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SYNTHESIS AND ANTI-HIV ACTIVITY OF PYRROLO-[1,2-d]-(1,4)-BENZODIAZEPIN-6-ONES

George V. De Lucca* and Michael J. Otto

Du Pont Merck Pharmaceutical Company
Experimental Station P.O. Box 80353
Wilmington, DE 19880-0353

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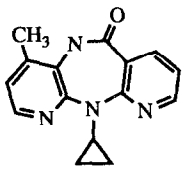
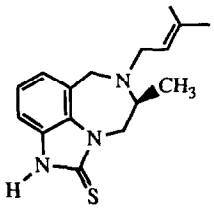
Abstract. The synthesis of novel pyrrolo annulated 1,4-benzodiazepines is described. These pyrrolo[1,2-d]-(1,4)-benzodiazepines have been found to have antiviral activity against HIV-1. Like other non nucleoside HIV-1 RT inhibitors, these compounds appear to be specific for HIV-1.

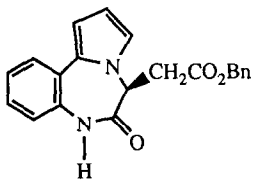
Therapeutics for the treatment of HIV infection are currently limited to AZT, ddC, and ddI, the only approved drugs for AIDS. These are nucleoside analogs which inhibit the reverse transcriptases (RT) of HIV-1[1] and other retroviruses. Unfortunately, these compounds suffer from a number of limitations including toxic side effects and the emergence of resistant strains[2]. Recently there have been several reports of non-nucleoside HIV-1 RT inhibitors. Janssen[3] has disclosed the discovery of the benzodiazepine **TIBO** compounds such as **R82150** and Boehringer-Ingelheim Pharmaceuticals[4] has disclosed a series of dipyridodiazepinones such as **BI-RG-587** both of which have potent antiviral activity for HIV-1 but are inactive against HIV-2.



Boehringer-Ingelheim
BI-RG-587
IC_{50} = 0.040 uM(Ref. 4)



Janssen Research
R82150
IC_{50} = 0.028 uM(Ref. 3)

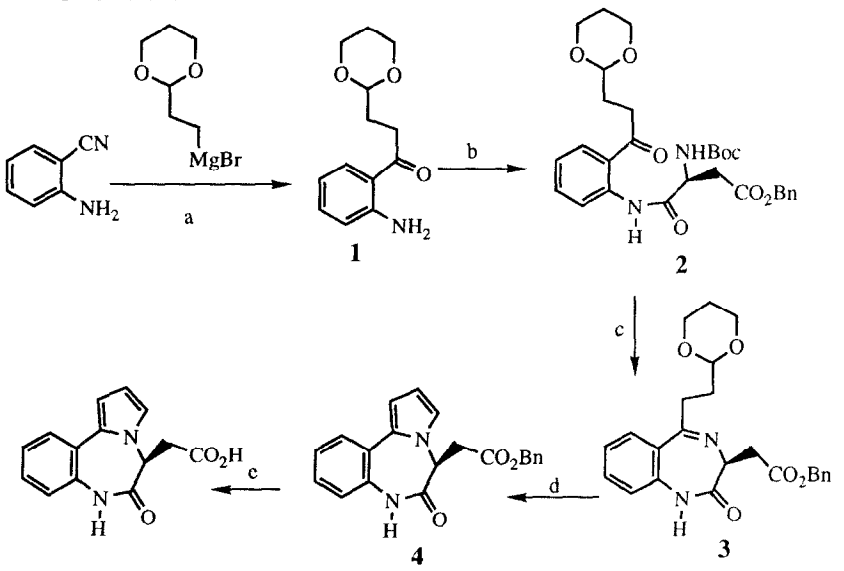


4
IC_{90} = 0.8 uM

From a previous program involving the design and synthesis of peptidomimetics we had on hand several novel benzodiazepine containing compounds. The report by the Janssen group prompted us to evaluate some of these for antiviral properties. Based on this screening process we have identified a series of novel[5] pyrrolo-benzodiazepines (such as **4**) that have antiviral activity against HIV-1. In this communication we report the synthesis and the biological activity of this class of compounds.

The synthesis of this class of compounds is outlined in **SCHEMES 1** and **2**. The reaction of an excess of the Grignard reagent derived from 2-(2-bromoethyl)-1,3-dioxane[6] with anthranilonitrile gives ketone **1**. This is then coupled[7] with the suitably protected aspartic acid derivative to provide amide **2**. The protecting group is removed by treating an ether solution of **2** with $HCl_{(g)}$ to give the HCl salt. Dissolving the salt in MeOH and neutralizing with 1N NaOH to give the benzodiazepine **3**[8]. Treatment of **3** with an aqueous solution of oxalic acid at reflux gives the desired pyrrolo-benzodiazepine **4**[9]. Catalytic hydrogenation gives the free acid.

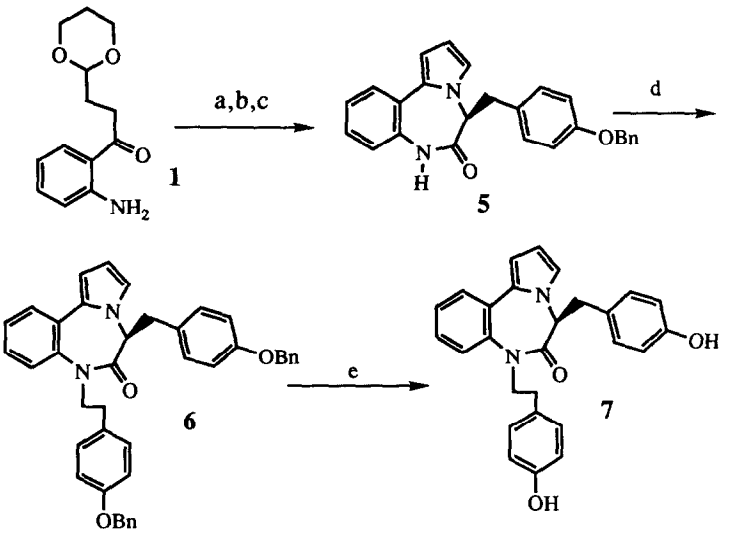
SCHEME 1



a. THF/reflux/1 h. b. N-Boc-Asp-β-benzyl ester, NMM, isobutylchloroformate/THF/rt 24h. c. 1. $HCl_{(g)}$/ether/0°C/1 h. 2. MeOH/1N NaOH/rt 1 h. d. oxalic acid/H_2O/reflux 1 h. e. H_2/10%Pd-C

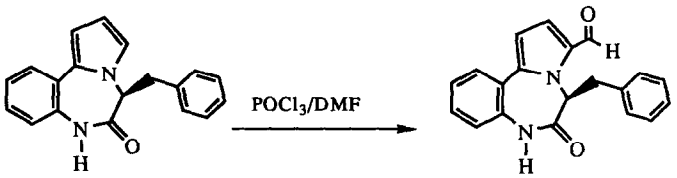
The amide nitrogen of the BZD ring can be substituted using standard alkylation conditions[10] as shown in **Scheme 2**. Starting with the ketone amine **1** and the appropriate tyrosine derivative, the pyrrolo-benzodiazepine **5** is prepared using the reaction sequence outlined in **Scheme 1**. Treatment of **5** with NaH in DMF gives the sodium salt which can be alkylated with a variety of electrophiles, such as p(O-benzyl)phenethyl bromide, to give the substituted pyrrolo-benzodiazepine **6**. The benzyl protecting groups can be removed by treatment with trimethylsilyliodide in refluxing chloroform to give the free phenol analog **7**.

SCHEME 2



a. N-Boc-OBn-Tyr, NMM, isobutylchloroformate/THF/rt 24h.
b. 1. $HCl_{(g)}$/ether/0°C/1 h. 2. MeOH/1N NaOH/rt 1 h.
c. Oxalic acid/H_2O/reflux 1 h.
d. 1. NaH/DMF/rt 2. $BrCH_2CH_2C_6H_4OBn$/50°C/2 h.
e. $CHCl_3$/TMSI/reflux/2 h.

The pyrrole ring can be further elaborated using standard pyrrole chemistry. For example the treatment of pyrrolo-benzodiazepines under the usual Vilsmeier-Haack reaction conditions gives good yields of the formyl derivatives[11].



The antiviral activity of various pyrrolo-benzodiazepines were determined using cell protection and yield reduction assays according to the protocols described previously[12]. The results are summarized in **TABLE 1** and **2**. The most potent pyrrolo-benzodiazepine that we found from our screening work is **4** with an IC_{90} of 0.29 ug/mL. The mechanism of action of **4** may be the same as the other benzodiazepine type of non-nucleoside RT inhibitors that have been reported recently.[3,4] Specifically **4** is an HIV-1 RT inhibitor (IC_{50} = 0.04

ug/ml). It is more active against HIV-1 than HIV-2, and it does not inhibit HIV-1 protease. Thus, **4** seems to have a similar profile and compares favorably with **TIBO** type compounds when tested under the same assay conditions.

In conclusion, we have described the synthesis of novel pyrrolo-(1,2-d)-(1,4)-benzodiazepin-ones such as **4** which have been found to possess antiviral activity against the HIV-1 virus. This novel ring system furthers the scope of compounds containing a benzodiazepine nucleus having anti-HIV-1 activity. The compounds disclosed in this communication may have the same mechanism of action as other non nucleoside RT inhibitors recently described in the literature.

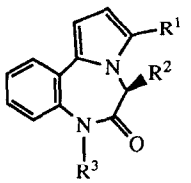


TABLE 1. ANTIVIRAL ACTIVITY AGAINST HIV-1(3b) in MT-2 CELLS

R1	R2	R3	IC_{90}ug/ml	TC_{50}ug/ml
H	H	H	>50	>50
CHO	H	H	>50	>50
CHO	$CH_2C_6CH_5$	H	>2.5	2.5
H	$CH_2C_6CH_5$	H	>21	21
H	$CH_2C_6CH_4OH$	H	>23	23
H	$CH_2C_6CH_4OH$	CH_3	>5.2	5.2
H	$CH_2C_6CH_4OH$	$CH_2CH_2C_6CH_4OH$	0.8	10
H	CH_2CO_2Bn	H	0.29	10
H	CH_2CO_2H	H	>50	>50
H	$CH_2C_6CH_4OH$	$(CH_2)_4NPth$	>23	23
H	$CH_2C_6CH_4OH$	$(CH_2)_5NPth$	2.6	>50

IC_{90} = Concentration required to protect 90% of the cells (MT-2) from HIV-1 (3b) induced cell death.
TC_{50} = Concentration required to inhibit cell number/viability by 50%

TABLE 2. COMPARISON OF COMPOUND 4 WITH TIBO ANALOG

TESTS		COMPOUNDS ug/ml	
		4	TIBO[a]
Yield Reduction in MT-2 cells:	HIV-1 (rf)	IC_{90} = 0.29	IC_{90} = 0.15
	HIV-2 (rod)	IC_{50} = 3.0	IC_{50} > 50
Cell Protection in MT-2 cells:	HIV-1 (3b)	IC_{90} =0.3	IC_{90} = 1 5
Cell Toxicity in MT-2 Cells		TC_{50} = 10	TC_{50} = 55

a.(+)-s-4,5,6,7-Tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)-imidazo[4,5,1-jk]1,4-benzodiazepine-2-thione obtained from Pharmatech International, Inc. West Orange, NJ.

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5. The pyrrolo[1,2-d]-(1,4)benzodiazepine-6-one ring system is not very common. A search of the open and patent literature showed only one example of this ring system: a. Aiello, E.; Dattolo, G.; Cirrincione, G.; Plescia, S.; Daidone, G. *J. Heterocyclic Chem.* **1979**, *16*, 209. b. Dattolo, G.; Cirrincione, G.; Aiello, E. *J. Heterocyclic Chem.* **1980**, *17*, 701. For a related ring system see: c. Matsumura, H.; Hashizume, H.; Matsushita, A.; Eigyo, M. *U.S. Patent* **4,923,984**, 1990.

6. Stowell, J.C. *J. Org. Chem.* **1976**, *41*, 560.

7. For standard mixed anhydride coupling conditions see: Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1984; pp109-110.

8. For similar reaction conditions see method B in: Evans, B.E.; Rittle, K.E.; Bock, M.G.; DiPardo, R.M.; Freidinger, R.M.; Whitter, W.L.; Gould, N.P.; Lundell, G.F.; Homnick, C.F.; Veber, D.F.; Anderson, P.S.; Chang, R.S.L.; Lotti, V.J.; Cerino, D.J.; Chen, T.B.; King, P.J.; Kunkel, K.A.; Springer, J.P.; Hirshfield, J. *J. Med. Chem.* **1987**, *30*, 1229.

9. Physical properties: mp 141-142 °C, ^{1}H NMR ($CDCl_3$) δ 3.1 (1 H, bm), 3.4 (1 H, bm), 5.05 (1 H, bm), 5.1 (2 H, s), 6.34 (1 H, m), 6.49 (1 H, dd, 3.6 and 1.5 Hz), 6.79 (1 H, dd, 2.6 and 1.8 Hz), 6.79 (1 H, m), 7.12 (1 H, dd, 1.2 and 7.4 Hz), 7.2 (2 H, m), 7.6 (1 H, dd, 1.8 and 7.3 Hz), 9.5 (1 H, bs), ms (NH_3, CI) $(M+H)^+$ = 347.

10. For an example of alkylation conditions see Method D reference 8.

11. For examples of typical reaction conditions see: *Org. Syntheses Coll Vol.* **3**,

1963, p 831. (also pp. 331 and 539).

12. Smallheer, J.M.; Otto, M.J., Amaral-Ly, C.A., Earl, R.A., Myers, M.J , Pennev, P., Montefiori, D.C., Wuonola, M.A.; *Antiviral Chemistry and Chemotherapy*, 1992, in press. A short description of the assays is given below:

CELL PROTECTION ASSAY. Test compounds were dissolved in DMSO to 5 mg/ml and serially diluted into RPMI medium to ten times the desired final concentration. MT-2 cells (5 x 10E4/0.1ml) were added to each well of a 96 well culture plate and 0.02ml of the appropriate test compound solution was added to the cells such that each compound concentration was present in two wells. The cells and compounds were allowed to sit for 30 minutes at room temperature. HIV(3b) or HIV(Rf) (5 x 10E5 plaque forming units/ml) was diluted in medium and added to the cells and compound mixtures to give a multiplicity of infection of 0.1 plaque forming unit/cell. The mixtures were incubated for 3 days at 36 °C, during which time the virus replicated and caused the death of unprotected cells. The percentage of cells protected from virus induced cell death was determined by the degree of metabolism of the tetrazolium dye, XTT. In living cells XTT was metabolized to a colored formazan product which was quantitated spectrophotometrically at 450nm The amount of colored formazan was proportional to the number of cells protected from virus by the test compound. The concentration of compound protecting either 50% (IC_{50}) or 90% (IC_{90}) with respect to uninfected cell culture was determined.

YIELD REDUCTION ASSAY. Test compounds were dissolved in DMSO to 5 mg/ml and serially diluted into RPMI medium to ten times the desired final concentration MT-2 cells (5 x 10E4/ml) in 2.3 ml were mixed with 0.3 ml of the appropriate test compound solution and allowed to sit for 30 minutes at room temperature. HIV(3b) or HIV(Rf) (5 x 10E5 plaque forming units/ml) in 0.375 ml was added to the cells and compound mixtures and incubated for 1 hour at 36 °C. The mixtures were centrifuged at 1000 rpm for 10 minutes and the supernatants containing unattached virus were discarded. The cell pellets were suspended in fresh RPMI containing the appropriate concentrations of test compound and placed in a 36 °C, 4% CO2 incubator. Virus was allowed to replicate for 3 days. Cultures were centrifuged for 10 minutes at 1000 rpm and the supernatants containing cell free progeny virus was removed for plaque assay. The virus titers of the progeny virus produced in the presence or absence of test compounds was determined by plaque assay. Progeny virus suspensions were serially diluted in RPMI and 1 0 mL of each dilution was added to 9 mL of MT-2 cells in RPMI. Cells and virus were incubated for 3 hours at 36°C to allow for efficient attachment of the virus to the cells. Each virus and cell mixture was aliquoted equally to two wells of a six well poly-L-lysine coated culture plate and incubated overnight at 36°C, 4% CO_2. Liquid and unattached cells were removed prior to addition of 1.5 mL of RPMI with 0.75% (w/v) Seaplaque agarose (FMC Corp) and 5% FCS. Plates were incubated for 3 days and a second RPMI/agarose overlay was added. After an additional 3 days at 36°C, 4% CO_2, a final overlays of phosphate-buffered saline with 0.75% Seaplaque agarose and 1 mg MTT/mL was added. The plates were incubated overnight at 36°C. Clear plaques on a purple background were counted and the number of plaque forming units of virus was calculated for each sample. The antiviral activity of test compounds was determined by the percent reduction in the virus titers with respect to virus grown in the absence of any inhibitors.