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SYNTHESIS AND ANTI-HIV ACTIVITIES OF UREA-PETT ANALOGS BELONGING TO A NEW CLASS OF POTENT NON-NUCLEOSIDE HIV-1 REVERSE TRANSCRIPTASE INHIBITORS

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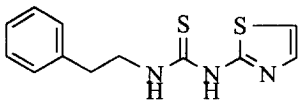
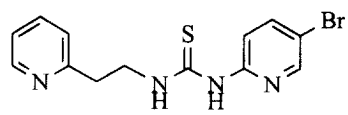
Abstract: A series of potent specific HIV-1 RT inhibitory compounds is described. The compounds are urea analogs of PETT (PhenylEthylThiazoleThiourea) derivatives and the series includes derivatives with an ethyl linker (**1-6**) and conformationally restricted analogs (**7-13**). The antiviral activity is determined both at the RT level and in cell culture on both native and mutant forms of HIV-1. Many compounds display activity in the nM range against wt-RT.

The AIDS epidemic caused by the HIV virus is a serious health problem throughout the world. In the search for therapeutic drugs against this disease, the main interest has been focused on two key enzymes in the HIV machinery namely HIV reverse transcriptase (RT) and HIV protease (PR).[1] There are 5 clinically approved nucleoside RT inhibitors (AZT, ddI, ddC, d4T and 3TC). These agents must first be phosphorylated before they can bind to the catalytic site. Another type of RT inhibitor is the non-nucleoside RT inhibitors (NNRTI's) which bind to an allosteric site of HIV-1 RT. The PETT compounds belong to this type of inhibitor as do the two clinically approved compounds nevirapine and delavirdine. Recently, PR inhibitors such as ritonavir, saquinavir, indinavir and nelfinavir, have been approved as AIDS drugs. However, since modern AIDS therapy is moving towards combination therapy there is a great need for new compounds having high activity against wild type and mutated forms of HIV-virus, with low toxicity and good pharmacokinetics.

The first compounds in the PETT series were reported in 1993 followed by full papers in 1995.[2] The lead compound in the series was LY73497. The first generation of PETT compounds resulted in trovirdine which was selected for clinical trials on the basis of good antiviral activity and a favourable pharmacokinetic and toxicological profile.



LY 73497
ED_{50} 1.3 μM
IC_{50} 0.9 μM



Trovirdine
ED_{50} 20 nM
IC_{50} 15 nM

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Different approaches have been used to further optimize the PETT series. This publication describes one part of the synthesis program, the synthesis and anti HIV-1 activity of a series of urea-PETT analogs.[3]

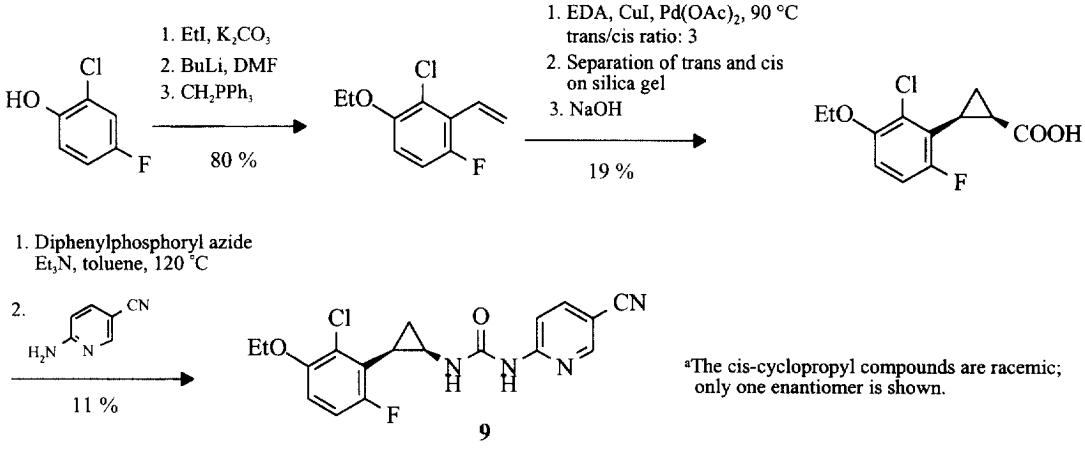
Chemistry

The preparation of the compounds described in the Table are based on two general methods depicted in Schemes 1 and 2.

The key steps in Scheme 1 involve, for the restricted analogs, a cyclopropanation reaction and a Curtius rearrangement of an appropriate acid. In the cyclopropanation reaction, an appropriate styrene derivative reacts with ethyl diazoacetate (EDA) in the presence of catalytical amounts of copper(I)iodide and palladium acetate. The trans/cis ratio is generally about 3 and the overall yield about 60 %. Subsequent silica gel chromatography to separate cis and trans followed by basic hydrolysis afforded diastereomeric pure cis cyclopropyl acids. In forthcoming papers we will show how to obtain mostly the cis isomers by using a chiral ligand and copper(I)triflate and running the reactions at 0 °C, resulting in trans/cis ratios of about 0.3 and high enantiomeric excesses.[4]

The outcome of the Curtius reaction is quite variable. In the particular example in Scheme 1 the unoptimized yield is quite low, although in other reactions yields between 40-60 % have been observed.

Scheme 1. Representative synthesis of compound **9**[a].

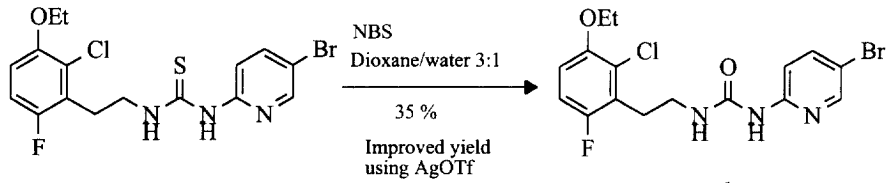


[a]The cis-cyclopropyl compounds are racemic; only one enantiomer is shown.

In the other general scheme to the urea-PETT compounds, a transformation of the thiourea moiety of a PETT compound to a urea function is the key step. This was originally achieved with N-bromosuccinimide (NBS) in water and dioxane. Later it was found that silver triflate is a better reagent for this reaction. For the use of mercury(II) acetate and other reagents for conversion of thioureas to

ureas see reference 5. The urea PETT compounds were purified by silica gel chromatography and carefully analyzed. It was shown that no thiourea compounds were present in the samples when testing for antiviral activity. This is very important since thiourea compounds are known as highly potent HIV-1 inhibitors.[2]

Scheme 2. Representative synthesis of compound **6**.



6

Biological Results and Discussion

The anti-HIV activity of the compounds **1-13** were assessed both on recombinant HIV-RT and in cell culture. At the enzyme level the inhibiting effect was determined on wild type HIV-1 or HIV-1 RT with an Ile in position 100 and with a Cys in position 181.[6] The antiviral activity in cell culture was determined in MT 4 cells using an XTT assay which contains 10 % fetal calf serum.[7] Some compounds were tested in the presence of 15 % human AB serum. Clone 118 has a mutation Leu100 to Ile100 and clone 90 has Tyr181 to Cys181. Trovirdine, 9-Cl-TIBO,[8] nevirapine[9] and L-697,661[10] are all known non-nucleoside RT inhibitors and were used as reference compounds. The IC_{50} and ED_{50} values from these experiments are shown in Table 1.

The reported SAR concerning thiourea PETT compounds have shown that optimal activity is obtained by di(2,6)-or tri(2,3,6)-substituents like halogens or alkoxy in the phenyl moiety of the molecule and 5-substituted (Cl, Br or CN) pyridines at the opposite side of the thiourea moiety.[2] Furthermore, it was postulated that an internal hydrogen bond between the pyridine nitrogen and the thiourea-hydrogen adjacent to the ethyl linker was essential for activity.[2b] The use of conformationally restricted thiourea compounds has been communicated.[11] These studies show that an optimal activity is achieved with *cis*-cyclopropyl analogs. Some enantioselectivity using different enantiomeric *cis*-cyclopropyl compounds has been reported.[4]

The above SAR work for the thiourea PETT compounds has guided us in the design of the urea compounds reported in this paper. The urea analog of trovirdine was not synthesized but for comparison the thiourea and urea 3-fluoro-4-methoxy (in the ethyl-pyridine part of the molecule) analogs of trovirdine were prepared and tested. These results showed that the thiourea compound (IC_{50}=1.3 nM and ED_{50}=0.5 nM) is more active than trovirdine and also much more active than the urea compound (IC_{50}=50 nM and ED_{50}=75 nM). However, the activities of one of the first synthesized urea analog i.e. compound **1** and of the above mentioned compound were promising. Also these urea

compounds might have better toxicological and pharmacokinetic properties and it was found later that the urea-PETT compounds maintain their antiviral activity in cell culture in the presence of added human serum much better than the thiourea compounds. These points justified a SAR program in which different urea-PETT analogs were synthesized and evaluated. The compounds shown in Table 1 are (i) ethyl linked analogs (**1-6**) and (ii) conformationally restricted cyclopropyl analogs (**7-13**). When comparing the antiviral activity between these analogs there seems to be a general trend that the cyclopropyl compounds are more potent than the ethyl linked compounds especially on mutants. For example, the cyclopropyl compound **11** is 10 times more active on clone 118 than the ethyl compound **3** and compound **7** is 10 times more active than the ethyl compound **1** at the enzyme level using RT with an isoleucine in the 100 position. A comparison of the activity on wt and mutant models of compound **6** and its cyclopropyl and bromo (R_4) analog **8** show that the activity both on mutant enzymes and in the clones decrease much more for the ethyl compound than for the cyclopropyl analog. We speculate that the more flexible ethyl derivatives might have problems in adopting conformations that fit in the mutant enzymes compared to the more restricted cyclopropyl analogs. Furthermore, in cell culture other factors such as transportation into the cells might be of importance in explaining these differences.

In the ethyl series where R_3 is methoxy the activity decreased when an acetyl was introduced as R_1 substituent (**5**) compared with the alkoxy substituted compounds (**3** and **4**). The 2,6-dihalo compound **6** is a potent compound which is also the case for the PETT thiourea derivatives.[4] In order to enhance water solubility, the amino compound **2** was made but this was sub-optimal in that the antiviral activity decreased substantially. In the cyclopropyl series better activity, especially on the mutant enzymes, was achieved with the 2,6-dihalo-3-alkoxy compounds compared with the 2-halo-3,6-dialkoxy compounds, cf. the activity of compound **11** and **13** against compounds **8** and **9**. Earlier SAR studies with thiourea PETT compounds have shown that the preferred 5 substituents on the pyridine ring are bromo, chloro or cyano.[2] This is also true for the urea compounds (data not shown) and the overall most active compounds in this series are the cyano analog **9** and the chloro analogs **8** and **12.**

The anti-HIV-1 activity of the most active compounds in this study i.e compounds **8**, **9** and **12** are by far superior than the studied reference compounds. Furthermore, although the antiviral activity for the urea-PETT compounds **8**, **9** and **12** declines on clones 90 and 118, it is much higher than the reference compounds, especially on Tyr181→Cys181 mutants.

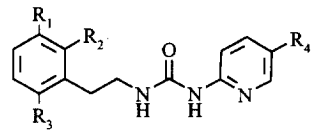
There is a reasonably good correlation between the activity in cell culture and at the enzyme level supporting that these compounds from a mechanistic viewpoint are inhibitors of RT. Kinetic experiments for the corresponding thiourea derivatives show that they are non-competitive reversible RT-inhibitors supporting the hypothesis that these non-nucleoside HIV-1 RT inhibitors bind to an allosteric site/sites of RT.[6,12] X ray crystallography studies with nevirapine have shown that it binds to

an allosteric site.[13] For some compounds eg. **3** and **4** there is a fairly large difference between the activity in cell culture and at the enzyme level. A reasonable explanation for this discrepancy might be difficulties for the compounds in penetrating into the cell or interaction with serum proteins used in the cell culture assay, see below.

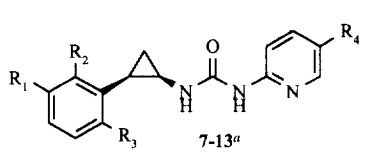
An indirect measure of the protein binding of a compound is to test it in the presence of human serum. This was performed for some compounds and a decrease in the ED_{50} values was noted. However, for the high activity compounds **7** and **8**, the activity only decreased by a factor of 6 and 3, respectively. The best compound in this respect was the cyano compound **9** which only loses its activity with a factor of 2 in the presence of human serum. This indicates that compound **9** might not be heavily protein bound *in vivo*.

The relatively high activity on the mutant enzymes both in cell culture and on the RT level together with high activity in the presence of 15% serum make these compounds attractive as potential drugs against HIV/AIDS and further optimization is ongoing in our laboratories.

Table 1. Inhibition of HIV-1; urea-PETT compounds and reference compounds.



1-6



7-13[a]

						Anti HIV-1 activity						
						IC_{50} (μM)[b]			ED_{50} (μM)[c]			
No.	R_1	R_2	R_3	R_4	Route	wt	Ile 100	Cys 181	wt	wt[d]	clone 118	clone 90
1	H	F	F	Cl	II	0.003	0.42	0.48	0.03	0.85	32	>32
2	NMe_2	F	F	Br	I	0.025	>2.5	1.5	0.5	NT[e]	>25	>25
3	OMe	F	OMe	Cl	I	0.003	0.14	0.14	0.011	0.09	17	3
4	OEt	F	OMe	Cl	I	0.008	2.5	0.5	0.13	NT	14	>27
5	COMe	F	OMe	Cl	II	0.1	11	10	0.2	NT	>27	>27
6	OEt	Cl	F	Br	I	0.004	1.7	0.24	0.07	NT	15	8
7	H	F	F	Cl	I	0.002	0.03	0.12	0.006	0.01	NT	NT
8	OEt	Cl	F	Cl	I	0.008	0.08	0.05	0.016	0.06	0.1	NT
9	OEt	Cl	F	CN	II	0.004	0.08	0.07	0.01	0.02	0.27	0.53
10	OEt	F	F	Cl	I	0.0015	0.08	0.015	0.008	0.03	0.75	0.27
11	OMe	F	OMe	Cl	I	0.002	0.27	0.27	0.012	0.1	1.6	2.7
12	OEt	F	Cl	Cl	I	0.0015	0.05	0.02	0.008	0.1	0.1	0.1
13	OEt	F	OMe	Br	II	0.007	0.24	0.09	0.025	0.4	NT	NT
Trovirdine						0.015	0.7	3.2	0.02	5	0.8	>5
9-Chloro-TIBO						0.2	31	12	0.25	NT	>22	124
Nevirapine						0.2	13	160	0.15	0.12	0.62	22
L-697,661						0.1	1.6	15	0.065	NT	0.85	>11

[a]The cis-cyclopropyl compounds are racemic; only one enatiomer is shown. [b]The assay used rCdG as the template and dGTP as the substrate as described in ref 6. The concentration producing 50 % inhibition (IC_{50}) is stated as the mean of at least two experiments. [c]The cell culture assay used MT4 cells infected with $HIV\text{-}1_{IIIB}$. The concentration which reduced the cytopathic effect caused by the virus (ED_{50}) is stated as the mean of at least two experiments. [d]The assay contains 15 % human AB serum. [e]Not tested.

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