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IMIDAZO[2',3':6,5]DIPYRIDO[3,2-b:2',3'-e]-1,4-DIAZEPINES: NON-NUCLEOSIDE HIV-1 REVERSE TRANSCRIPTASE INHIBITORS WITH GREATER ENZYME AFFINITY THAN NEVIRAPINE

Nicholas K. Terrett,* Dejan Bojanic, James R. Merson, and Peter T. Stephenson
Pfizer Central Research, Sandwich, Kent, CT13 9NJ

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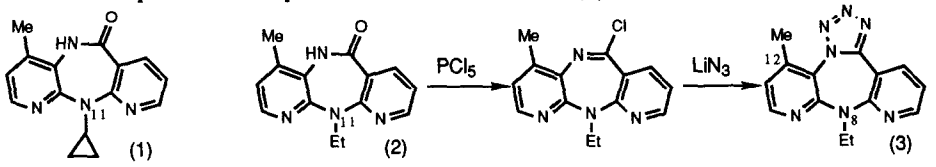
Abstract: The chemistry and SAR of a new series of imidazo[2',3':6,5]dipyrido[3,2-b:2',3'-e]-1,4-diazepines is described. These compounds show improved affinity for HIV-1 RTase and antiviral activity in vitro over nevirapine, which has undergone clinical trials.

In the search for more effective and safe chemotherapeutic agents for the treatment of the acquired human immunodeficiency syndrome (AIDS), there has been considerable interest recently in non-nucleoside inhibitors of the HIV-1 enzyme, reverse transcriptase (RTase). Nucleoside inhibitors of RTase, including azidothymidine (AZT)[1], and dideoxyinosine(ddI)[2] have demonstrated clinical utility in the therapy of HIV-mediated disease, but the usefulness of AZT has been limited by serious toxic side effects[3] and possibly by the emergence of resistant viral strains[4]. Non-nucleoside structures with RTase inhibitory and anti-HIV-1 activity have recently been reported from Boehringer-Ingelheim[5], Mitsubishi[6], Merck[7], Upjohn[8], and Janssen[9]. As these inhibitors interact with an allosteric site specific to HIV-1, it is anticipated that the affinity for mammalian DNA polymerases will be considerably lower than nucleoside inhibitors that interact with the active site, and that toxic side effects will be minimized.

In this communication we describe the chemistry and SAR of imidazo[2',3':6,5]dipyrido[3,2-b:2',3'-e]-1,4-diazepines. These compounds show improved affinity for HIV-1 RTase and antiviral activity in vitro over nevirapine[5], which has undergone clinical trials.

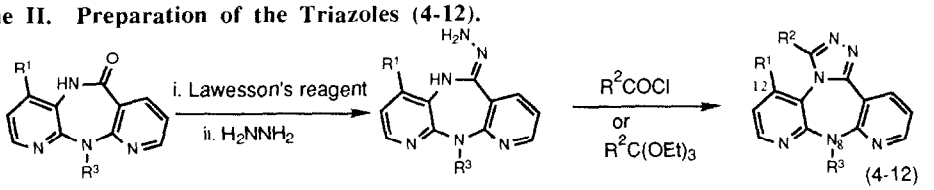
Chemistry: Extension of the diazepinone ring by the addition of various five-membered heterocycles such as imidazole or triazole, is a common bioisosteric modification[10]. Nevirapine contains a diazepinone ring and we investigated the effect that fusion of tetrazole, triazole and imidazole had on RTase inhibitory activity and anti-HIV activity. The new compounds were prepared by standard procedures from known diazepinones. For example, conversion of the dipyridodiazepinone[11] (2) to the chloroimine using phosphorous pentachloride, followed by reaction with lithium azide (prepared in situ) gave the fused tetrazole (3).

Scheme I. Nevirapine and Preparation of the Tetrazole (3).



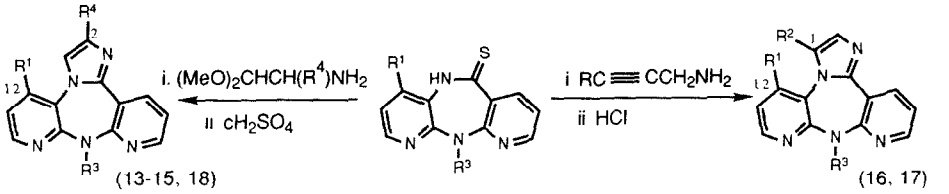
However, the tetrazole provides no option to add sidechains, and thus to explore the effect of substitution on enzyme affinity and antiviral activity, we instead attempted the preparation of triazoles and imidazoles. For preparation of the triazoles, the dipyridodiazepinones were initially converted to thiolactams using Lawesson's reagent and subsequent condensation with hydrazine gave key intermediate hydrazones.

Scheme II. Preparation of the Triazoles (4-12).



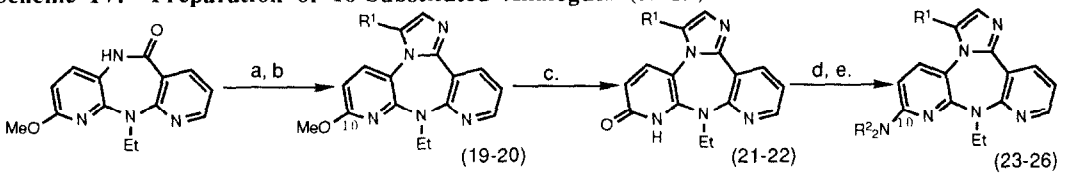
Cyclization was achieved with a number of acyl chlorides or orthoesters, allowing variation of the triazole substituent (4-12). Alternatively, reaction of thiolactams with a number of propargylamines or aminoacetaldehyde acetals, followed by acid-catalysed cyclization gave a number of variously substituted imidazoles (13-18).

Scheme III. Preparation of the Imidazoles (13-18).



The effect of introducing substituents onto the pyridine rings has been extensively explored previously[12,13], and it has been shown that lipophilic groups on the 10- and 12-position enhance affinity of the inhibitors for the RTase enzyme. In addition to preparing compounds containing a 12-methyl group (Schemes I-III), we also investigated modification of the 10-position. Commencing with the methoxydipyridodiazepinone (prepared by the method of Ref.13), the methoxy group was transformed as shown in Scheme IV in the imidazole series. Hydrobromic acid cleavage gave pyridones (21-22) and conversion to 10-amino derivatives (23-26) was achieved by the nucleophilic displacement of an intermediate triflate.

Scheme IV. Preparation of 10-Substituted Analogues (19-29).



a. Lawesson's reagent, b. as scheme III, c. HBr, d. Tf_2O, e. $R^2{}_2NH$.

Results and Discussion: The large majority of compounds prepared in this study possess an 8-ethyl substituent, whereas the corresponding substituent in nevirapine (1) is a cyclopropyl group. Thus, to assess the effect of our modifications to the lactam bond, comparison should be made with 11-ethylnevirapine (2) (the 11-position in nevirapine tricyclic structures is equivalent to the 8-position in the tetracyclic analogues). However, results on the few cyclopropyl analogues (e.g. 7 and 14, Table 1) we prepared suggest that ethyl and cyclopropyl on the 8-position confer similar enzyme affinities.

The activity of new compounds was determined by measuring their ability to inhibit RTase-catalysed incorporation of tritiated thymidine onto either an RNA molecule of mixed nucleotides (poly(rN)-oligo(dN) template-primer) or an RNA molecule containing purely adenine base (poly(rA)-oligo(dT) template-primer). As previously reported[14], the nature of the template-primer employed has a considerable effect on the IC_{50} values determined, possibly due to a major conformational effect on the allosteric binding site of the inhibitors. However, the relative activity of these types of inhibitor appear to be unaffected by the template-primer used, and a good correlation of activities between the two substrates we employed was observed.

When tested for its propensity to inhibit HIV-1 RTase, the tetrazole (3) was found to have enzyme affinity clearly inferior to nevirapine (see Table 1). Likewise, the unsubstituted triazoles (4 and 5) have similarly weak RTase affinity. Removal of the pyridine 12-methyl group (5) reduces enzyme affinity, but this group may be satisfactorily replaced by a methyl group on the triazole (6) with recovery of inhibitory activity. Consideration of simple models demonstrates that a similar region of space is occupied by either methyl group. The compound containing both methyl substituents (8) had poorer RTase affinity, as steric interactions possibly affect the preferred active conformation of the molecule. There appears to be a requirement for a small lipophilic group on the triazole, as the larger ethyltriazole (9) is marginally less active than the methyl analogue (6). Isopropyl (10) is weaker still and substitution with phenyl (11) or dimethylaminomethyl substituents (12) essentially abolishes enzyme affinity.

Table 1. HIV-1 Reverse Transcriptase Inhibitory Activity.

Example[b]			IC_{50} Values in μM[a]:	poly(rA)	poly(rN)
1		nevirapine		6.44	2.11
2		11-ethylnevirapine		10.3	1.50
tetrazole					
3				75.1	6.72
triazoles	R1	R2	R3		
4	Me	H	Et	44.5	NT[c]
5	H	H	Et	72.8	NT
6	H	Me	Et	38.9	NT
7	H	Me	cyclopropyl	11.5	7.82
8	Me	Me	Et	198	NT
9	H	Et	Et	56.6	20.2
10	H	iPr	Et	>100	62.6
11	H	Ph	Et	>100	NT
12	H	Me_2NCH_2	Et	>100	>100

Table 1 continued

imidazoles	R1	R2	R3	R4	poly(rA)	poly(rN)
13	Me	-	Et	H	1.62	0.245
14	Me	-	cyclopropyl	H	2.05	0.217
15	H	-	Et	H	11.2	0.775
16	H	Me	Et	-	1.22	0.425
17	H	Et	Et	-	5.63	1.10
18	H	-	Et	Me	11.2	2.75

[a]The HIV-1 RTase inhibition assay was carried out by measuring the HIV-1_{IIIB} reverse transcriptase (American Biotechnologies Inc.) catalysed incorporation of tritiated thymidine triphosphate onto either a biotinylated rA.dT or rN.dN template primer in the presence of varying concentrations of test compound. Tritiated thymidine incorporation was assessed using the streptavidin-based scintillation proximity assay (Amersham International). The IC_{50} values quoted are the means of two experiments.

[b]All new compounds had satisfactory spectroscopic (^{1}H NMR) and analytical (TLC, CHN) data

[c]NT - not tested

Replacing the lactam bond of the dipyridodiazepinone with an imidazole gives compounds with superior enzyme affinity to the parent diazepinone or triazoles and furthermore had potent antiviral activity in an in vitro HIV assay. Substitution on the imidazole follows the same SAR as the triazole series. The 1-methyl imidazole (16) exhibits high enzyme inhibitory activity, with the 1-ethyl (17) and the unsubstituted analogue (15) possessing less affinity. The 2-methylimidazole (18) is less well tolerated by RTase than the 1-methyl compound (16) and has reduced enzyme affinity.

As previously reported[12,13], modification of the 10-position on the pyridine ring can have beneficial effects on HIV RTase affinity of dipyridodiazepinones. We discovered that a 10-methoxy group had a potency enhancing effect in the imidazolo[2'3':6,5]dipyrido[3,2-b:2',3'-e]-1,4-diazepine series leading to exceptionally potent RTase inhibitors (e.g. 19-20, Table 2).

Table 2. HIV-1 Reverse Transcriptase Inhibitory Activity of 10-Substituted Analogues.

Example[b]		IC_{50} Values in μM[a] :	poly(A)	poly(N)
1	nevirapine		6.44	2.11
2	11-ethylnevirapine		10.3	1.50
	R^1			
19	H		0.598	0.258
20 (UK-129,485)	Me		0.190	0.156
21	H		23.9	6.98
22	Me		12.2	3.20
	R^1	$R^2{}_2N$		
23	H	pyrrolidine	3.90	2.00
24	H	Me_2N	3.90	2.19
25	Me	pyrrolidine	2.70	1.77
26	Me	Me_2N	2.40	1.10

See footnote *a* to Table 1 for assay description.

[b]All new compounds had satisfactory spectroscopic (^{1}H NMR) and analytical (TLC, CHN) data

That a lipophilic group is preferred in this position was suggested by the drop in enzyme affinity following conversion to the pyridones (21-22). However, the potency advantage conferred by the imidazole ring allowed the pyridone derivative (22) to have enzyme affinity similar to nevirapine. Nevirapine-like activity was also

obtained by the introduction of lipophilic 10-amino substituents (23-26). Compounds with potent RTase inhibitory activity were evaluated for antiviral activity and cytotoxicity in a human T-cell line (H9) infected with HIV-1_{III-B}(Table 3). A good correlation between enzyme inhibitory activity and antiviral activity, as assessed by p24-IC_{90} values, was observed. Furthermore, as would be expected, marked inhibition of p24 protein production correlated with low values of infectious titre (IC_{100}). None of the analogues had profound cytotoxicity.

Table 3. *In Vitro* Antiviral Activities of Selected Imidazoles (μM).

Compound	RTase IC_{50} poly(A)	[a]p24 IC_{90}	[b]Infectious Titre IC_{100}	[c]Cell Toxicity
1	6.44	0.078	0.1-0.5	>10
13	1.62	0.55	0.1-1	>100
15	11.2	0.070	0.1-0.5	50
16	1.22	0.018	0.01-0.1	>100
19	0.598	0.0080	0.1	>10
20 (UK-129,485)	0.190	0.0023	0.01	>10
22	12.2	0.11	0.1-1	>100
23	3.90	0.082	0.1-1	100
24	3.90	0.041	0.1	10
26	2.40	0.25	0.1-1	>100

[a]. The antiviral activity was assessed by incubating test compound at 0.001, 0.01, 0.1, 1, 10 and 100 μM with a human T-cell line (H9) infected with HIV-1_{IIIB}. The IC_{90} values are the lowest test concentrations of compound required to reduce tissue culture supernatant p24 levels on day 7 by 90% relative to untreated controls.

[b]. Infectious virus was quantitated by incubating serial dilutions of the supernatant with C8166 cells (human T-cell line) for 7 days after which cultures were examined for syncytia formation. The infectious titre (IC_{100}) is the lowest test concentration affording complete protection to the culture.

[c]. Concentrations of compound that were determined to be cytotoxic (reduced cell number as compared with controls), were recorded after 7 days incubation in the H9/HIV-1_{IIIB} assay system.

Amongst the analogues tested, the most potent antiviral compounds were the imidazo[2',3':6,5]dipyrido[3,2-b:2',3'-c]-1,4-diazepines, 16, 19 and 20. These three compounds were at least equivalent to nevirapine in our assays, with the 10-methoxy derivative, UK-129,485 (20) being a clearly superior anti-HIV-1 agent when tested in vitro.

In summary, we have investigated a number of new nevirapine analogues with fused tetrazole, triazole and imidazole rings replacing the lactam bond of the dipyridodiazepinone. Many of these compounds have good HIV-1 RTase inhibitory activity and antiviral activity in vitro and structure-activity relationships suggest a requirement for a small lipophilic 1-substituent on the fused heterocycle. Furthermore, there is a suggestion that a hydrogen-bond to the oxygen of nevirapine, or to the corresponding ring nitrogen in these new analogues may be important for good RTase affinity. Amongst the new compounds tested, UK-129.485, a 10-methoxy derivative in the imidazo[2',3':6,5]dipyrido[3,2-b:2',3'-e]-1,4-diazepine series (20) has clearly superior RTase inhibition and antiviral activity to nevirapine.

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