction mixture was treated with H_2O, made acidic with 1 N HCl, and extracted with ethyl acetate and the combined ethyl acetate extracts were dried (Na_2SO_4) and evaporated to afford analytically pure acid **6** (482 mg, 71%) as a white foam: TLC R_f = 0.35, methanol/chloroform/acetic acid (5:95:0.1); ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.93 (12H, m), 1.7−2.3 (6H, m), 3.65 (1H, m), 3.96 (1H, m), 4.06 (0.4H, m), 4.58 (2 H, m), 4.63 (0.6H, t, J = 8.2 Hz), 6.91 (0.2H, m), 7.40 (0.2H, m), 7.99 (4H, m), 8.61 (0.6H, m), 8.73 (1H, d, J = 7.7 Hz). Anal. ($C_{24}H_{30}N_3F_3O_6 \cdot 1.0H_2O$) C, H, N.

Method T: (3*RS*)-(4-Hydroxybenzoyl)-L-valyl-*N*-[3-(1,1,1-trifluoro-4-methyl-2-oxopentyl)]-L-prolinamide (44). A solution of acetate **41** (820 mg, 1.56 mmol) and H_2O (2 mL) in methanol (12 mL) was treated with K_2CO_3 (excess) stirred at room temperature for 18 h and evaporated. The residue was acidified with 1 N HCl and extracted with ethyl acetate. The combined ethyl acetate extracts were washed with brine, dried ($MgSO_4$), and evaporated to afford analytically pure phenol **44** (462 mg, 62%) as a white foam: TLC R_f = 0.64 and 0.72, diethyl ether/ethyl acetate (1:1); ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.95 (12H, m), 1.72−2.38 (6H, m), 3.65 (1H, m), 3.96 (1H, m), 4.08 (0.4H, m, CH α to CF_3 carbonyl for hydrate), 4.48 (2.3H, m), 4.64 (0.3H, d, J = 6.0 Hz, CH α to CF_3 carbonyl for one of two ketone diastereomers), 6.82 (2H, d, J = 8.60 Hz), 7.81 (2H, d, J = 8.60 Hz). Anal. ($C_{23}H_{30}N_3F_3O_5 \cdot 0.55\ H_2O$) C, H, N.

Method U: (3*RS*)-[[2-[3-(Hydroxycarbonyl)naphthyl]]carbonyl]-L-valyl-*N*-[3-(1,1,1-trifluoro-4-methyl-2-oxopentyl)]-L-prolinamide (11). A solution of benzyl ester **16** (420 mg, 0.64 mmol) in ethanol (20 mL) was hydrogenated over 10% Pd−C (50 mg, dry) at 50 psi for 8 h, filtered, and evaporated to afford acid **11** (255 mg, 70%) as an analytically pure white solid: HPLC t_R = 7.82 and 8.86, H_2O/CH_3CN/THF/TFA (55:35:15:0.1), flow rate = 1 mL/min, Phenomenex Zorbax C-8 analytical column (4.6 mm × 35 cm); ^{1}H NMR (300 MHz, DMSO-d_6/TFA) δ 1.02 (12H, m) 1.77−2.37 (6H, m), 3.69 (1H, m), 3.97 (1H, m), 4.11 (0.4H, m, CH α to CF_3 carbonyl for hydrate), 4.57 (2.6H, m), 7.67 (2H, m), 7.98 (1H, s), 8.07 (2H, m), 8.39 (1H, s). Anal. ($C_{28}H_{32}F_3N_3O_6$) C, H, N.

***In Vivo* Assay—Acute Lung Injury Model (ALIM).** The ALIM has previously been described in detail.[5] Male Syrian hamsters (90−110 g) were anesthetized with Brevital sodium (30 mg/kg, ip) and the trachea surgically exposed. A dose of HNE (100 μg) in phosphate-buffered saline (0.3 mL, 0.01 M) was injected into the exposed trachea via a 1/2 in., 23 ga. needle. The incision was closed with stainless steel surgical staples, and the animals were allowed to recover. Twenty-four hours after the injection of HNE, the animals were killed with an overdose of pentobarbital sodium. The lungs and heart were resected and the lungs and trachea carefully cleaned of extraneous material. Following measurement of wet lung weight, the tracheas were cannulated and lavaged three times with PBS (2 mL). The recovered lavages were pooled for each animal, and the volume was recorded. Total red and white cells were determined using a Coulter counter. The data are expressed as lung weight/100 g of body weight and total cells recovered (white or red, cells/mL × volume recovered). The values for wet lung weights, total lavagable red cells, and total lavagable white cells are elevated in a dose-dependent manner following administration of HNE. Test compounds are evaluated in this model for their ability to reduce this effect of HNE when they are administered as a solution orally (solution in 10% PEG/PBS) 30 min prior to administration of HNE.

***In Vivo* Assay—Acute Hemorrhagic Assay (AHA)**. The AHA used was a minor modification of a published procedure[30] that measures protection against the lung hemorrhage induced in anesthetized hamsters by 50 mg of intratracheally administered HNE, as determined by spectrophotometrically measuring the amount of hemoglobin present in lung lavage after 4 h. Test compounds were administered orally (solution in 10% PEG/PBS) 30 min prior to administration of HNE.

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References

(1) Abbreviations: AHA, acute hemorrhagic assay; ALIM, acute lung injury model; HNE, human neutrophil elastase; PPE, porcine pancreatic elastase; Ac, acetyl; Cbz, benzyloxycarbonyl; TFMK, trifluoromethyl ketone; PFEK, perfluoroethyl ketone; CAN, ceric ammonium nitrate; TEA, triethylamine; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; DMAP, (dimethylamino)pyridine; DCC, dicyclohexylcarbodiimide; MCPBA, *m*-chloroperbenzoic acid; EDAC, 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole monohydrate; TBAF, tetra-*n*-butylammonium fluoride; TFA, trifluoroacetic acid; DMF, dimethylformamide; NMM, *N*-methylmorpholine; DMP, Dess-Martin periodinane, 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one; MeO, methoxy; Suc, succinyl; *p*Na, *p*-nitroanilide; PEG, polyethylene glycol; PBS, phosphate-buffered saline; ip, intraperitoneal; po, oral; it, intratracheal.

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