Novel Non-Nucleoside Inhibitors of HIV-1 Reverse Transcriptase. 2. Tricyclic Pyridobenzoxazepinones and Dibenzoxazepinones

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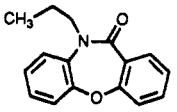
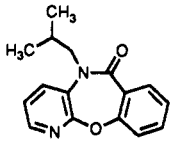
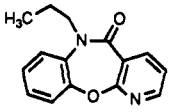
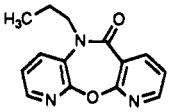
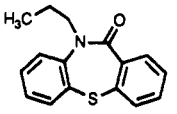
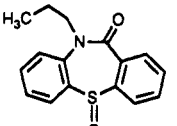
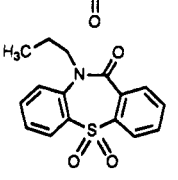
Dibenz[*b,f*][1,4]oxazepin-11(10*H*)-ones (III), pyrido[2,3-*b*][1,4]benzoxazepin-6(5*H*)-ones (IV), and pyrido[2,3-*b*]-[1,5]benzoxazepin-5(6*H*)-ones (V) were found to inhibit human immunodeficiency virus type 1 reverse transcriptase with IC_{50} values as low as 19 nM. A-ring substitution has a profound effect on activity, with appropriate substituents at the positions ortho and para to the lactam nitrogen providing dramatically enhanced potency. Substitution in the C-ring is generally neutral or detrimental to activity. Although a C-ring amino substituent at the position meta to the lactam carbonyl is generally beneficial to activity, it has essentially no effect when the A-ring is optimally substituted. Like the dipyridodiazepinone nevirapine, compounds III–V are specific for HIV-1 RT, exhibiting no inhibitory activity against HIV-2 RT or other virial reverse transcriptase enzymes.

Since the discovery of the human immunodeficiency virus type 1 (HIV-1)[1] and its causative role in the development of acquired immunodeficiency syndrome (AIDS), considerable research effort has been directed toward a full elucidation of the viral life cycle and the identification of potential targets for clinical intervention.[2] Among the many approaches being pursued in the design of new AIDS therapies, inhibition of the viral enzyme reverse transcriptase (RT) represents a particularly attractive strategy.[3] Unique to the RNA viruses, RT catalyzes the transcription of single-stranded viral genomic RNA into double-stranded DNA, a process essential for retroviral replication. The vast majority of known RT inhibitors are nucleoside analogues, which require phosphorylation by cellular enzymes in order to function as active inhibitors. These compounds operate by a complex mechanism of action, potentially serving both as competitive inhibitors and as chain terminators.[4] The dideoxynucleoside zidovudine (AZT)[5] was the first drug approved for use in the treatment of AIDS, and ddI[6] has recently received approval for patients who are intolerant to AZT. Several others (ddC, d4T, FLT) are currently in clinical trials. Despite apparent selectivity for RT, these substrate analogues also inhibit mammalian DNA polymerases α, β, γ, and δ, and often exhibit significant toxic side effects. The emergence of viral strains that are resistant to AZT[7] is an additional concern associated with its use.

Recently, novel non-nucleoside reverse transcriptase inhibitors have been reported.[8,9] The dipyridodiazepinone nevirapine is a potent and exquisitely specific inhibitor of HIV-1 RT, exhibiting no inhibitory activity against HIV-2 RT or against mammalian DNA polymerases. Its specificity, extremely low cytotoxicity, and potent antiviral activity against all viral strains tested, including those resistant to AZT, make nevirapine an especially promising clinical candidate for the treatment of HIV-1 infection. Since it functions by a mechanism[10] distinct from that of the nucleoside substrate analogues, it is anticipated that nevirapine will avoid the clinical toxicities associated with many of them. The compound is currently undergoing Phase II clinical evaluation.

The discovery of nevirapine was the result of intensive lead optimization following a high capacity screening program specifically designed to identify a non-nucleoside inhibitor of HIV-1 RT. The synthesis and structure–activity relationships (SAR) of pyridobenzo- (I) and dipyridodiazepinone (II) RT inhibitors have been reported previously.[11] In parallel to those efforts, an investigation

Table I

		% inhibn at 1 μM[a]	IC_{50} (nM)[a]
1		72	375
2		31	ND[b]
3		29	ND
4		5	ND
5		77	400
6		20	ND
7		31	ND

[a] Inhibition of HIV-1 RT. See Experimental Section. [b] ND = not determined.

† Department of Medicinal Chemistry.
‡ Department of Biochemistry.

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of analogous series of dibenz- and pyridobenzoxazepinones (III–V) was also undertaken, the results of which are re-

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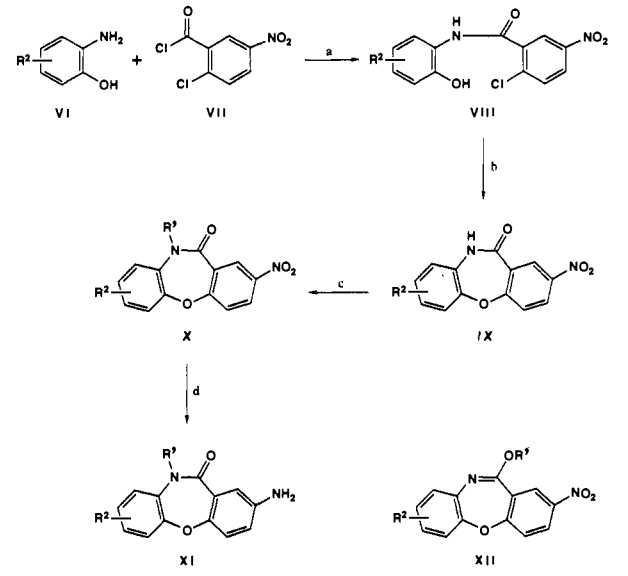
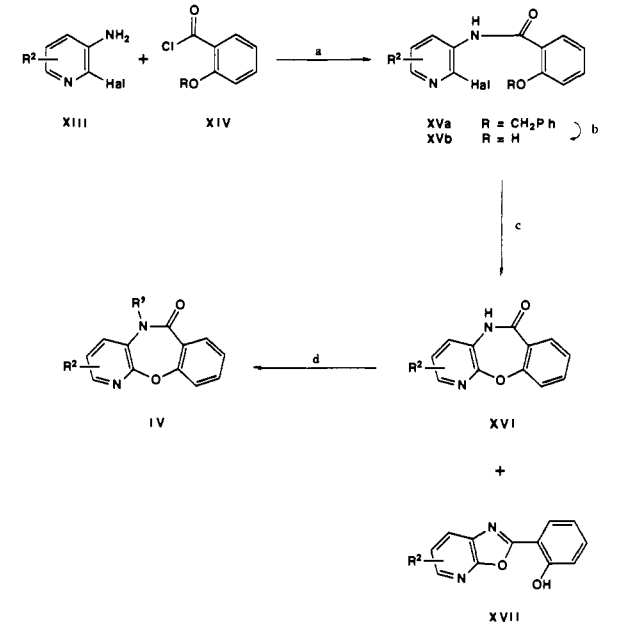
Scheme I[a]



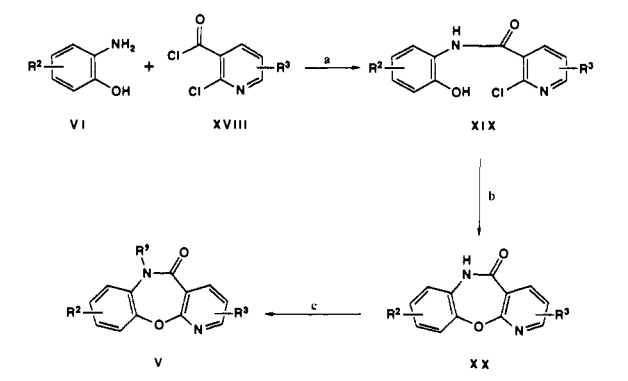
[a] (a) $(i\text{-Pr})_2$NEt, THF; (b) aqueous NaOH, reflux; (c) NaH, R^1X, DMF; (d) H_2, Pd/C, EtOH; or $SnCl_2$, HCl, HOAc.

Scheme II[a]



[a] (a) Et_3N, toluene; (b) HBr, HOAc; (c) $NaOCH_3$, tetraglyme, 220 °C; (d) R^1X, NaH, DMF.

Scheme III[a]



[a] (a) $(i\text{-Pr})_2$NEt, EtOAc; (b) pyridine, 90 °C; or NaOH, DMF, 100 °C; (c) R^1X, NaH, DMF.

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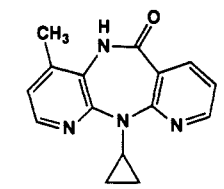
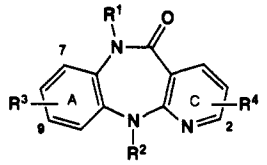
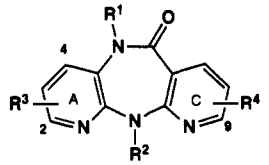


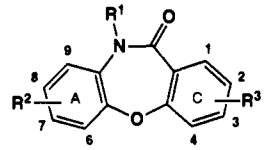
Nevirapine



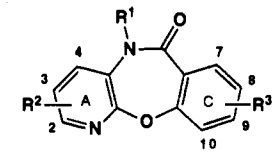
I



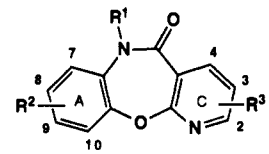
II



III



IV

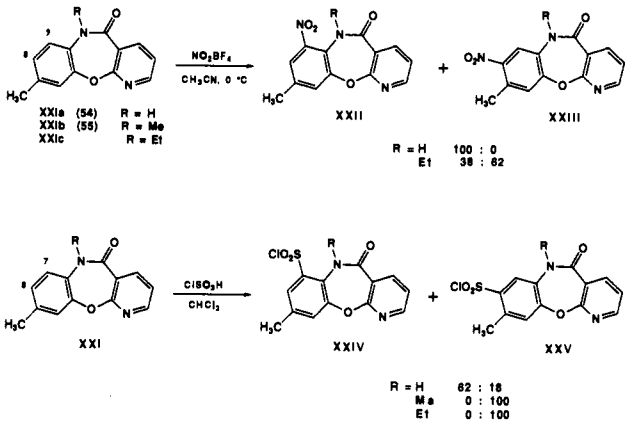


V

Chemistry

The synthesis of 2-aminodibenz[*b,f*][1,4]oxazepin-11-(10*H*)-ones XI was accomplished according to literature procedures,[12] as illustrated in Scheme I. Thus, condensation of 2-aminophenols VI with 2-chloro-5-nitrobenzoyl chloride (VII) afforded carboxamides VIII. Although THF was employed as the solvent for many of these reactions, we have since discovered that EtOAc generally provides the best results. With the nitro substituent serving to activate the C-ring, ring closure was conveniently effected by treatment of VIII with hot aqueous sodium hydroxide. Amide alkylation (NaH, R^1X, DMF) and nitro reduction (H_2, Pd/C, EtOH; or $SnCl_2$, HCl, HOAc) then afforded 2-aminodibenz[*b,f*][1,4]oxazepin-11(10*H*)-ones XI. O-Alkylation of the amide to give XII was a persistent side-reaction, especially when the 9-position was substituted.

Scheme IV



Compounds XI were the focus of considerable attention, due to their potent inhibitory activity against HIV-1 RT (vide infra). In addition, the amino substituent, via the corresponding diazonium ion, served as a convenient precursor for the introduction of other substituents into the 2-position of III. Although yields were only moderate, diazotization of the appropriate amines, followed by treatment with $Cu(NO_3)_2 \cdot 2.5H_2O$ and Cu_2O,[13] afforded 2-hydroxy compounds **39** and **44**. Similarly, 2-cyano compounds **17**, **40**, and **43** were prepared by Sandmeyer reactions of **19**, **27**, and **37**, respectively. Nitrile reduction (Raney nickel, H_2, EtOH) of **17** provided the corresponding aminomethyl compound **18**. Reduction of the intermediate diazonium ion was also possible; deamination of **37** and **27** with isoamyl nitrite in DMF[14] produced compounds **42** and **25**. Alternatively, these compounds were prepared by a route analogous to that outlined in Scheme I, utilizing 2-chlorobenzoyl chloride in place of 2-chloro-5-nitrobenzoyl chloride. In the absence of the activating nitro substituent, more stringent conditions (KOMe, tetraglyme, 275 °C)[15]

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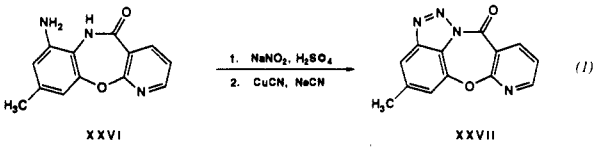
were required to induce ring closure.

Pyrido[2,3-*b*][1,4]benzoxazepin-6(5*H*)-ones (IV)[16] were prepared by an adaptation of a published procedure[16a] (Scheme II). Amide condensation was accomplished by the addition of salicyloyl chloride (XIV; R = H) to a toluene solution of 3-amino-2-halopyridine XIII, though it often proved advantageous to use a protected salicyloyl chloride (XIV; R = CH_2Ph). Deprotection was then effected with HBr/HOAc to give XVb. Treatment of XVb with sodium methoxide in tetraglyme afforded the desired tricyclic oxazepinone (XVI), which was alkylated by conventional methods. Pyridoxazoles XVII were often obtained as byproducts from the ring closure reaction.

Pyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-ones (V)[17] were obtained by methods analogous to those already discussed, as shown in Scheme III. Simple heating in pyridine (90–100 °C) was sufficient to effect ring closure of XIX when a nitro substituent was present in the position para to the leaving group. Without nitro-activation of the C-ring, ring closure was achieved with powdered sodium hydroxide in DMF (120 °C).

The chemical reactivity of the intact tricyclic oxazepinone V has also been explored. Although conventional nitration procedures were not successful, treatment of XXIa with nitronium tetrafluoroborate[18] afforded exclusively the 7-nitro derivative in 85% yield, whereas the 8-nitro isomer was the major product when the *N*-ethyl compound XXIc was nitrated under similar conditions (Scheme IV). Chlorosulfonation of XXIa also occurred predominantly at the 7-position, but in this case the 8-isomer was also obtained (Scheme IV). As noted for the nitration of XXI, the regioselectivity of chlorosulfonation also shifted substantially toward the 8-position when the amide was alkylated. Bromination of XXIa could be effected with $Br_2/AgNO_3$,[19] but the reaction was not regioselective and was accompanied by numerous side reactions, including nitration and dibromination.

The 7-nitro derivative of XXIa served as a key synthetic intermediate for the exploration of SAR at the 7-position. Amide alkylation and nitro reduction afforded the corresponding amines **79** and **75**. Sandmeyer reaction ($NaNO_2$, CuCN, NaCN) of **79** proceeded smoothly to afford the nitrile **77** in 83% yield. Interestingly, Sandmeyer reaction of **75** was much less efficient, providing the desired nitrile **76** in very poor yield (<10%). An attempt to circumvent this problem by performing the Sandmeyer reaction prior to amide alkylation was unsuccessful, resulting only in formation of triazene XXVII by intramolecular trapping of the diazonium ion (eq 1). Conversion of nitrile **77** to



XXVI XXVII (1)

the corresponding amide **81**, acid **83**, ester **80**, and imidate **82** was accomplished by standard procedures, and the hydroxymethyl compound **84** was prepared by reduction of the acid (EtCOCl, $NaBH_4$).

Because chlorosulfonation of XXIa occurred predominantly at the 8-position, alternative routes were required for the preparation of pyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-ones bearing sulfur substituents at the 7-position. Diazotization of **79**, followed by treatment with sodium thiomethoxide, afforded the corresponding methyl sulfide, which was oxidized (*m*-CPBA, $CHCl_3$) to afford sulfone **85**.

Results and Discussion

All compounds were assayed in vitro for inhibitory activity against HIV-1 reverse transcriptase. Presented in Table I is a comparison of the results obtained for representative examples of several related ring systems. In general, dibenzoxazepinones (III) are more potent inhibitors of HIV-1 RT than are monopyridooxazepinones (IV and V), whereas dipyridooxazepinones[20] (e.g., **4**) are only weakly active. Interestingly, this trend is exactly the reverse of that observed in the diazepinone series.[11] 10-*n*-Propyldibenz[*b*,*f*][1,4]-thiazepin-11(10*H*)-one (**5**)[21] proved to have inhibitory activity nearly equivalent to that of the corresponding dibenzoxazepinone **1**. However, oxidation at sulfur resulted in a dramatic reduction in potency. The generally poor solubility of thiazepinones was an additional concern, and these compounds were not pursued further. Synthetic efforts therefore focused on the benzopyrido- and dibenzoxazepinones III–V.

A preliminary investigation of SAR at the lactam nitrogen revealed in all three series a preference for lipophilic substituents of intermediate size (e.g., *n*-Pr, *i*-Pr, allyl). By comparison, a methyl-substituted amide proved to be optimal in the diazepinone series.[11] Apparently the enzyme is able to accommodate greater steric bulk around the amide of the oxazepinones, perhaps a result of the fact that the other heteroatom of the lactam ring is unsubstituted. Hydroxamic acid **15** and methyl hydroxamate **16**[22] are less active than the corresponding *N*-alkyl dibenzoxazepinones **1** and **10–12**.

Substitution in the A-ring of III and V, particularly at the 7- and 9-positions, causes a shift in preference toward smaller amide substituents. However, an unsubstituted oxazepinone lactam (R^1 = H) invariably results in a dramatic loss in activity, regardless of A-ring substitution (e.g., **31**, **41**, **54**). This result stands in sharp contrast to the previous finding that an unsubstituted diazepinone lactam is strongly preferred when the 4-position of II is substituted.[11] Removal of the amide carbonyl (**14**) or conversion to a thioamide (**13**, **73**) is detrimental to activity. By contrast, diazepinethiones are generally more active than the corresponding diazepinones.[11]

Dibenz[*b*,*f*][1,4]oxazepin-11(10*H*)-ones (III). A comparison of **9** to compounds **17–26** (Table II) provides an initial indication of the effects of A- and C-ring sub-

(16) (a) Schmidt, G. Production of Pyrido[2,3-*b*][1,4]benzoxazepine-6-(5*H*)-ones. Canadian Patent, 790,597, 1968. (b) Hargrave, K. D.; Schmidt, G. Pyrido[2,3-*b*][1,4]benzoxazepin (and thiazepin)-6(5*H*)-ones and -thiones and Their Use in the Prevention or Treatment of AIDS. Eur. Patent Appl. EP 417,534, 1991.

(17) Hargrave, K. D.; Schmidt, G. Pyrido[2,3-*b*][1,5]benzoxazepin (and thiazepin)-5(6*H*)-ones and -thiones and Their Use in the Prevention or Treatment of HIV Infection. Eur. Patent Appl. EP 415,303, 1991.

(18) Kuhn, S. J.; Olah, G. A. Aromatic Substitution. VII. Friedel–Crafts Type Nitration of Aromatics. *J. Am. Chem. Soc.* **1961**, *83*, 4564–4571.

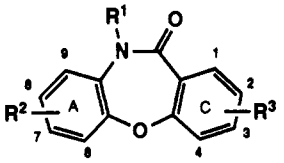
(19) Fleifel, A. M. Bromination and Iodination of Some *p*-Alkylbenzoic Acids. *J. Org. Chem.* **1960**, *25*, 1024–1025.

(20) Compound **4** was prepared by a route analogous to that depicted in Scheme III. See: Hargrave, K. D. Dipyrido[3,2-*b*:2′,3′-*e*][1,4]oxazepin (and thiazepin)-6(5*H*)-ones and -thiones and Their Use in the Prevention or Treatment of AIDS. Eur. Patent Appl. EP 415,304, 1991.

(21) Dibenz[*b*,*f*][1,4]thiazepin-11(10*H*)-one, the precursor to **5**, was prepared according to the procedure of Bélanger et al. Bélanger, P. C.; des Ormeaux, D.; Rokach, J.; Laval, J. S. 10,11-Dihydro-dibenzo[*b*,*f*][1,4]-thiazepin Derivatives. U.S. Patent 4,728,735, 1988.

(22) Compound **15** was obtained in low yield by oxidation of **1** (NaH, MoOPH, THF). Methylation of **15** (K_2CO_3, MeI, DMF) afforded **16**.

Table II. Dibenz[*b,f*][1,4]oxazepin-11(10*H*)-ones



no.	R^1	R^2	R^3	mp, °C	formula	% inhibn at 1 μM[a]	IC_{50} (nM)[a]
8[b]	H	H	H	211–213	$C_{13}H_9NO_2$	30	ND[f]
9[c]	CH_3	H	H	79–81	$C_{14}H_{11}NO_2$	20	ND
10[c]	CH_2CH_3	H	H	52–52.5	$C_{15}H_{13}NO_2$	54	560
11	$CH_2CH{=}CH_2$	H	H	85	$C_{16}H_{13}NO_2$	58	465
1	$CH_2CH_2CH_3$	H	H	oil	$C_{16}H_{15}NO_2$	72	375
12	$CH(CH_3)_2$	H	H	102–103	$C_{16}H_{15}NO_2$	73	340
13	$CH_2CH_2CH_3$ (thioamide)	H	H	oil	$C_{16}H_{15}NOS$	46	2200
14	CH_2CH_3 (reduced amide)	H	H	54–56	$C_{15}H_{15}NO$	12	ND
15	OH	H	H	101–102	$C_{13}H_9NO_3$	15	ND
16	OCH_3	H	H	oil	$C_{14}H_{11}NO_3$	36	ND
17	CH_3	H	2-CN	152.5–153.5	$C_{15}H_{10}N_2O_2$	12	ND
18	CH_3	H	2-CH_2NH_2	oil	$C_{15}H_{14}N_2O_2{\cdot}0.25H_2O$	0	ND
19[d]	CH_3	H	2-NH_2	133–136	$C_{14}H_{12}N_2O_2$	73	180
20	CH_2CH_3	H	2-NH_2	165–168	$C_{15}H_{14}N_2O_2$[e]	82	145
21	CH_2CH_3	H	2-NO_2	142–143	$C_{15}H_{12}N_2O_4$	23	ND
22	CH_3	H	2-$NHCH_3$	130–135	$C_{15}H_{14}N_2O_2$	40	ND
23[d]	CH_3	H	3-NH_2	195.5–198	$C_{14}H_{12}N_2O_2$	19	ND
24[d]	CH_3	7-NH_2	H	194–196	$C_{14}H_{12}N_2O_2$	20	ND
25	CH_3	7-CH_3	H	104–107	$C_{15}H_{13}NO_2$	66	600
26	CH_3	9-CH_3	H	111–113	$C_{15}H_{13}NO_2$	36	ND
27	CH_3	7-CH_3	2-NH_2	185–187	$C_{15}H_{14}N_2O_2$	96	30
28	$CH_2CH_2CH_3$	7-CH_3	2-NH_2	146–148	$C_{17}H_{16}N_2O_2$	84	130
29[d]	CH_2CH_3	8-CH_3	2-NH_2	110–111.5	$C_{15}H_{14}N_2O_2$	76	240
30	$CH_2CH_2CH_3$	8-CH_3	2-NH_2	129–132	$C_{17}H_{16}N_2O_2$	53	ND
31	H	9-CH_3	2-NH_2	235–237	$C_{14}H_{12}N_2O_2$	31	ND
32	CH_2CH_3	9-CH_3	2-NH_2	153–154.5	$C_{16}H_{16}N_2O_2$	90	60
33	$CH_2CH_2CH_3$	9-CH_3	2-NH_2	185–187	$C_{17}H_{16}N_2O_2$	70	260
34	$CH_2CH_2CH_3$	7,8-$(CH_3)_2$	2-NH_2	209–212	$C_{18}H_{20}N_2O_2$	87	165
35	$CH_2CH_2CH_3$	8,9-$(CH_3)_2$	2-NH_2	133–135	$C_{18}H_{20}N_2O_2$	63	670
36	$CH_2CH_2CH_3$	6,8-$(CH_3)_2$	2-NH_2	135–136	$C_{18}H_{20}N_2O_2$	52	900
37	CH_3	7,9-$(CH_3)_2$	2-NH_2	225–229	$C_{16}H_{16}N_2O_2$	95	20
38	CH_2CH_3	7,9-$(CH_3)_2$	2-NH_2	174–176	$C_{17}H_{16}N_2O_2$	95	43
39	CH_3	7-CH_3	2-OH	198–200	$C_{15}H_{13}NO_2$	58	640
40	CH_3	7-CH_3	2-CN	232–234	$C_{16}H_{12}N_2O_2$	69	280
41	H	7,9-$(CH_3)_2$	H	238–240	$C_{15}H_{13}NO_2$	39	ND
42	CH_3	7,9-$(CH_3)_2$	H	124–126	$C_{16}H_{15}NO_2$	86	57
43	CH_3	7,9-$(CH_3)_2$	2-CN	184–186	$C_{17}H_{14}N_2O_2$	77	50
44	CH_3	7,9-$(CH_3)_2$	2-OH	198–200	$C_{16}H_{15}NO_3$	80	63

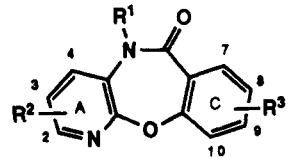
[a] Inhibition of HIV-1 RT. See Experimental Section. [b] Sample obtained from Aldrich Chemical Co. [c] Hey, D. H.; Leonard, J. A.; Rees, C. W.; Todd, A. R. *J. Chem. Soc. C* **1967**, 1513–1518. [d] See ref 12b. [e] C: calcd, 70.85; found, 69.64. [f] ND = not determined.

stituents on inhibitory activity. As previously noted for the analogous position 4 in the dipyridodiazepinone (II) series,[11] methyl substitution at position 9 of III increases activity. However, a 7-methyl substituent has a more pronounced beneficial effect, whereas substitution at the corresponding position 2 of II was previously reported to have a neutral effect on inhibitory activity. Especially noteworthy in the dibenzoxazepinone (III) series is the dramatic enhancement in potency provided by an amino substituent at the 2-position. This effect is substantially reduced upon alkylation of the amine, while amino substitution at other ring positions is detrimental to activity (e.g., **23**, **24**). These findings prompted a more thorough investigation of 2-aminodibenz[*b,f*][1,4]oxazepin-11(10*H*)-ones (XI), as exemplified by compounds **27–38**. Most of the 2-amino compounds were prepared first with an *n*-propyl substituent at N-10, based on the SAR exhibited by compounds **1** and **8–12**. However, as mentioned above, the SAR at N-10 is strongly dependent upon the aromatic substitution pattern; where available, data is provided both for the *N*-propyl compound and for the compound bearing the optimal substituent at N-10. As expected, 7-methyl and 9-methyl substitution produced compounds (**27** and **32**) with increased potency relative to **19** and **20**, respectively, whereas an 8-methyl substituent was detrimental to activity (**29**). The effect of two methyl substituents also followed predictable trends (**34–38**), with 7,9-dimethyl substitution providing the best activity. In fact, 7,9,10-trimethyldibenz[*b,f*][1,4]oxazepin-11(10*H*)-one (**37**) is one of the most potent oxazepinones tested.

Despite the promising antiviral activity of 2-aminodibenz[*b,f*][1,4]oxazepin-11(10*H*)-ones (XI), work on this series was halted when it was found that one of the compounds (**27**) provided a positive result in the Ames test for mutagenicity. In light of the reported carcinogenicity of aniline compounds,[23] analogues of III that would retain inhibitory activity against HIV-1 RT in the absence of a

(23) (a) Williams, G. M.; Weisburger, J. H. Chemical Carcinogenesis. In *Casarett and Doull's Toxicology*; Amdur, M. O., Doull, J., Klaassen, C. D., Eds.; Pergamon Press: New York, 1991; pp 127–200. (b) Weisburger, E. K.; Russfield, A. B.; Homburger, F.; Weisburger, J. H.; Boger, E.; Van dongen, C. G.; Chu, K. C. Testing of Twenty-one Environmental Aromatic Amines or Derivatives for Long-Term Toxicity or Carcinogenicity. *J. Environ. Pathol. Toxicol.* **1978**, *2*, 325–356.

Table III. Pyrido[2,3-*b*][1,4]benzoxazepin-6(5*H*)-ones



no.	R^1	R^2	R^3	mp, °C	formula	% inhibn at 1 μM[a]	IC_{50} (nM)[a]
45[b]	CH_3	H	H	137–139	$C_{13}H_{10}N_2O_2$	2	ND[c]
46	CH_3	2-Cl	H	170–171	$C_{13}H_9ClN_2O_2$	21	ND
47	CH_2CH_3	2-Cl	H	128.5–129.5	$C_{14}H_{11}ClN_2O_2$	35	ND
48	$CH_2CH_2CH_3$	2-Cl	H	97–99.5	$C_{15}H_{13}ClN_2O_2$	68	210
2	$CH_2CH(CH_3)_2$	H	H	145–148	$C_{16}H_{16}N_2O_2$	29	ND
49	$CH_2CH_2SCH_3$	2-Cl	H	125–126	$C_{15}H_{13}ClN_2O_2S$	79	72
50	CH_3	2,4-$(CH_3)_2$	H	163–164.5	$C_{15}H_{14}N_2O_2$	25	ND

[a] Inhibition of HIV-1 RT. See Experimental Section. [b] See ref 12d. [c] ND = not determined.

2-amino substituent were therefore sought. These efforts were largely successful, as exemplified by compounds **42–44**. Whereas deamination of **27** produced a compound (**25**) with a 20-fold decrease in activity, **42** is only slightly less potent than **37**. It is possible that the 2-amino substituent provides access to an additional or alternative binding element that greatly enhances binding affinity for otherwise weakly active compounds. Optimal substitution at positions 7 and 9, however, appears to alter the enzyme–inhibitor binding conformation in such a way that this interaction becomes less significant.

Compounds **42–44** were further tested for their ability to block the proliferation of HIV-1 infection in vitro (Table V). All three compounds inhibited syncytia formation in cultures of $CD4^+$ human T-cells (c8166) infected with $HIV\text{-}1_{IIIB}$, with potencies comparable to those exhibited in the enzymatic assay. Although compounds **42–44** possess good inhibitory activity against HIV-1 RT and are effective antiviral agents in cell culture, all three proved to be very poorly water soluble.

Pyrido[2,3-*b*][1,4]benzoxazepin-6(5*H*)-ones (IV) and Pyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-ones (V). In the pyrido[2,3-*b*][1,4]benzoxazepin-6(5*H*)-one series (IV) (Table III), a 2-chloro substituent enhanced activity. Compound **49** is a surprisingly potent inhibitor of HIV-1 RT, but all attempts at further structure optimization led to a decrease in activity. Of note is the fact that 2,4-dimethyl substitution, which corresponds to 7,9-disubstitution in III and V, does not translate into increased potency.

The isomeric pyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (V) series were examined in more detail (Table IV). The 7- and 9-positions were the primary focus of SAR exploration, in light of the results obtained for compounds III. As evidenced by compounds **55–61**, electron-donating substituents are well-tolerated at the 9-position, with methyl substitution at this position once again proving to be optimal. However, strongly hydrophilic groups lead to a decrease in activity, and electron-withdrawing substituents are also detrimental. Requirements at the 7-position appear to be somewhat more flexible (compounds **62–64** and compounds **72–85**); surprisingly, this is one position in which nitro substitution produces an enhancement in activity.

As in the dibenz[*b*,*f*][1,4]oxazepin-11(10*H*)-one series, a C-ring amino substituent at the position meta to the lactam carbonyl generally provides an increase in activity (compounds **66–71**). Of the 3-amino analogues, the 7,9-dimethyl compounds **70** and **71** exhibit the best potency, although 8,9-dimethyl compounds **68** and **69** are also quite active. Once again, however, the amino substituent is not required if the A-ring is optimally substituted. In fact, **71** and **72** are equipotent, and **76** is the most potent oxazepinone tested. Unfortunately, attempts to increase solubility relative to the nitrile **76** by the introduction of other hydrophilic substituents at position 7 led, for the most part, to inactive compounds (**81–85**). The in vitro antiviral activity of compounds V is exemplified by compounds **70** and **71** (Table V).

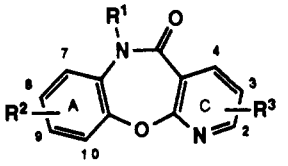
Conclusions

Like the diazepinone class of reverse transcriptase inhibitors, the oxazepinones described here are specific for HIV-1, exhibiting no inhibitory activity against HIV-2 RT or against mammalian DNA polymerases. Photoaffinity labeling experiments have established that compounds I–V all bind at the same allosteric site of HIV-1 RT,[24] a binding site shared also by other reported non-nucleoside inhibitors of HIV-1.[9b,10] Despite several striking differences, SAR in the oxazepinone series of RT inhibitors is in some respects quite similar to that observed in the diazepinone series. In particular, A-ring substitution has a profound effect on activity, with appropriate substituents at the positions ortho and para to the amide nitrogen providing dramatically enhanced potency. Substitution in the C-ring, on the other hand, is generally neutral or detrimental to activity. Although a C-ring amino substituent at the position para to the oxygen atom is generally beneficial to oxazepinone activity, it has essentially no effect when the A-ring is optimally substituted. Significant differences between the oxazepinone SAR presented here and previously reported diazepinone SAR[11] include (1) a reversal in ring system preference (i.e. III > V > IV, whereas II > I), and (2) an absolute requirement in the oxazepinone series for a substituted lactam nitrogen. Molecular modeling techniques are currently being utilized in an attempt to better understand the similarities and differences between oxazepinones and diazepinones as related to inhibitory activity against HIV-1 RT.

As a class, oxazepinones are inherently less potent inhibitors of HIV-1 RT than are diazepinones, a problem that has been at least partially solved by substituent optimization. In addition, oxazepinones suffer the disadvantage of being less soluble than diazepinones. This problem is exacerbated by the fact that dibenzoxazepinones are more potent than the mono- or dipyrido compounds, whereas the reverse is true in the diazepinone series. Attempts to optimize solubility have been thwarted by the general requirement of the enzyme binding site for lipophilic substituents. Thus, despite the identification of potent (IC_{50} < 50 nM) oxazepinone inhibitors of HIV-1

(24) Wu, J. C. Unpublished results.

Table IV. Pyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-ones



no.	R^1	R^2	R^3	mp, °C	formula	% inhibn at 1 μM[a]	IC_{50} (nM)[a]
51	CH_3	H	H	114–115	$C_{13}H_{10}N_2O_2$	23	ND[c]
52	CH_2CH_3	H	H	83–84	$C_{14}H_{12}N_2O_2$	25	ND
3	$CH_2CH_2CH_3$	H	H	oil	$C_{15}H_{14}N_2O_2$	31	ND
53	$CH_2CH{=}CH_2$	H	H	59–63	$C_{15}H_{12}N_2O_2$	40	ND
54	H	9-CH_3	H	241–243	$C_{13}H_{10}N_2O_2$	5	ND
55	CH_3	9-CH_3	H	165–168	$C_{14}H_{12}N_2O_2$	43	ND
56	CH_3	9-F	H	160–162	$C_{13}H_9FN_2O_2$	3	ND
57	CH_3	9-NO_2	H	132.5–136	$C_{13}H_9N_3O_4$	0	ND
58	CH_3	9-NH_2	H	207–209	$C_{13}H_{11}N_3O_2$	0	ND
59	CH_3	9-$N(CH_3)_2$	H	128.5–129.5	$C_{15}H_{15}N_3O_2$	34	ND
60	CH_3	9-OH	H	252–253.5	$C_{13}H_{10}N_2O_3$	14	ND
61	CH_3	9-OCH_3	H	157–158.5	$C_{14}H_{12}N_2O_3$	24	ND
62	CH_3	7-CN	H	207–210	$C_{14}H_9N_3O_2$	29	ND
63	CH_2CH_3	7-NO_2	H	128–131	$C_{14}H_{11}N_3O_4$	58	343
64	CH_2CH_3	7-NH_2	H	244–247	$C_{14}H_{13}N_3O_2$	33	ND
65	CH_3	9-CH_3	3-NO_2	197–198	$C_{14}H_{11}N_3O_4$	0	ND
66	CH_3	9-CH_3	3-NH_2	191–193	$C_{14}H_{13}N_3O_2$	57	250
67	CH_3	8,9-$(CH_3)_2$	3-NH_2	178–180	$C_{15}H_{15}N_3O_2$	72	322
68	CH_2CH_3	8,9-$(CH_3)_2$	3-NH_2	193–195	$C_{16}H_{17}N_3O_2$	83	71
69	$CH(CH_3)_2$	8,9-$(CH_3)_2$	3-NH_2	235–237	$C_{17}H_{19}N_3O_2$	89	45
70	CH_3	7,9-$(CH_3)_2$	3-NH_2	234–236	$C_{15}H_{15}N_3O_2$	92	46
71	CH_2CH_3	7,9-$(CH_3)_2$	3-NH_2	236–238	$C_{16}H_{17}N_3O_2$	93	27
72	CH_2CH_3	7,9-$(CH_3)_2$	H	105–107	$C_{16}H_{16}N_2O_2$	92	29
73	CH_2CH_3 (thioamide)	7,9-$(CH_3)_2$	H	142–144	$C_{16}H_{16}N_2OS$	82	90
74	CH_2CH_3	7-NO_2, 9-CH_3	H	120.5–122	$C_{15}H_{13}N_3O_4$	85	31
75	CH_2CH_3	7-NH_2, 9-CH_3	H	233–235	$C_{15}H_{15}N_3O_2$	73	132
76	CH_2CH_3	7-CN, 9-CH_3	H	149.5–151.5	$C_{16}H_{13}N_3O_2$[b]	96	19
77	CH_3	7-CN, 9-CH_3	H	159.5–160.5	$C_{15}H_{11}N_3O_2$	88	55
78	CH_3	7-NO_2, 9-CH_3	H	132–135	$C_{14}H_{11}N_3O_4$	85	23
79	CH_3	7-NH_2, 9-CH_3	H	230–231.5	$C_{14}H_{13}N_3O_2$	53	1000
80	CH_3	7-CO_2CH_3, 9-CH_3	H	179–180	$C_{16}H_{14}N_2O_4$	95	22
81	CH_3	7-$CONH_2$, 9-CH_3	H	319–320	$C_{15}H_{13}N_3O_3$	31	ND
82	CH_3	7-$C(OCH_3){=}NH$, 9-CH_3	H	177–178.5	$C_{16}H_{15}N_3O_3$	0	ND
83	CH_3	7-CO_2H, 9-CH_3	H	269–173	$C_{15}H_{12}N_2O_4$	0	ND
84	CH_3	7-CH_2OH, 9-CH_3	H	157–159	$C_{15}H_{14}N_2O_3$	39	ND
85	CH_3	7-SO_2CH_3, 9-CH_3	H	213–215	$C_{15}H_{14}N_2O_4S$	27	ND

[a] Inhibition of HIV-1 RT. See Experimental Section. [b] C: calcd, 68.81; found, 69.88. [c] ND = not determined.

Table V. Inhibitory Effect of Selected Compounds On HIV-1_{IIIB} Replication in c8166 Cells

no.	IC_{50} (nM)[a]
42	38
43	31
44	88
70	115
71	54

[a] Inhibitory concentration of compound producing a 50% reduction in centers of syncytia. See ref 8.

RT, poor solubility continues to be a critical impediment to the development of this series of compounds.

Experimental Section

General. Short-path chromatography was performed with EM-Science silica gel 60 (finer than 230 mesh). Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Bruker WM-250 (250 MHz) spectrometer and ^{13}C NMR spectra were recorded on a Bruker AC-270 (69.2 MHz) spectrometer with Me_4Si as the internal standard. Mass spectra were recorded on a Finnegan 4023 GC/MS/DS spectrometer. Elemental analyses were determined by Midwest Laboratories, Indianapolis, IN, and are within 0.4% of theoretical values unless otherwise noted.

2-Aminophenols either were commercially available and used as received or were prepared by nitration of the appropriately substituted phenol and/or reduction of the nitrophenol.

***N*-(2-Hydroxy-4-methylphenyl)-2-chloro-5-nitrobenzenecarboxamide (VIII; R^2 = 4-CH_3).** A slurry of 2-chloro-5-nitrobenzoic acid (10.0 g, 0.05 mol) and $SOCl_2$ (12 mL, 0.14 mol), containing 2 drops of DMF, was heated at reflux to obtain a yellow-brown solution. Excess $SOCl_2$ was removed in vacuo, and the residue was dissolved in THF (25 mL). The resultant acid chloride solution was added dropwise over 30 min to a solution of 6-amino-*m*-cresol (6.11 g, 0.05 mol) and $(i\text{-Pr})_2NEt$ (17.3 mL, 0.10 mol) in THF (25 mL) at 0 °C. The reaction mixture was allowed to warm to 25 °C. After 48 h, the reaction mixture was diluted with Et_2O (200 mL), washed with 1 N HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated to a yellow oil. Trituration with Et_2O afforded the title compound (13.48 g, 89%): mp 190–193 °C; ^1H NMR (d_6-DMSO) δ 9.89 (br s, 1 H), 9.65 (br s, 1 H), 8.43 (d, *J* = 3 Hz, 1 H), 8.31 (dd, *J* = 3, 9 Hz, 1 H), 7.84 (d, *J* = 9 Hz, 1 H), 7.64 (d, *J* = 8 Hz, 1 H), 6.73 (s, 1 H), 6.65 (d, *J* = 8 Hz, 1 H), 2.23 (s, 3 H). Anal. ($C_{14}H_{11}ClN_2O_4$) C, H, N.

7-Methyl-2-nitrodibenz[*b*,*f*][1,4]oxazepin-11(10*H*)-one (IX; R^2 = 7-CH_3). A solution of *N*-(2-hydroxy-4-methylphenyl)-2-chloro-5-nitrobenzenecarboxamide (53.08 g, 0.17 mol) and 2 N NaOH (95 mL, 0.19 mol) in water (1.5 L) was heated at reflux for 10 h. The resultant slurry was allowed to stand overnight at room temperature. The product was collected by suction filtration and washed with copious amounts of water. Air-drying afforded the title compound (39.9 g, 85%): mp 274–277 °C; ^1H NMR (d_6-DMSO) δ 10.79 (s, 1 H), 8.52 (d, *J* = 3 Hz, 1 H), 8.45 (dd, *J* = 3, 8.8 Hz, 1 H), 7.60 (d, *J* = 8.8 Hz, 1 H), 7.23 (s, 1 H), 7.04–7.11 (m, 2 H), 2.27 (s, 3 H). Anal. ($C_{14}H_{10}N_2O_4$) C, H, N.

7,10-Dimethyl-2-nitrodibenz[*b*,*f*][1,4]oxazepin-11(10*H*)-one (III; $R^1 = CH_3$; $R^2 = 7\text{-}CH_3$; $R^3 = 2\text{-}NO_2$). To a suspension of NaH (50% oil dispersion, 0.68 g, 0.014 mol) in DMF (60 mL) was added in one portion 7-methyl-2-nitrodibenz[*b*,*f*][1,4]oxazepin-11(10*H*)-one (3.5 g, 0.013 mol). The mixture was warmed to 50 °C to produce a clear red solution. The reaction mixture was cooled to room temperature and CH_3I (1.6 mL, 0.026 mol) was added. Stirring was continued overnight. The reaction mixture was diluted with ice–water, and the resultant precipitate was collected by suction filtration, washed with water and petroleum ether (30–60 °C), and air-dried to afford the title compound (3.46 g, 94%) as a brown powder, suitable for use in the next reaction. Its properties upon recrystallization from EtOAc are as follows: mp 162.5–163.5 °C; ^{1}H NMR (d_6-DMSO) δ 8.78 (d, J = 3 Hz, 1 H), 8.31 (dd, J = 3, 9 Hz, 1 H), 7.33 (d, J = 9 Hz, 1 H), 7.15 (d, J = 8 Hz, 1 H), 7.05–7.09 (m, 2 H), 3.59 (s, 3 H), 2.34 (s, 3 H). Anal. ($C_{15}H_{12}N_2O_4$) C, H, N.

2-Amino-7,10-dimethyldibenz[*b*,*f*][1,4]oxazepin-11(10*H*)-one (27). A solution of 7,10-dimethyl-2-nitrodibenz[*b*,*f*][1,4]oxazepin-11(10*H*)-one (3.4 g, 0.012 mol) in EtOH (150 mL) was hydrogenated at 50 psi over 10% Pd/C (0.3 g). After 16 h, the reaction mixture was filtered through Celite and concentrated. The residue was dissolved in 1% MeOH–CH_2Cl_2 and passed through a short plug of silica gel. Concentration and recrystallization of the residue from CH_2Cl_2–hexanes afforded **27** (2.68 g, 88%): mp 185–187 °C; ^{1}H NMR (d_6-DMSO) δ 7.29 (d, J = 8.2 Hz, 1 H), 7.09 (d, J = 1.5 Hz, 1 H), 7.04 (dd, J = 1.5, 8 Hz, 1 H), 6.96 (d, J = 8.6 Hz, 1 H), 6.89 (d, J = 3 Hz, 1 H), 6.68 (dd, J = 3, 8.6 Hz, 1 H), 5.18 (s, 2 H, N*H*$_2$), 3.42 (s, 3 H), 2.27 (s, 3 H). Anal. ($C_{15}H_{14}N_2O_2$) C, H, N.

2-Cyano-6,7,9-trimethyldibenz[*b*,*f*][1,4]oxazepin-11(10*H*)-one (43). To 2-amino-6,7,9-trimethyldibenz[*b*,*f*][1,4]oxazepin-11(10*H*)-one (**37**) (1.0 g, 3.7 mmol) in a 250-mL three-necked flask was added 2 N HCl (6 mL). The hydrochloride salt precipitated. The mixture was cooled to 0 °C, and a solution of $NaNO_2$ (0.27 g, 3.9 mmol) in 3 mL of water was added slowly dropwise to produce a clear yellow solution. As stirring continued at 0 °C, the diazonium salt precipitated. A mixed cyanide solution was prepared by adding a solution of KCN (1.1 g, 16.9 mmol) in 4 mL of water to a hot solution of $CuSO_4 \cdot 5H_2O$ (0.92 g, 3.7 mmol) in 3 mL of water. The resultant clear solution was cooled to room temperature and added by pipet to the diazonium ion mixture. The foamy reaction mixture was warmed to room temperature and then heated at 50–60 °C for 1 h. The reaction mixture was diluted with EtOAc and filtered through Celite. The organic layer was washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated to give an orange oil. Purification by short-path chromatography (elution with 10–30% EtOAc–hexanes) and recrystallization from EtOAc–hexanes afforded **43** (0.36 g, 35%) as yellow-orange needles: mp 184–186 °C; ^{1}H NMR (d_6-DMSO) δ 8.12 (d, J = 2 Hz, 1 H), 8.02 (dd, J = 2, 8 Hz, 1 H), 7.52 (d, J = 8 Hz, 1 H), 7.11 (br s, 1 H), 7.00 (br s, 1 H), 3.34 (s, 3 H), 2.27 (s, 3 H), 2.25 (s, 3 H); MS (CI) m/z 279 (MH^+). Anal. ($C_{17}H_{14}N_2O_2$) C, H, N.

2-Hydroxy-6,7,9-trimethyldibenz[*b*,*f*][1,4]oxazepin-11(10*H*)-one (44). The amine **37** (0.54 g, 2.0 mmol) was dissolved with heating in 35% H_2SO_4 (3 mL). Water (2 mL) was added and the solution was cooled in an ice-bath. The sulfate salt precipitated as a gummy solid, to which a solution of $NaNO_2$ (0.18 g, 2.6 mmol) in water (3 mL) was added dropwise, producing a clear yellow solution. Stirring was continued at 0 °C for 30 min, during which time the diazonium ion precipitated. To the reaction mixture was added a solution of $Cu(NO_3)_2 \cdot 2.5H_2O$ (7.2 g, 31 mmol) in 70 mL of water. The ice-bath was removed and Cu_2O (0.29 g, 2.0 mmol) was added in one portion with vigorous stirring. After 15 min, the reaction mixture was diluted with Et_2O and the entire mixture was filtered through Celite. The aqueous layer was extracted with additional Et_2O. The combined organic extract was washed with water and saturated aqueous NaCl, filtered through Florisil, and concentrated to give a yellow oil. Immediate purification by short-path chromatography (10–50% EtOAc–hexanes) afforded **44** (0.13 g, 24%): mp 198–200 °C; ^{1}H NMR (d_6-DMSO) δ 9.66 (s, 1 H, O*H*), 7.08 (d, J = 9 Hz, 1 H), 6.99 (d, J = 3 Hz, 1 H), 7.00 (s, 1 H), 6.92 (s, 1 H), 6.85 (dd, J = 3, 9 Hz, 1 H), 3.29 (s, 3 H), 2.24 (s, 6 H); MS (CI) m/z 270 (MH^+). Anal. ($C_{16}H_{15}NO_3$) C, H, N.

2-Chloro-3-cyano-4,6-dimethylpyridine. A mixture of 3-cyano-4,6-dimethyl-2-hydroxypyridine (7.5 g, 0.05 mol) and $POCl_3$ (15 mL, 0.16 mol) was heated at reflux for 3 h. The reaction mixture was cooled to room temperature, transferred to a separatory funnel, and added dropwise to water. Ice was added as necessary to maintain the temperature below 35 °C. The resultant precipitate was collected by suction filtration and washed with water. Air-drying afforded the title compound (8.05 g, 97%) as a white powder, suitable for use in the next reaction. Its properties upon recrystallization from hexanes are as follows: mp 99–100 °C; ^{1}H NMR ($CHCl_3$) δ 7.08 (s, 1 H), 2.57 (s, 3 H), 2.54 (s, 3 H). Anal. ($C_8H_7ClN_2$) C, H, N.

2-Chloro-4,6-dimethylnicotinamide. A solution of 2-chloro-3-cyano-4,6-dimethylpyridine (7.0 g, 0.042 mol) in concentrated H_2SO_4 (10 mL) was heated at 120 °C for 2 h. The reaction mixture was cooled to room temperature and poured into ice–water. The solution was neutralized with solid $NaHCO_3$, and the resultant precipitate was collected by suction filtration and washed with water. Air-drying afforded the title compound (7.76 g, 100%), which was used without further purification: mp 173–175 °C; ^{1}H NMR (d_6-DMSO) δ 7.98 (br s, 1 H), 7.75 (br s, 1 H), 7.18 (s, 1 H), 2.41 (s, 3 H), 2.26 (s, 3 H).

3-Amino-2-chloro-4,6-dimethylpyridine (XIII; $R^2 = 4,6\text{-}(CH_3)_2$; Hal = Cl). Bromine (2.4 mL, 0.05 mol) was added to a solution of sodium hydroxide (6.1 g, 0.15 mol) in 60 mL of water at −5 °C. 2-Chloro-4,6-dimethylnicotinamide (7.4 g, 0.04 mol) was added in one portion. The reaction mixture was stirred at 0 °C for 1 h and then at 70 °C for 45 min. The reaction mixture was then cooled to room temperature and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried ($MgSO_4$), and concentrated. Recrystallization of the residue (Et_2O–petroleum ether (30–60 °C)) afforded the title compound (4.68 g, 75%) as tan needles: mp 58–60 °C; ^{1}H NMR (d_6-DMSO) δ 6.87 (s, 1 H), 5.01 (s, 2 H), 2.23 (s, 3 H), 2.12 (s, 3 H).

***N*-(2-Chloro-4,5-dimethyl-3-pyridyl)salicylamide (XVb; $R^2 = 4,6\text{-}(CH_3)_2$; Hal = Cl).** A solution of salicyloyl chloride[25] (0.203 g, 1.29 mmol) in toluene (2 mL) was added, under N_2, dropwise to a stirred suspension of 3-amino-2-chloro-4,6-dimethylpyridine (0.203 g, 1.30 mmol) and Et_3N (0.129 g, 1.28 mmol) in toluene (5 mL). After the addition was complete, the mixture was heated at reflux for 3 h. The reaction mixture was then cooled to ambient temperature and shaken well with 1 N NaOH (2 × 10 mL). The aqueous layer was collected, adjusted to pH 5 with 1 N HCl, and extracted with EtOAc (3 × 20 mL). The combined organic extract was washed with water and saturated aqueous NaCl, and dried ($MgSO_4$). The solvent was removed in vacuo, and the residue was crystallized from EtOH–water to afford the title compound (0.127 g, 35%): mp 91–93 °C; ^{1}H NMR (d_6-DMSO) δ 11.50–12.40 (v br s, 1 H, O*H*), 10.37 (br s, 1 H, N*H*), 8.00 (dd, J = 2, 7 Hz, 1 H), 7.45–7.51 (m, 1 H), 7.28 (s, 1 H), 6.95–7.01 (m, 2 H), 2.44 (s, 3 H), 2.23 (s, 3 H). Anal. ($C_{14}H_{13}ClN_2O_2$) C, H, Cl, N.

2,4-Dimethylpyrido[2,3-*b*][1,4]benzoxazepin-6(5*H*)-one (XVI; $R^2 = 4,6\text{-}(CH_3)_2$). Sodium methoxide (0.123 g, 2.28 mmol) was added to a solution of *N*-(2-chloro-4,5-dimethyl-3-pyridyl)-salicylamide (0.420 g, 1.52 mmol) in tetraethyleneglycol dimethyl ether (4 mL), and the stirred mixture was heated at 220 °C for 3 h. The reaction mixture was then cooled to room temperature, diluted with water (25 mL), and neutralized with 1 N HCl. The resultant solid was collected and crystallized from EtOH–water to afford the title compound (0.118 g, 32%): mp 242–244.5 °C. Extraction of the aqueous solution with Et_2O yielded an additional 75 mg (21%) of the desired product: ^{1}H NMR ($CHCl_3$) δ 8.02 (s, 1 H), 7.91 (dd, J = 1.7, 7.8 Hz, 1 H), 7.56 (ddd, J = 1.7, 7.3, 8.1 Hz, 1 H), 7.43 (dd, J = 1, 8.1 Hz, 1 H), 7.28 (apparent dt, J = 1, 7.5 Hz, 1 H), 6.90 (s, 1 H), 2.45 (s, 3 H), 2.37 (s, 3 H).

2,4,5-Trimethylpyrido[2,3-*b*][1,4]benzoxazepin-6(5*H*)-one (50). Sodium hydride (0.022 g, 0.93 mmol) was added to a solution of 2,4-dimethylpyrido[2,3-*b*][1,4]benzoxazepin-6(5*H*)-one (0.160 g, 0.67 mmol) in dry DMF (5 mL), and the reaction mixture was stirred at room temperature under N_2 for 1.5 h. Methyl iodide

(25) Von Schönenberger, H.; Holzheu-Eckardt, J.; Bamann, E. Vergleichende Untersuchungen über die Herstellung von isomeren Oxybenzamiden. *Arzneim.-Forsch.* **1964**, *14*, 324.

(0.285 g, 2.0 mmol) was then added, and stirring was continued overnight. The reaction mixture was diluted with water (50 mL) and filtered. The filtrate was extracted with Et_2O (3 × 25 mL), the organic extract was dried ($MgSO_4$), and the solvent was removed under reduced pressure. The solid residue was purified by gravity chromatography on silica gel (elution with 20% EtOAc–hexanes). First to elute was the O-alkylated compound, 2,4-dimethyl-6-methoxy[2,3-*b*][1,4]benzoxazepine (0.017 g, 7%): ^{1}H NMR (d_6-DMSO) δ 8.15 (dd, *J* = 2, 8 Hz, 1 H), 7.47–7.54 (m, 1 H), 7.06–7.13 (m, 2 H), 7.01 (s, 1 H), 4.01 (s, 3 H), 2.66 (s, 3 H), 2.62 (s, 3 H). The desired compound **50** was obtained as the major product (0.117 g, 65%): mp 163–164.5 °C; ^{1}H NMR ($CHCl_3$) δ 7.84 (dd, *J* = 1.8, 7.7 Hz, 1 H), 7.45 (ddd, *J* = 1.8, 7, 8.1 Hz, 1 H), 7.35 (dd, *J* = 1.3, 8.1 Hz, 1 H), 7.24 (apparent dt, *J* = 1.3, 7 Hz, 1 H), 6.91 (s, 1 H), 3.41 (s, 3 H), 2.45 (s, 3 H), 2.33 (s, 3 H). Anal. ($C_{15}H_{14}N_2O_2$) H, N; C: calcd, 70.85; found, 70.40.

2-Chloro-5-nitronicotinic Acid. Fuming nitric acid (10.0 mL, 0.24 mol) was added to a mixture of 2-hydroxynicotinic acid (14.0 g, 0.1 mol) and concentrated H_2SO_4 (40 mL). The reaction mixture was slowly heated to 50 °C and maintained at that temperature for 5 h. Care should be taken that the temperature does not exceed 60 °C, or a reduction in yield may result. The reaction mixture was then poured into ice–water, and the resultant precipitate was collected by suction filtration, washed with cold water, and air-dried to give 14.9 g of a light orange solid. Recrystallization from water afforded 2-hydroxy-5-nitronicotinic acid (13.3 g, 72%), as a pale yellow crystalline powder: mp 240 °C (lit.[26] mp 247–248 °C); ^{1}H NMR (d_6-DMSO) δ 13.0 (br s, 1 H, COO*H*), 9.02 (d, *J* = 3 Hz, 1 H), 8.72 (d, *J* = 3 Hz, 1 H).

A mixture of 2-hydroxy-5-nitronicotinic acid (18.4 g, 0.1 mol) and $POCl_3$ (50 mL) was heated at reflux for 3 h. The reaction mixture was cooled to ambient temperature and added cautiously from a separatory funnel to 300 mL of water, maintaining the temperature below 40 °C. Ice was added as necessary to cool the solution. The mixture was stirred for 30 min, and then was extracted with THF–Et_2O (1:2). The organic layer was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated. Recrystallization (Et_2O–petroleum ether (30–60 °C)) afforded 2-chloro-5-nitronicotinic acid (15.86 g, 78%): mp 142–143 °C; ^{1}H NMR (d_6-DMSO) δ 14.40 (br s, 1 H, COO*H*), 9.34 (d, *J* = 2.7 Hz, 1 H), 8.87 (d, *J* = 2.7 Hz, 1 H). Anal. ($C_6H_3ClN_2O_4$) C, H, N, Cl.

***N*-(2-Hydroxy-4-methylphenyl)-2-chloro-5-nitro-3-pyridinecarboxamide (XIX; R^2 = 4-CH_3; R^3 = 5-NO_2).** A suspension of 2-chloro-5-nitronicotinic acid (6.1 g, 0.03 mol) in $SOCl_2$ (15 mL) was heated at reflux until a clear solution was obtained (3 h). Excess $SOCl_2$ was removed by rotary evaporation, and the residue was dissolved in THF (150 mL). The resultant solution was added slowly dropwise to a solution of 6-amino-*m*-cresol (4.0 g, 0.03 mol) and (*i*-Pr)$_2$NEt (7.0 mL, 0.04 mol) in THF (150 mL) at 0 °C under argon. The reaction mixture was allowed to warm to room temperature. After 2 h, the reaction mixture was diluted with water, concentrated to half-volume, and extracted with Et_2O. The organic layer was washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated to give a gummy orange solid. Stirring with CH_2Cl_2 (50 mL) produced a precipitate, which was collected by suction filtration, washing with additional CH_2Cl_2. Air-drying afforded the title compound (6.3 g, 68%) as a bright orange powder, suitable for use in the next reaction: mp 195–196.5 °C dec; ^{1}H NMR (d_6-DMSO) δ 10.03 (s, 1 H), 9.74 (s, 1 H), 9.30 (d, *J* = 2.7 Hz, 1 H), 8.84 (d, *J* = 2.7 Hz, 1 H), 7.77 (d, *J* = 8 Hz, 1 H), 6.73 (s, 1 H), 6.66 (d, *J* = 8 Hz, 1 H), 2.23 (s, 3 H).

9-Methyl-3-nitropyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (V; R^2 = 9-CH_3; R^3 = 3-NO_2). A solution of *N*-(2-hydroxy-4-methylphenyl)-2-chloro-5-nitro-3-pyridinecarboxamide (6.3 g, 0.02 mol) in pyridine (100 mL) was heated at 90 °C under argon for 2.5 h. The reaction mixture was allowed to cool to room temperature. Dilution with water produced a yellow-orange precipitate, which was collected by suction filtration and washed with water. The collected solid was stirred in hot water (100 mL) for 45 min, cooled to room temperature, filtered, and washed with EtOH and Et_2O to give a tan-brown solid. Recrystallization from DMF–H_2O afforded the title compound (3.76 g, 69%) as a fluffy, tan solid. Concentration of the filtrate and purification of the residue by short-path chromatography (elution with EtOAc–CH_2Cl_2) afforded an additional 0.32 g (6%) of the desired product: mp 285–288 °C; ^{1}H NMR (d_6-DMSO) δ 10.97 (br s, 1 H, N*H*), 9.30 (d, *J* = 3 Hz, 1 H), 8.83 (d, *J* = 3 Hz, 1 H), 7.23 (s, 1 H), 7.11 (AB, 2 H), 2.28 (s, 3 H). Anal. ($C_{13}H_9N_3O_4$) C, H, N.

6,9-Dimethyl-3-nitropyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (65). 9-Methyl-3-nitropyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (3.75 g, 0.014 mol) was added in one portion to a suspension of sodium hydride (50% oil dispersion, 0.73 g, 0.015 mol) in DMF (75 mL) at room temperature under argon. After bubbling had subsided, CH_3I (1.3 mL, 0.021 mol) was added to the clear orange-red solution. Stirring was continued at room temperature overnight. The reaction mixture was then diluted with ice–water, and the resultant precipitate was collected by suction filtration and washed with water and petroleum ether (30–60 °C). Recrystallization (EtOAc) afforded **65** (3.14 g, 79%) as a fluffy, pale yellow solid: mp 197–198 °C; ^{1}H NMR (d_6-DMSO) δ 9.27 (d, *J* = 3 Hz, 1 H), 8.81 (d, *J* = 3 Hz, 1 H), 7.44 (d, *J* = 8 Hz, 1 H), 7.26 (apparent singlet, 1 H), 7.20 (dd, *J* = 1, 8 Hz, 1 H), 3.51 (s, 3 H), 2.30 (s, 3 H); MS (CI) *m/z* 286 (MH^+). Anal. ($C_{14}H_{11}N_3O_4$) C, H, N.

3-Amino-6,9-dimethylpyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (66). To a suspension of **65** (1.6 g, 5.6 mmol) in HOAc (30 mL) was added a solution of stannous chloride dihydrate (10 g, 44 mmol) in concentrated HCl (13 mL). The reaction mixture turned warm and all starting material dissolved. After several hours, a precipitate formed. The reaction mixture was filtered, washing the collected solid with Et_2O. The resultant yellow powder was dissolved in water, the solution was made basic with 2 N NaOH, and the milky mixture was extracted with several portions of Et_2O and EtOAc. The combined extract was dried over $MgSO_4$ and concentrated. Recrystallization of the residue (EtOH–hexanes) afforded **66** (0.82 g, 57%) as tan needles: mp 191–193 °C; ^{1}H NMR (d_6-DMSO) δ 7.70 (d, *J* = 3 Hz, 1 H), 7.36 (d, *J* = 3 Hz, 1 H), 7.33 (d, *J* = 8 Hz, 1 H), 7.06–7.12 (m, 2 H), 5.48 (s, 2 H, N*H*$_2$), 3.44 (s, 3 H), 2.27 (s, 3 H); MS (EI) *m/z* 256 (M^+). Anal. ($C_{14}H_{13}N_3O_2$) C, H, N.

***N*-(2-Hydroxy-4-methylphenyl)-2-chloro-3-pyridinecarboxamide (XIX; R^2 = 4-CH_3; R^3 = H).** To a solution of 6-amino-*m*-cresol (15.5 g, 0.13 mol) and (*i*-Pr)$_2$NEt (35 mL, 0.2 mol) in EtOAc (250 mL) at 0 °C, under argon, was added a solution of 2-chloronicotinoyl chloride (22.2 g, 0.126 mol) in EtOAc (250 mL). Stirring was continued for 2 h after addition was complete. Water (300 mL) was added, and the reaction mixture was stirred for 45 min. The mixture was filtered, washing the collected solid with water. Air-drying afforded the desired product (21.75 g, 64%) as an off-white powder. The filtrate was extracted with EtOAc, and the organic layer was dried ($MgSO_4$) and concentrated. The residue was suspended in a mixture of EtOH (125 mL) and 0.1 N NaOH (25 mL) and stirred for 2 h. The EtOH was removed, the residue was diluted with water, and the solution was neutralized with 2 N HCl. The resultant precipitate was collected by suction filtration, washed with water, and air-dried to give an additional 9.16 g (27%) of the title compound: mp 218 °C; ^{1}H NMR (d_6-DMSO) δ 9.83 (br s, 1 H), 9.65 (s, 1 H), 8.50 (dd, *J* = 1.9, 4.8 Hz, 1 H), 8.03 (dd, *J* = 1.9, 7.6 Hz, 1 H), 7.67 (d, *J* = 8.1 Hz, 1 H), 7.53 (dd, *J* = 4.8, 7.6 Hz, 1 H), 6.72 (s, 1 H), 6.64 (d, *J* = 8.1 Hz, 1 H), 2.23 (s, 3 H). Anal. ($C_{13}H_{11}N_2ClO_2$) C, H, N.

9-Methylpyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (V; R^1 = H; R^2 = 9-CH_3; R^3 = H). To a solution of *N*-(2-hydroxy-4-methylphenyl)-2-chloro-3-pyridinecarboxamide (30.9 g, 0.12 mol) in DMF (130 mL) was added powdered sodium hydroxide (4.9 g, 0.12 mol), and the resultant solution was heated at 120 °C under argon. After 5 h, the heat was removed and the reaction mixture was allowed to stand at room temperature overnight, during which time a precipitate formed. Water was added, and the precipitate was collected by suction filtration, washing with additional water. Air-drying afforded 21.68 g (80%) of the title compound. An analytically pure sample was obtained by recrystallization from acetone–DMF (6:1): mp 239.5–240 °C; ^{1}H NMR (d_6-DMSO) δ 10.68 (br s, 1 H, N*H*), 8.51 (dd, *J* = 2, 4.8 Hz, 1 H), 8.27 (dd, *J* = 2, 7.6 Hz, 1 H), 7.46 (dd, *J* = 4.8, 7.6 Hz, 1 H), 7.18 (s, 1 H), 7.09 (d, *J* = 8.1 Hz, 1 H), 7.14 (dd, *J* = 1.6, 8.1 Hz, 1 H), 2.28 (s,

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3 H). Anal. ($C_{13}H_{10}N_2O_2$) C, H, N.

9-Methyl-7-nitropyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (V; R^1 = H; R^2 = 7-NO_2, 9-CH_3; R^3 = H). Nitronium tetrafluoroborate (85%, 2.9 g, 0.019 mol) was added in one portion to a solution of 9-methylpyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (3.4 g, 0.015 mol) in CH_3CN (50 mL) at 0 °C under argon. After 15 min, the clear red solution was poured into ice–water. The resultant precipitate was collected by suction filtration, washing with water, EtOAc, and Et_2O. Air-drying afforded 3.5 g (85%) of the crude product, which was spectroscopically pure and suitable for use in the next reaction. Analytically pure 9-methyl-7-nitropyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one was obtained as brown needles by recrystallization from EtOH: mp 197–201 °C; ^{1}H NMR (d_6-DMSO) δ 9.88 (br s, 1 H, N*H*), 8.53 (dd, *J* = 2, 4.8 Hz, 1 H), 8.36 (dd, *J* = 2, 7.6 Hz, 1 H), 7.88 (d, *J* = 1.5 Hz, 1 H), 7.61 (d, *J* = 1.5 Hz, 1 H), 7.37 (dd, *J* = 4.8, 7.6 Hz, 1 H), 2.42 (s, 3 H); MS (CI) *m/z* 272 (MH^+). Anal. ($C_{13}H_9N_3O_4$) C, H, N.

7-Amino-9-methylpyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (V; R^1 = H; R^2 = 7-NH_2, 9-CH_3; R^3 = H). A solution of 9-methyl-7-nitropyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (0.27 g, 1.0 mmol) and iron powder (0.25 g) in HOAc (20 mL) was heated at 50 °C for 5 h. The reaction mixture was then cooled to room temperature, diluted with water, and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated. The resultant solid was stirred in hot CH_2Cl_2, the suspension was cooled to room temperature, and a yellow powder was collected by suction filtration. Air-drying gave 0.16 g (66%) of the title compound, suitable for use in the next reaction: mp 259–262 °C; ^{1}H NMR (d_6-DMSO) δ 9.80 (s, 1 H, N*H*), 8.46 (dd, *J* = 2, 4.8 Hz, 1 H), 8.20 (dd, *J* = 2, 7.6 Hz, 1 H), 7.43 (dd, *J* = 4.8, 7.6 Hz, 1 H), 6.39 (s, 2 H), 5.30 (s, 2 H, N*H*$_2$), 2.13 (s, 3 H); MS (CI) *m/z* 242 (MH^+).

7-Amino-6,9-dimethylpyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (79). Sodium hydride (50% oil dispersion, 0.3 g, 6.3 mmol) was added to a solution of 7-amino-9-methylpyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (2.3 g, 5.5 mmol) in DMF (20 mL) at room temperature under argon. After 1 h, CH_3I (0.34 mL, 5.5 mmol) was added, and stirring was continued for 4 h. The reaction mixture was diluted with ice–water and extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated. Trituration with Et_2O produced a beige crystalline solid. Recrystallization from EtOAc–hexanes afforded **79** (0.89 g, 63%) as cream-colored crystals: mp 230–231.5 °C; ^{1}H NMR (d_6-DMSO) δ 8.42 (dd, *J* = 2, 4.8 Hz, 1 H), 8.15 (dd, *J* = 2, 7.6 Hz, 1 H), 7.44 (dd, *J* = 4.8, 7.6 Hz, 1 H), 6.45 (d, *J* = 1.7 Hz, 1 H), 6.41 (d, *J* = 1.7 Hz, 1 H), 5.40 (s, 2 H, N*H*$_2$), 3.32 (s, 3 H), 2.16 (s, 3 H). Anal. ($C_{14}H_{13}N_3O_2$) C, H, N.

7-Cyano-6,9-dimethylpyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (77). Compound **79** (0.51 g, 2.0 mmol) was dissolved in 35% H_2SO_4 (2 mL). Water (2 mL) was added, and the mixture was cooled to 0 °C. A solution of $NaNO_2$ (0.15 g, 2.2 mmol) in water (4 mL) was added slowly dropwise. After addition was complete, the clear yellow solution was stirred at 0 °C for 30 min. The cold solution was then neutralized to pH 7 with aqueous Na_2CO_3. The resultant mixture was added by pipet to a solution of CuCN (0.19 g, 2.1 mmol) and NaCN (0.21 g, 4.3 mmol) in water (2 mL). The foamy solution was stirred at room temperature for 30 min, and then at 50 °C for 30 min. The reaction mixture was cooled to room temperature and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated. Purification of the residue by short-path chromatography (elution with CH_2Cl_2–hexanes) afforded **77** (0.44 g, 83%) as a yellow-orange solid. Further purification by recrystallization from EtOAc–hexanes produced yellow-orange needles: mp 159.5–160.5 °C; ^{1}H NMR (d_6-DMSO) δ 8.49 (dd, *J* = 1.9, 4.8 Hz, 1 H), 8.29 (dd, *J* = 1.9, 7.5 Hz, 1 H), 7.69 (s, 1 H), 7.66 (s, 1 H), 7.50 (dd, *J* = 4.8, 7.5 Hz, 1 H), 3.59 (s, 3 H), 2.35 (s, 3 H); MS (CI) *m/z* 266 (MH^+). Anal. ($C_{15}H_{11}N_3O_2$) C, H, N.

7-Carbamoyl-6,9-dimethylpyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (81). Compound **77** (0.27 g, 1.0 mmol) was dissolved in concentrated H_2SO_4 (10 mL) and heated at 120 °C for 1.5 h. The reaction mixture was cooled to room temperature, poured into ice–water, and neutralized with saturated aqueous Na_2CO_3. The mixture was filtered, washing the collected solid with EtOAc. The filtrate was extracted with additional EtOAc, and the organic layer was washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated. The combined solids were suspended in water, stirred at 60 °C for 30 min, cooled to room temperature, and filtered. Air-drying gave spectroscopically pure **81** (0.21 g, 74%). Further purification by recrystallization from EtOH afforded a white crystalline powder: mp 319–320 °C; ^{1}H NMR (d_6-DMSO) δ 8.45 (dd, *J* = 2, 4.8 Hz, 1 H), 8.23 (dd, *J* = 2, 7.5 Hz, 1 H), 8.07 (br s, 1 H, N*H*), 7.70 (br s, 1 H, N*H*), 7.47 (dd, *J* = 4.8, 7.5 Hz, 1 H), 7.32 (d, *J* = 2 Hz, 1 H), 7.19 (d, *J* = 2 Hz, 1 H), 3.31 (s, 3 H), 2.31 (s, 3 H); MS (CI) *m/z* 284 (MH^+). Anal. ($C_{15}H_{13}N_3O_3$) C, H, N.

6,9-Dimethyl-7-(iminomethoxymethyl)pyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (82). A solution of **77** (0.21 g, 0.8 mmol) and *p*-toluenesulfonic acid monohydrate (0.15 g, 0.8 mmol) in MeOH (20 mL) was heated at 60 °C in a stoppered flask. After 5 h, heat was removed and the reaction mixture was allowed to stand at room temperature overnight. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated. Purification of the residue by short-path chromatography (elution with EtOAc–hexanes), followed by recrystallization (Et_2O–petroleum ether (30–60 °C)) afforded **82** (0.20 g, 84%), which exists in solution as a mixture of rotational isomers: mp 177–178.5 °C; ^{1}H NMR (d_6-DMSO) (16:1 ratio of rotational isomers; data provided for major isomer only) δ 10.55 (br s, N*H*), 8.04 (dd, *J* = 2, 5 Hz, 1 H), 7.68 (dd, *J* = 2, 7.3 Hz, 1 H), 7.00 (d, *J* = 1 Hz, 1 H), 6.89 (dd, *J* = 5, 7.3 Hz, 1 H), 6.81 (d, *J* = 1 Hz, 1 H), 3.70 (s, 3 H), 3.21 (s, 3 H), 2.15 (s, 3 H); MS (CI) *m/z* 298 (MH^+). Anal. ($C_{16}H_{15}N_3O_3$) C, H, N.

7-Carboxy-6,9-dimethylpyrido[2,3-*b*][1,5]benzoxazepin-5(6*H*)-one (83). A solution of **77** (0.20 g, 0.75 mmol) in concentrated H_2SO_4 (2 mL) was heated at 120 °C for 1 h to effect conversion to the amide. The reaction mixture was then cooled to 0 °C, and a solution of $NaNO_2$ (55 mg, 0.8 mmol) in water (4 mL) was added very slowly by capillary pipet. The ice bath was removed, and the reaction mixture was allowed to warm to room temperature. Effervescence was observed. When TLC showed no additional conversion, the reaction mixture was poured into ice–water. The resultant precipitate was collected by suction filtration, air-dried, redissolved in 1 N NaOH, and washed extensively with EtOAc. Concentration of the organic layer afforded ca. 40 mg of the intermediate amide. The aqueous layer was acidified with 2 N HCl and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated. Recrystallization of the residue from EtOH–hexanes afforded **83** (50 mg, 23%): mp 269–273 °C; ^{1}H NMR (d_6-DMSO) δ 13.64 (br s, 1 H, O*H*), 8.46 (dd, *J* = 2, 4.8 Hz, 1 H), 8.26 (dd, *J* = 2, 7.6 Hz, 1 H), 7.44–7.45 (m, 3 H), 3.28 (s, 3 H), 2.33 (s, 3 H); MS (CI) *m/z* 285 (MH^+). Anal. ($C_{15}H_{12}N_2O_4$) C, H, N.

HIV-1 Reverse Transcriptase Enzyme Assay. Recombinant HIV-1 reverse transcriptase was obtained from T. Steitz (Yale University). This enzyme was homogeneous (>95%) p66/p51 heterodimer. Reverse transcriptase was assayed by a modification of the previously described procedure.[8] The reaction mixture consisted of 50 mM Tris (pH 7.8), 50 mM glutamic acid, 1 mM DTT, 2 mM $MgCl_2$, 0.02% CHAPS, 0.8 μg/mL poly(rC):oligo(dG) (Pharmacia), and 500 nM [^{3}H]dGTP (NEN Du Pont), adjusted to a final volume of 60 μL. Inhibitors were assayed at a concentration of 1 μM in the presence of RT (0.5 nM). The inhibitors, which were dissolved in DMSO and diluted many-fold with buffer, were added as solutions, and reaction was initiated by the addition of enzyme. After 1 h at room temperature 50 μL each of ice-cold 10% aqueous trichloroacetic acid and 2% aqueous sodium pyrophosphate were added, and the mixture was cooled at 4 °C for 15 min. Acid-insoluble products were harvested onto no. 30 glass fiber filters (Schleicher and Schuell) by means of a Skatron cell harvester. Dried filters were counted in an LKB 1205 Betaplate liquid scintillation counter. Inhibition was determined by comparison of the amount of product in reactions run with and without test compound. Plots of percent inhibition versus log [compound] yielded the concentration at which half-maximal inhibition was observed (IC_{50}).

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Registry No. 1, 135810-41-2; 2, 14417-38-0; 3, 134894-43-2; 4, 134364-78-6; 5, 135810-44-5; 6, 140412-81-3; 7, 140412-82-4; 8, 3158-85-8; 9, 17296-47-8; 10, 17296-50-3; 11, 135810-46-7; 12, 135810-42-3; 13, 135810-43-4; 14, 140412-83-5; 15, 140412-84-6; 16, 140438-10-4; 17, 140412-85-7; 18, 140412-86-8; 19, 2474-62-6; 20, 23474-61-5; 21, 140412-87-9; 22, 135810-50-3; 23, 23474-52-4; 24, 23474-51-3; 25, 140412-88-0; 26, 140412-89-1; 27, 135810-45-6; 28, 140412-90-4; 29, 23474-58-0; 30, 140412-91-5; 31, 140412-92-6; 32, 140412-93-7; 33, 140412-94-8; 34, 140412-95-9; 35, 140412-96-0; 36, 140412-97-1; 37, 140412-98-2; 37·HCl, 140412-99-3; 38, 140413-00-9; 39, 140413-01-0; 40, 140413-02-1; 41, 140413-03-2; 42, 140413-04-3; 43, 140413-05-4; 44, 140413-06-5; 45, 14527-81-2; 46, 140413-07-6; 47, 140413-08-7; 48, 140413-09-8; 49, 134369-51-0; 50, 140413-10-1; 51, 134894-49-8; 52, 134894-50-1; 53, 134894-51-2; 54, 140413-11-2; 55, 134894-53-4; 56, 140413-12-3; 57, 140413-13-4; 58, 140413-14-5; 59, 140413-15-6; 60, 140413-16-7; 61, 140413-17-8; 62, 140413-18-9; 63, 140413-19-0; 64, 134894-56-7; 65, 134894-60-3; 66, 134894-45-4; 67, 140413-20-3; 68, 140413-21-4; 69, 140413-22-5; 70, 134894-46-5; 71, 134894-47-6; 72, 140413-23-6; 73, 140413-24-7; 74, 140413-25-8; 75, 140413-26-9; 76, 140413-27-0; 77, 140413-28-1; 78, 140413-29-2; 79, 140413-30-5; 80, 140413-31-6; 81, 140413-32-7; 82, 140413-33-8; 83, 140413-34-9; 84, 140413-35-0; 85, 140413-36-1; III ($R^1 = CH_3$, $R^2 = 7\text{-}CH_3$, $R^3 = 2\text{-}NO_2$), 135810-40-1; V ($R^2 = 9\text{-}CH_3$, $R^3 = 3\text{-}NO_2$), 134894-59-0; V ($R^1 = H$, $R^2 = 7\text{-}NO_2$, $9\text{-}CH_3$, $R^3 = H$), 140413-37-2; V ($R^1 = H$, $R^2 = 7\text{-}NH_2$, $9\text{-}CH_3$, $R^3 = H$), 140413-38-3; VIII ($R^2 = 4\text{-}CH_3$), 140413-39-4; IX ($R^2 = 7\text{-}CH_3$), 135810-39-8; XIII ($R^2 = 4,6\text{-}(CH_3)_2$, Hal = Cl), 140413-40-7; XVb ($R^2 = 4,6\text{-}(CH_3)_2$, Hal = Cl), 140413-41-8; XVI ($R^2 = 4,6\text{-}(CH_3)_2$), 140413-42-9; XIX ($R^2 = 4\text{-}CH_3$, $R^3 = 5\text{-}NO_2$), 134894-58-9; XIX ($R^2 = 4\text{-}CH_3$, $R^3 = H$), 140413-43-0; 2-chloro-5-nitrobenzoic acid, 2516-96-3; 6-amino-*m*-cresol, 2835-98-5; 3-cyano-4,6-dimethyl-2-hydroxypyridine, 769-28-8; 2-chloro-3-cyano-4,6-dimethylpyridine, 14237-71-9; 2-chloro-4,6-dimethylnicotinamide, 140413-44-1; salicyloyl chloride, 1441-87-8; 2,4-dimethyl-6-methoxypyrido-[2,3-*b*][1,4]benzoxazepine, 140413-45-2; 2-hydroxynicotinic acid, 609-71-2; 2-hydroxy-5-nitronicotinic acid, 42959-38-6; 2-chloronicotinoyl chloride, 49609-84-9.

Substrate Specificity of Isopenicillin N Synthase

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Highly purified isopenicillin N synthase (IPNS) from two sources (naturally occurring in *Penicillium chrysogenum* and that expressed in *Escherichia coli* via a cloned gene derived from *Cephalosporium acremonium*) have been isolated and utilized in vitro to test synthetic modifications of the natural substrate, (L-α-amino-δ-adipyl)-L-cysteinyl-D-valine (ACV). A very sensitive procedure utilizing the ability of β-lactams to induce the synthesis of β-lactamase was employed to determine whether an ACV analogue could serve as a substrate for IPNS. A wide variety of amino and carboxyl terminal tripeptide substitutions were examined and found to elicit positive β-lactamase induction profiles. However, none of these modifications were found to function as efficiently as a substrate as ACV. One of the β-lactam products which was formed from the reaction of IPNS and the tripeptide analogue was independently synthesized and evaluated for antibacterial activity. Modification of the L-cysteine residue in the second position of ACV resulted in tripeptides that were unable to serve as substrates. Conversion of the D-valine residue in the third position of ACV to an aromatic amino acid or to a highly electronegative residue such as trifluorovaline resulted in elimination of substrate activity and creation of an inhibitor of the enzyme.

Isopenicillin N synthase (IPNS) is the enzyme responsible for the oxidative conversion of the tripeptide (L-α-amino-δ-adipyl)-L-cysteinyl-D-valine (ACV) (8) to the bicyclic β-lactam antibiotic, isopenicillin N (Scheme I). The recent cloning and expression of the enzymes responsible for the biosynthesis of the penicillin and cephalosporin antibiotics[1-7] has opened the way for a systematic investigation of substrate specificity of these novel enzymes. Use of synthetic analogues of the naturally occurring peptide substrate (L-α-amino-δ-adipyl)-L-cysteinyl-D-valine [Aad(–Cys–D–Val) or ACV (8)] would allow us to evaluate the ability of IPNS to interact with and convert these analogues into β-lactam products. This information could be instrumental in understanding the mechanism of action of this unique biosynthetic enzyme. Furthermore, if IPNS proved to be flexible in its ability to accept modified substrates, we would be able to readily generate new β-lactam antibiotics which might exhibit novel antibacterial profiles.

Chemistry

Tripeptide analogues of ACV can be prepared by a number of methods, several of which have been previously

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