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Thiourea Derivatives: A Promising Class Against HIV/TB Co-Infection

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1. Introduction

Nowadays, the Human Immunodeficiency Virus (HIV), which is the causative agent of Acquired Immune Deficiency Syndrome (AIDS), represents a serious public health problem. According to the World Health Organization (WHO), in 2009 there were 33.3 million people living with HIV worldwide, and more particularly in sub-Saharan Africa, where the overwhelming majority (67%) of cases appear. Furthermore, 2.6 million people have been recently infected with the virus in 2009, when HIV/AIDS was estimated to have caused 1.8 million deaths (United Nations Program on HIV/AIDS [UNAIDS], 2010).
Due to the impairment of their immune system, HIV bearers are more susceptible to opportunistic infections, such as Tuberculosis (TB), which is a leading cause of HIV-related deaths worldwide. The risk for TB is 20-37-fold greater among HIV-infected individuals, depending on the status of the HIV epidemic. According to WHO, one-third of people living with HIV are infected with TB, and there was an estimate of 1.4 million new TB cases per year among said population. Moreover, one in four TB deaths occurs in HIV-positive patients, while TB was responsible for 23% of AIDS-related deaths (WHO, 2010a).
This situation becomes especially alarming in view of the number of challenges in the control and management of TB in HIV-infected individuals, such as the difficulties to conclude a TB diagnosis, as well as the complexity involved in the treatment of HIV infection-related TB. Due to their great relevance to the subject matter of this work, the above factors will be emphasized in the next section.

2. Challenges in the management of HIV infection-related TB

2.1 Diagnosis of TB in HIV-infected individuals

Within the context of lung diseases, there are some aspects that may constitute a bar to the diagnosis of TB (Box **1**): HIV-infected patients are minimally symptomatic or asymptomatic, as they present few or less specific classic symptoms of TB (productive cough, chest pain, fever, night sweats, weight loss, hemoptysis); patients with low $CD4^+$ T lymphocyte counts

have atypical chest radiograph findings, with lower prevalence of cavitary disease, while the findings could be normal in up to 22% of HIV-infected individuals; the main method used worldwide for TB detection, namely the microscopic examination of Ziehl-Neelsen-stained sputum smears, has low sensitivity among HIV-infected individuals, as they develop acid-faster smear negative diseases with higher frequency than HIV-uninfected people (Sterling et al., 2010).

Furthermore, HIV-infected individuals present more often the subclinical form of TB, said factor leading to a delay in the diagnosis and treatment. Another difficulty for the TB diagnosis in HIV-infected individuals is the increased risk (10-20% in HIV-uninfected individuals, compared to 40-80% in HIV-infected persons) to develop extrapulmonary TB, whose most prevalent forms are pleural effusion, lymphadenopathy, pericardial disease, miliary disease, meningitis and disseminated TB (Chaisson et al., 1987).

Box 1. Usual difficulties involved in the diagnosis of TB in HIV-infected individuals

- Minimally symptomatic or asymptomatic patients;
- Atypical chest radiograph findings;
- Acute prevalence of acid--fast smear negative disease ;
- High frequency of subclinical TB form;
- Increased risk of development of extrapulmonary TB.

2.2 TB treatment in HIV bearers

In addition to diagnostic difficulties, the treatment of HIV infection-related TB also presents several challenges, such as duration and frequency, determining the precise moment to start antiretroviral therapy (ART), management of drug interactions, as well as several side effects from therapy.

As regards the duration of TB therapy, WHO recommends a 6-month rifampicin-based treatment (2HRZE/4HR, Table **1**), applying to both HIV-infected and uninfected individuals. Nevertheless, Perriens and collaborators showed that, after providing a 12-month therapy with 2HRZE/4HR, the recurrence rate at the 18th month shall be lower than those observed at the standard regime (Perriens et al., 1995). Furthermore, another study performed by Fitzgerald et al indicated that the administration of isoniazid for 1 year upon termination of the therapy under standard regime reduces the recurrence of TB only among HIV-infected patients (Fitzgerald et al., 2000). It is worthy to mention that, in both studies, patients had no access to antiretroviral therapy, which contributes to extend the beneficial effects from TB treatment, without presenting the risks that are inherent to the combination of TB-HIV drugs.

Phase	Duration (months)	Dosing Frequency	Drugs
Intensive	2	Daily	HRZE
Continuous	4	Daily or three times per week[a]	HR

[a] For the continuation phase, the optimal dosing frequency may be also daily; should the administration of such a dosage be impossible, three times per week is a suitable alternative.
H = isoniazid, R= rifampicin, Z = pyrazinamide, E= ethambutol

Table 1. TB treatment for HIV bearers, as recommended by WHO.

Providing another relevant instruction on TB treatment in HIV-infected patients, WHO also recommends a daily TB therapy within, at least, the intensive phase (WHO, 2010b). Such orientation is based on a recent study, which showed that the incidence of relapse and failure among HIV-positive TB patients who were treated with intermittent TB therapy throughout treatment was 2–3 times higher, in comparison with patients who received a daily intensive therapy (Kahn et al., 2010). Moreover, another study indicated that, among HIV-positive patients, the risk of acquired resistance to rifampicin is higher when failing a three times weekly short-course intermittent regime (Kahn et al., 2010).

In relation to HIV treatment (Box **2**), WHO recommends that the first-line ART regime should comprise a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI), as said drugs are effective, available at low costs in the market, and present generic and fixed-dose combinations (FDCs). Therefore, protease inhibitors (PIs) should be kept for second-line regimes (WHO, 2010b).

Box 2. Combined regime for both HIV and TB treatment.

- First-line regime: **2NRTIs + 1NNRTI**
  - [AZT or TDF] and [3TC or FTC] plus [EFV or NVP];
  - ART regime containing EFV is preferred, since interactions with anti-TB drugs are minimal;
  - EFV should not be administrated to women during the first trimester pregnancy, due to its teratogenic effects .
- **In cases of EFV intolerance, of HIV resistant to EFV, or contraindications to an EFV-based regime:** [AZT + 3TC + NVP] or TDF + [3TC or FTC] + NVP or the triple NRTI regime [AZT + 3TC + ABC] or [AZT + 3TC + TDF]
- **ART regime containing a boosted protease inhibitor** (PIs)
  - If available, a rifabutin-based TB treatment should be preferable.
  - Hepatitis among healthy adults is recurrent.

AZT= zidovudine, TDF= tenofovir, 3TC= lamivudine, FTC= emtricitabine, EFV= efavirenz , NVP= nevirapine.

2.3 Optimal timing to start the ART treatment

A critical issue among HIV-infected individuals diagnosed with TB is to determine the precise moment to initiate ART. Although ART improves the survival of HIV-positive patients, including those with TB, the optimal deadline from the onset of TB to start such a treatment is a matter that still requires further clarification. According to WHO, the TB treatment should be initiated in all HIV bearers showing active TB disease, regardless of their $CD4^+$ lymphocyte count. TB treatment should be started first, and, then, followed by ART as soon as possible, and preferably within the first weeks from the beginning of the TB treatment (WHO, 2010b). However, the concomitant treatment of both diseases results in several disadvantages, whose examples are listed and discussed at the Table **2**.

In spite of a number of clinical trials intended to determine the optimal timing for ART administration in bearers of HIV infection-related TB, the question still requires further studies (Piggot & Karakousis, 2011). Among the above referred works, Abdool Karim and collaborators have been performing the Starting Antiviral Therapy at Three Points in TB (SAPIT), which is an open-label, randomized and controlled trial conducted in Durban,

South Africa, with 642 HIV-positive patients with a CD4 count <500/mm^3 and with smear-positive TB (Abdool et al., 2010). The preliminary findings of SAPIT showed a decreased mortality rate among individuals who had started ART during anti-TB regime, in comparison with those who initiated ART only upon completion of the anti-TB therapy.

Disadvantage	Comments
High pill burden	More than 10 pills daily; discourage treatment adherence.
Overlapping of adverse effects	Hepatotoxicity promoted by H, R and Z and also by PI and NNRTI; increased risk of hepatitis C virus infection (Velasco et al., 2009). Gastrointestinal upset and rash are common in both therapies Peripheral neuropathy promoted by H, didanosine and stavudine
Immune reconstitution inflammatory syndrome (IRIS)	Although ART triggers the regeneration of immune cells, the immune system unexpectedly produces an overwhelming inflammatory response that unmasks or worsens the co-infection symptoms (TB is the most common among them); The risk of IRIS is the main ground to initiate TB treatment prior to ART, whenever it is possible. Its frequent symptoms comprise fever, swollen lymphonodes, skin lesions and rashes, changes in breathing, pneumonia, hepatitis, abscesses and eye inflammation. They often appear within 2–6 weeks from the beginning of HIV therapy (Antonelli et al., 2010).
Drug-drug interactions	**See Table 3**

Table 2. Disadvantages from the concomitant treatment of TB and HIV.

Following these conclusions, another study performed by Blanc and collaborators provided strong evidences for an early start of ART administration (Blanc et al., 2010). The Cambodian Early versus Late Introduction of Antiretrovirals (CAMELIA) was an open label, prospective, randomized controlled trial, registering HIV-positive patients with a CD4 count <200/mm^3 and smear-positive TB living in Cambodia. This study demonstrated that the group treated with ART within 2 weeks from the beginning of the TB therapy showed a relevant decline of 34% at the mortality rate, when compared with patients who received ART only 8 weeks after the initiation of TB treatment. In both studies, an increased incidence of IRIS was observed in patients who were early treated with ART. Nevertheless, the data shown by the SPIT trial, namely a lack of mortality or changes in antiretroviral regime attributable to IRIS, also corroborate the early ART initiation in HIV infection-related TB patients.

2.4 Management of drug-drug interactions between antiretroviral and anti-TB agents

The most delicate kind of interactions involves the concomitant use of rifamycins (among which the most usual are rifampicin and rifabutin), NNRTIs and PIs, given that these last two classes of antiretroviral drugs are essentially metabolized through cytochrome P450 (CYP) 3A4 enzymes, whose expression is induced by rifamycins. Consequently, the plasma concentration and exposure of NNRTIs and PIs are significantly reduced, when they are concomitantly administrated with rifamycins (Burman et al., 2001). Furthermore, rifampicin improves the activity of the efflux multidrug transporter P-glicoprotein, which promotes the elimination of PIs (Kim et al., 1998; Schuetz et al., 1996). Due to the reduction of NNRTIs and PIs at the plasma concentration, as a result from the simultaneous administration of rifamycins, the HIV treatment can fail, thus giving rise to the emergence of drug resistance.

The Table **3** below summarizes the most relevant interactions between rifamycins and antiretroviral agents (Centers for Disease Control and Prevention [CDC], 2007).

Antiretroviral agent	**Effects on pharmacokinetics parameters and comments**	
	Rifampicin	**Rifabutin**
NNRTIs		
EFV	EFV AUC↓ by 22%	Rifabutin AUC↓ by 38% **Increase Rifabutin dose to 450-600mg (daily or intermittent)**
NVP	NVP AUC↓ 37-58% and Cmin↓ 68% with 200mg 2x/day dose	NVP and Rifabutin AUC are not significantly changed
DLV	DLV AUC↓ 95% **Simultaneous use of such drugs should be avoided.**	DLV AUC↓ 95% Rifabutin AUC↑100% **Simultaneous use of such drugs should be avoided.**
PIs		
Ritonavir	Ritonavir AUC*↓ by 35% **Monitor for antiretroviral activity of ritonavir**	
Fos-Amprenavir	**Simultaneous use of such drugs should be avoided.**	**Rifabutin dose ↓ to 150mg/day or 300mg 3x/week**
Atazanavir	Atazanavir AUC↓ by >95% **Simultaneous use of such drugs should be avoided.**	Rifabutin AUC ↑ by 250% **Rifabutin dose ↓ to 150mg every other day or 3x/week**
Indinavir	Indinavir AUC↓ by 89% **Simultaneous use of such drugs should be avoided.**	Rifabutin AUC ↑ by 34% **Rifabutin dose ↓ to 150mg/day or 300mg 3x/week**
Nelfinavir	Nelfinavir AUC↓ by 82% Simultaneous use of such drugs should be avoided.	Rifabutin AUC ↑ by 207% **Rifabutin dose ↓ to 150mg/day or 300mg 3x/week**
Saquinavir	Saquinavir AUC↓ by 84% **Simultaneous use of such drugs should be avoided.**	
Saquinavir + Ritonavir	**Caution. The use of this combination could cause hepatitis.**	
Lopinavir + Ritonavir	**Caution. The use of this combination could cause hepatitis.**	Rifabutin AUC ↑ by 303% **Rifabutin dose ↓ to 150mg every other day or 3x/week**
Fusion Inhibitors		
Enfuvritide	No interaction. No dose adjustment.	No interaction. No dose adjustment.
CCR5 receptor antagonists		
Maraviroc	Maraviroc Cmin↓ by 78% **Increase Maraviroc to 600mg twice-daily**	**Change Maraviroc dose to 300mg twice daily and rifabutin to 300mg daily**
Integrase Inhibitors		
Raltegravir	Raltegravir concentratios ↓ by 40-61%	Rifabutin trough ↓ by 20% Raltegravir AUC is not affected **Change Rifabutin dose to 300mg daily and Raltegravir to 400mg twice daily**

*AUC= area under the plasma concentration time curve; estimated bioavailability.

Table 3. Management of interactions among anti-TB and anti-HIV drugs.

3. Thiourea derivatives: A promising class against HIV/TB co-infection

Due to such a number of complications that may possibly arise in the course of treatment of HIV-related TB, as described above, the development of new drugs against HIV and TB should be mandatory. Said medications should produce relevant effects, such as the improvement of patient well-being by means of the reduction of pill burden, as well as by the careful management of the overlapping toxicity resulting from the treatment of TB and HIV infections. Therefore, an alternative could be the development of drugs that might be able to simultaneously act in the treatment of both diseases. In this context, thiourea derivatives appear as a promising class of compounds. For instance, the tetrahydroimidazobenzodiazepinthiones (TIBO) derivative **9-Cl-TIBO** and the phenylethylthiazolylthiourea (PETT) derivatives **LY73497** and **trovirdine (TRV)** play a significant role in the inhibition of HIV reverse transcriptase (Fig. 1). On the other hand, the compound **isoxyl** (**ISO**, thiocarlide), another thiourea derivative, is known by its strong anti-TB activity (Fig. 1). By the way, although used as part of the TB clinical treatment since the 1960's, it may be pointed out that the relevance of **ISO** emerged from recent researches, and particularly from studies in the field of new treatments against MDR-TB. Therefore, the main purpose of this chapter is to highlight the importance of thioureas for the TB-HIV drug discovery, and to proceed with a review of data from recent literature, by focusing the most relevant contributions to the development of new prototypes containing this promising scaffold.

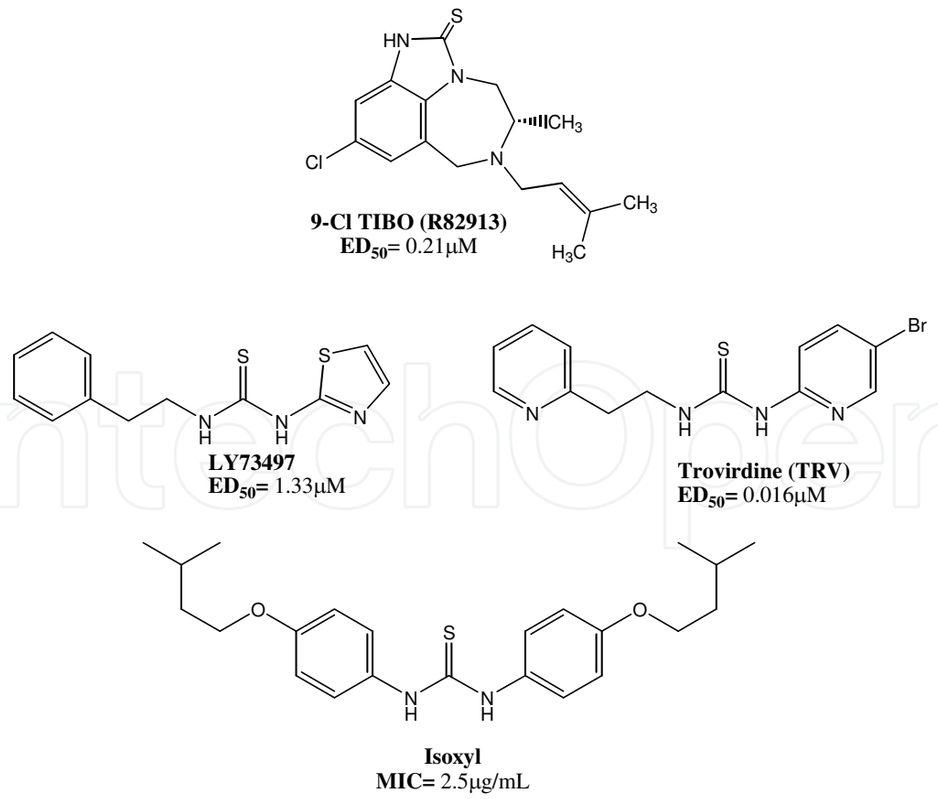


Fig. 1. Active thioureas against HIV (**9-Cl TIBO, LY73497** and trovirdine) or *M. tuberculosis* (**ISO**).

3.1 Thiourea derivatives showing a potential activity against HIV

3.1.1 TIBO derivatives

TIBO and HEPT [1-(2-hydroxyethoxymethyl)-6-(phenylthio)thymine] derivatives, yet discovered independently from each other, can be reputed a landmark in the history of the antiretroviral therapy. These compounds were the first congeners from a new category of anti-HIV drugs, currently known as NNRTIs (De Clercq, 2004). At the beginning of the 1990s, the only drug that had been approved for AIDS treatment was AZT, so that patients had to live at imminent risk to develop resistant mutant virus. Therefore, it becomes quite clear that the identification of a different class of antiretroviral drugs brought new perspectives in the treatment of AIDS.

The development of TIBO derivatives started from a screening program from the library of Janssen Research Foundation, working with 600 compounds that were selected due to their failure in producing effects on the standard pharmacological assays, and to their low toxicity in rodents as well (De Clercq, 2004). Upon evaluation of the biological activity of these compounds against HIV-1/$HTLV_{IIIB}$ in MT-4 cells, researchers identified the tetrahydro-benzodiazepine derivative **R14458** (Fig. 2), which presented a moderate anti-HIV-1 activity (IC_{50}= 62μM) (Pauwels et al., 1990). Using this substance as a lead compound, they started a program aiming at the improvement of its anti-HIV properties. Thereafter, several analogues were synthesized, thus allowing the performance of an extensive structure-activity relationship (SAR) study of this class of compounds.

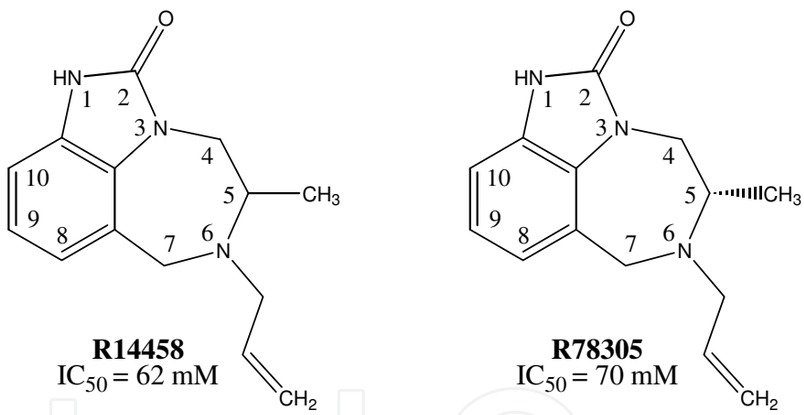


Fig. 2. Structure of TIBO derivatives **R4458** and **R78305**.

3.1.1.1 SAR of TIBO derivatives

Considering that the compound **R14458** is a racemic mixture, the authors initially investigated the role of stereochemistry in the biological activity of this substance. Although the two optical isomers were synthesized and tested against HIV-1, only the enantiomer **R78305** (Fig. 2) with *S* configuration was found to be active, showing that this configuration is required for the anti-HIV-1 properties of TIBOs (Pauwels et al., 1990). The subsequent studies aimed at the evaluation of systematic alterations in the structure of the lead compound, and its scaffold was independently modified in four different portions: the substituent bonded to the 6-positon nitrogen of the diazepine ring (Kukla et al., 1991a), the 5-ring urea portion (Kukla et., 1991b), the 7-membered ring portion (Breslin et al., 1995) and the substituent of the aromatic ring (Ho et al., 1995). The results obtained in these studies are described in the next sections.

3.1.1.1.1 Modifications in the substituent bonded to the 6-position nitrogen of the diazepine ring

Initially, it was verified that the presence of bulky groups attached to this position was mandatory to trigger anti-HIV-1 activity, since compounds containing hydrogen, ethyl or a linear propargyl substituent were inactive. The strongest activities were observed whenever an unsaturated allyl group was attached to the 6-positon nitrogen, such as in the lead compound, and it was verified that the substitution at the 2-position of this group led to more active compounds. The improvement degree of said activity varied in accordance with the following sequence: ethyl > methyl = vinyl = bromo > H. On the other hand, all compounds containing 2-propyl, phenyl, benzyl or fused cyclohexenyl were inactive. Substitutions at the 3-positon of the allyl group were also verified, and the dimethyl substituted compound showed the highest degree of activity found in these series. Introduction of bulky groups at this position usually led to completely inactive compounds. In view of the above results, an optimum size to the substituent for both 2- and 3-positions is required, since the substance loses its activity, as the length of the side chain increases or decreases. Moreover, the groups attached to the nitrogen which led to inactive compounds were the following: acetylene, alkyl groups containing heteroatom, and methylene attached to functional groups such as, nitrile, ketone, ester, alcohol, ether or a heteroaromatic pyrrole or imidazole (Kukla et al., 1991a).

3.1.1.1.2 Modification in the 5-ring urea portion

This portion of the tricyclic TIBO structures underwent several modifications, among which we can mention the replacement of carbonyl carbon by: nitrogen, sulfur dioxide or deletion of the carbonyl oxygen yielding, that are, respectively, a triazine, a sulfonamide and an imidazole. However, in the most part of trials, these modifications gave rise to inactive compounds. The most promising result was found by the replacement of the urea group by a thiourea, yielding a compound around one hundred times more active than the original prototype. The ring expansion, by insertion of a methylene or another carbonyl, led to a loss of anti-HIV-1 activity, and it was also verified that methylation of 1-positon nitrogen also gave rise to an inactive compound, probably in view of the need of NH to form hydrogen bonding (Kukla et., 1991b).

3.1.1.1.3 Modifications at the 7-membered ring portion

The demethylation of carbon in 5-position or introduction of bulky groups at this position yielded inactive or less active compounds, thus demonstrating that the size of the methyl group is optimum for the biological activity. The C-4 position showed a greater tolerance for larger groups, and the analogues presented a good anti-HIV-1 activity. The 7-position also underwent replacements, whereby high levels of activity were once more achieved. However, among the modifications performed at the 7-membered ring, none of them led to the discovery of compounds showing a better HIV inhibitory activity than the simplest 5-mono-methyl-substituted analogue (Breslin et al., 1995).

3.1.1.1.4 Evaluation of substituent effect on the aromatic ring

In order to evaluate the substituent effect on the aromatic ring, researchers used both urea and thiourea derivatives, and maintained the optimal conditions described by previous works, such as the attachment of a methyl group to the 5-position, and bonding of a dimethylallyl group to nitrogen at the 6-positon (except in case of compounds presenting

synthetic problems, where scientists had to use a cyclopropylmethyl, also able to lead to high levels of activity). The best results were found for 8-substituted analogues, which, in comparison to the lead compound, showed much higher potency, so that halogens reached the highest level of activities in the substituent group. Iodine was the only exception to the above conclusions, probably due to its larger volume. Compounds containing a methoxy or an acetylene group bounded at 8-positon displayed similar activities, when compared to the parent unsubstituted compound. Although the substitution of 8-methoxy group by 8-thiomethyl may have led to an improvement in the biological activity, the replacement of methoxy by the ethoxy group, which is larger, resulted in a decrease in anti-HIV activity. Amino, aminoacetyl, dimethylamino and nitro analogues remained inactive. Furthermore, the substitutions at 9-positon led to the formation of compounds, whose activities are similar to those of the parent unsubstituted compound, while 10-substituted compounds were found to be less active. The replacement of the aromatic ring by a heteroaromatic ring was also evaluated, but the derivatives were inactive (Ho et al., 1995).

3.1.1.1.5 Clinical trial with 9-Cl-TIBO (R82913)

Extensive SAR studies with TIBO derivatives allowed the identification of substances with similar or better activities than other known antiretroviral drugs, such as AZT, dideoxycytidine (ddC) and Dideoxyinosine (ddI). Among these substances, thiourea **9-Cl TIBO (R82913)** (Fig. 1) was selected for a phase I clinical trial. This study evaluated 22 patients, within the age group between 27-59 years old, who showed HIV infection in an advanced stage (Pialoux et al., 1991). The drug was administrated by daily intravenous injection through a peripheral or a central venous catheter. **R82913** had to be injected in a dose of 120-200mg, in order to reach the concentration observed *in vitro* (20-40ng/mL), which is required for the protection against HIV cytopathic effects. The measured half-life was of 3 days, and the pharmacokinetic profile of the substance was neither influenced by an increase of the dose, nor by its long-term administration. In spite of its side effects, which usually comprised phlebitis, drowsiness and fatigue, a general absence of toxicity could be attributed to **R82913**.

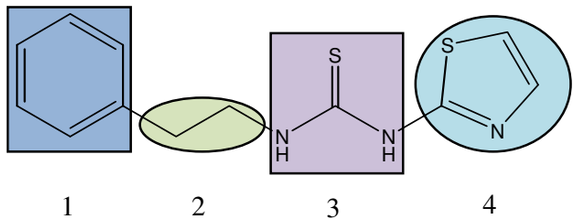
3.1.2 PETT derivatives

3.1.2.1 Discovery of PETT series and preliminary SAR studies

Phenethylthiazolylthiourea (PETT) analogues integrate the powerful class of NNRTIs, first described by researchers from Lilly laboratories in the second half of the 1990's (Ahgren et al., 1995). These compounds were discovered in an attempt to indentify the minimal structural elements that might be necessary for the development of the thiourea derivative **9-Cl TIBO** biological activity (Fig. 1). The researchers disconnected some bonds from the rigid tricyclic nucleus of this substance, thus producing simpler structures. The potential pharmacophores produced after the systematically disconnections were used to search similar structures in the organic compound database of Lilly Research Laboratories. The study disclosed approximately 250 substances, whose activity against HIV was duly evaluated. The *N*-(2-phenethyl)-*N*'-(2-thiazolyl)thiourea, **LY73497** (Fig. 3), was identified as the lead compound, and used in subsequent SAR studies (Bell et al., 1995).

SAR studies were performed by dividing **LY73497** into four portions and proceeding with an aleatory variation of each of them (Fig. 3) (Bell et al., 1995). Initially, the authors

modified the quadrant 1 of phenyl ring, introducing different substituents, or replacing it by other aromatic heterocycles. They observed that *meta* and *ortho* substitutions generally triggers better activities, when compared with the *para* one. As regards the electronic nature of *ortho* substituents, both small electron-donating and small electron-withdrawing groups presented good activities, and, among said elements, the preferred groups are the following: fluoro, chloro, azido and methoxy. A combination of alkoxy and halogen substitution resulted in compounds with improved activity. Although the introduction of an ethoxy group in *meta* position may have led to a good activity, the use of bulky alkoxy groups, such as propoxy and isopropoxy, seems to have been responsible for a reduction in the activities. Among the substances containing a heterocycle in replacement of the phenyl ring, the best activity was observed for the 2-pyridyl compound. Changes at the nitrogen atom for the 3- and 4-position induced a decrease in the activity.



LY73497

Fig. 3. SAR studies with PETTs derivatives.

The modifications in quadrant 2 were characterized by changes in the length of the alkyl linker. The main conclusion was the identification of an ethyl linker as optimal to the activities. It was also noted that the introduction of a methyl group in the benzylic position of ethylene linker enhanced the activity, while a methyl group in the phenethyl position led to its reduction.

The variations in quadrant 3 demonstrated the crucial role played by thiourea moiety at the anti-HIV activity of these compounds. In fact, the replacement of thiourea by urea resulted in an inactive compound and other isosters, such as cyanoguanidine derivatives, which appeared to be less potent. The methyl substitution at the nitrogen adjacent to the thiazole ring leads to a less active compound, while methyl substitution on the nitrogen adjacent to the phenethyl side chain provided compound with no activity at all. This result is attributed to presence of an internal hydrogen bond between the hydrogen bonded to nitrogen adjacent to the phenethyl side chain and the nitrogen of the thiazole nucleus in **LY73497**.

As regards quadrant 4, it was observed that a heterocycle in this position is determinant to reach a good activity. In general, substituted thiazoles were highly active, excepting the 4-carboxythiazolyl compound, whose lack of activity indicated that the allosteric site of the enzyme does not accept a polar group. When thiazole nucleus was replaced by another heterocyle, the 2-pyridyl analog showed the highest level of activity.

During a second phase of SAR studies, the authors combined the optimal substituents in quadrants 1-4, and observed that these parameters are additive, able to give rise to compounds with optimal activity. Among them, the compound **LY300046**, currently known as **trovirdine (TRV;** Fig. 1**)**, was selected for further pharmacological examinations, since the hydrochloride salt of this compound showed acceptable blood levels, when orally administrated in rats (Ahgren et al., 1995).

TRV displayed a comparable or a better ED_{50} than other known antiretroviral drugs, such as **9-Cl TIBO**, L697661, NVP, AZT, ddI and ddC, being capable of inhibiting the replication of HIV-1 in MT-4 cell culture, as well as the replication of various clinical HIV-1 isolates in MT-2 cells and PBL human cells. However, this substance showed no action against HIV-2. When it was tested against resistant isolates containing mutations in Ile-100, Cys-181, and Ile-100–H-188 reverse transcriptase (RT), the trials disclosed a cross resistance between trovirdine and other non-nucleoside compounds. Nevertheless, within the group of said non-nucleoside derivatives, trovirdine was found to present the highest level of activity against these mutants.

In the enzymatic assay with wild-type and mutant RT enzymes, **TRV** was also more active than the non-nucleosides **9-Cl TIBO**, L697661 and nevirapine, showing a lower IC_{50}.

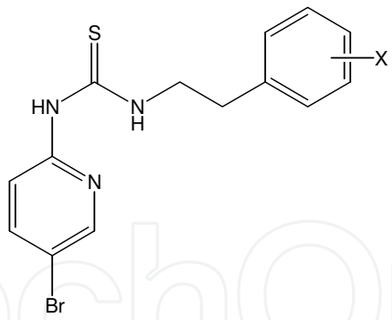
After **TRV** oral administration in rats (20mg/kg), a peak concentration in plasma of 3.5µg/mL was observed at 0.5h. The overall half-life was of 1 h, and the area under the concentration-time curve was 6.9µg/h/mL. The peak concentration in brain was 2.9µg/g, and the area under the concentration-time curve was 5.9µg/h/mL. This result shows that **TRV** crosses the blood-brain barrier, which is a desirable property of anti-retroviral agents, due to the risk of HIV-associated encephalopathy in contaminated patients (Ahgren et al., 1995).

Despite the promising outcomes related to the use of **TRV** as NNRTI, the clinical trials focused on this compound were suspended. Anyway, this substance is still considered a standard lead compound for the development of new PETT analogues, as it encouraged the course of an extensive serie of SAR studies based on modern approaches, such as crystallographic techniques and molecular modeling. These works will be discussed in the next section.

3.1.2.2 Rational design of new PETT analogues

This study was initiated when Vig and collaborators (Vig et al., 1998) proposed to synthesize series of novel PETT derivatives based on the structure of the non-nucleoside inhibitor (NNI) binding pocket of HIV-1 reverse transcriptase (RT). This composite binding pocket was built by superimposing nine individual crystal structures of RT-NNI complexes (Sudbeck et al., 1998). After having conducted docking studies with **TRV**, they verified the existence of multiple sites, which can be used for incorporation of larger functional groups, mainly surrounding the pyridyl ring, the ethyl linker and near the 5-bromo position. Hence, they proposed that a better use of these spaces by strategically designed functional groups could lead to a high-affinity binding and to the discovery of more potent anti-HIV agents. In view of the above, they decided to study the effects of introduction of several substituents in different positions of the phenyl ring, such as methoxy group, fluorine atom or chlorine atom (Table **4**).

These results disclosed by a preliminary SAR study attest to the potency of PETT derivatives phenyl ring substitutions on various positions (Fig. 4). After analyzing the composite NNI binding pocket, the authors identified three promising PETT derivatives with *ortho*-F **(HI-240)**, *ortho*-Cl (**HI-253**) and *metha*-F (**HI-241**) substituents on the phenyl ring, which showed potent anti-HIV activity (IC_{50} [p24] values of < 1nM), selectivity indexes (SI) of > 100,000, and were recognized to be more active than AZT or **TRV**. Among them, **HI-240** has been chosen as the lead compound, as it presented the highest level of activity against wide-type HIV RT. This finding could be grounded on the examination of a composite binding pocket model, showing that Wing 2 region is predominantly hydrophobic, except at the area nearby *ortho* positions on both sides of the phenyl ring, which would be compatible with polar groups, such as, for instance, halogen atoms.



Compound	X	IC_{50} rRT (μM)[a]	IC_{50} p24 (μM)[b]	SI[c]
HI-237	*o*-OMe	1.0	0.01	>1 x 10^4
HI-240	*o*-F	0.6	<0.001	>1 x 10^5
HI-253	*o*-Cl	0.7	<0.001	>1 x 10^5
HI-239	*m*-OMe	0.4	0.003	>3 x 10^4
HI-241	*m*-F	0.7	<0.001	>1 x 10^5
HI-254	*m*-Cl	3.1	N.D.	N.D.
1	*p*-OMe	0.9	0.015	>6 x 10^3
HI-242	*p*-F	6.4	N.D.	N.D.
HI-255	*p*-Cl	2.5	N.D.	N.D.
TRV	----	0.8	0.007	>1 x 10^4
AZT	----	>100	0.004	7 x 10^3

[a] Purified recombinant HIV RT assay.

[b] IC_{50} p24 values represent the inhibition of HIV-1 replication in relation to the virus control, as measured by p24 EIA.

[c] SI (selectivity index) = IC_{50}[MTA]/ IC_{50}[p24]. IC_{50}[MTA] values were >100μM.

Table 4. IC_{50} and SI values for series of PETT derivatives.

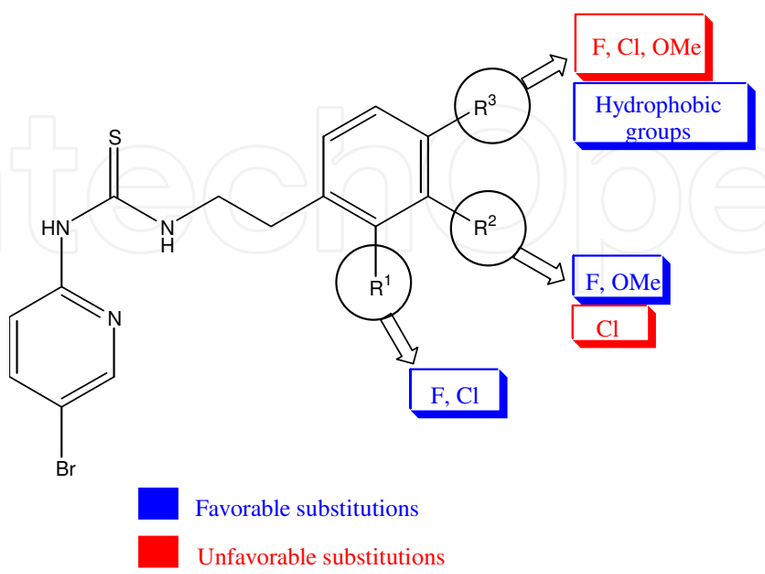


Fig. 4. Preliminary SAR of PETT derivatives.

The authors postulated that the lead compound **HI-240** would be effective against HIV RT mutants (Mao et al., 1999). This hypothesis was confirmed, since **HI-240** is three times more potent than **TRV** against the multiple-drug-resistant of HIV RT, thus emphasizing the relevance of a polar ring substituent, which could provide more favorable interactions with binding site residues (Table **5**).

During this SAR study, Mao and collaborators (Mao et al., 1999) rationally designed a novel PETT analogue (**HI-236**), by using the computer model of NNI binding pocket. This derivative was designed through the optimization of van der Waals contact with the binding pocket, mainly at Wing 2 region, that presents unrecognized spacious regions surrounding the phenyl ring. This strategy would improve the potency against wild-type RT, and also against Wing 2 mutants of RT. In view of the above, the authors proposed the synthesis of **HI-236**, whose 2,5-dimethoxy-substituted phenyl ring allows favorable contacts with Wing 2 region, and also decreases the unoccupied volume surrounding phenyl ring of **HI-240** by 25 $Å^3$. Therefore, **HI-236** showed a potent anti-HIV activity (IC_{50} < 0.001μM), which is lower than IC_{50} values for AZT (0.004 μM). This substance was not cytotoxic, and its calculated selectivity index was > 10^5.

Furthermore, **HI-236** was found to be highly effective against multidrug-resistant HIV-1 strain RT-MDR, whose multiple mutations involve several RT residues (Table **5**). These results are consistent with the prediction according to which **HI-236** would be more active than **HI-240**.

Compounds	IC_{50} p24	IC_{50} RT-MDR[a]	IC_{50} A17[b]	IC_{50} A17 variant[b]
HI-236	<0.001	0.005	0.1	11
HI-240	<0.001	0.005	0.2	41
DLV	0.009	0.4	50	>100
NVP	0.034	5	>100	>100
TRV	0.007	0.02	N.D.	N.D.
AZT	0.004	0.15	0.006	0.004

[a] V106A mutation

[b] Genotypic NNRTI-resistant HIV-strains (A17 and A17 variant) carrying clinically relevant mutations Y181C and K103N + Y181C, respectively.

Table 5. Inhibitory activity of **HI-236** and **HI-240** on p24 production in peripheral blood mononuclear cells infected with HIV strains HIV_{IIIB}, RT-MDR, A17 and A17 variant.

In another work, Mao and collaborators (Mao et al, 1998) proposed the synthesis of new PETT analogues through replacement of the planar pyridyl ring of TVR by a non-planar ring, such as a piperidinyl (**HI-172**) or piperazinyl ring (**HI-258**). This modification was based on the presence of unrecognized spacious regions surrounding the pyridyl ring of **TRV** (molecular volume (MV)= 160$Å^3$), which could be better filled than the spacious Wing 2 region of the butterfly-shaped NNI binding pocket. In comparison with the MV of **TRV**, **HI-172** and **HI-258** presented larger MVs (calculated in 276 and 272$Å^3$ respectively), being thus predicted to better fit into the potentially usable space of the binding site.

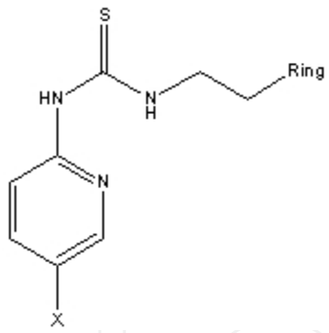
Furthermore, these heterocyclic rings are conformationally more flexible than the pyridyl ring, such a factor being likely to contribute to fit an uncompromising binding pocket in a more efficient way. Table **6** shows that both compounds were more potent than **TRV**, and that they inhibited HIV replication at nanomolar concentrations, without showing cytotoxicity. These findings indicate that, when compared to **TRV** analogues, double substitutions at axial or equatorial positions on these heterocyclic rings could lead to PETT derivatives with a broader range of curvatures, and that they would also better fit to Wing 2 region.

Compound	IC_{50} p24	IC_{50} [MTA]*	SI
HI-172	<0.001	>100	>1 x 105
HI-258	0.002	>100	>5 x 104
TRV	0.007	>100	>1 x 104
AZT	0.006	50	8 x 103

* MTA= Methyl tetrazolium assay

Table 6. IC_{50} and SI values for series of PETT derivatives.

Following their continuous program aiming at the development of new potent PETTs, Uckun and collaborators (Uckun et al., 1999a) decided to replace the pyridyl ring of **TRV** by an aciclyc cyclohexenyl, adamantly or *cis*-myrtanyl ring. Such a proposal of modifications was due to the fact that these chosen groups would fit well with Wing 2 region of the NNI binding pocket. Given the existence of a region compatible with polar atoms at Wing 1, the authors also suggested the replacement of bromine atom by chlorine or trifluoromethyl group (Table **7**). After a biological evaluation, they observed that the replacement of the pyridyl ring of **TRV** by the adamantly (**HI-504**) or *cis*-myrtanyl (**HI-444**) rings resulted in a complete loss of RT inhibitory function. In another important finding, bromine (**HI-346**) / chlorine (**HI-445**) atoms were found to reach the best biological result, thanks to their capacity of making more hydrophobic contacts at the binding pocket, when compared to trifluoromethyl group (**HI-347**). Moreover, the lead compounds **HI-346** and **HI-445** showed a significant activity against the multidrug resistant (MDR) strain, without presenting cytotoxicity, when administrated at effective concentrations (Table **7**).



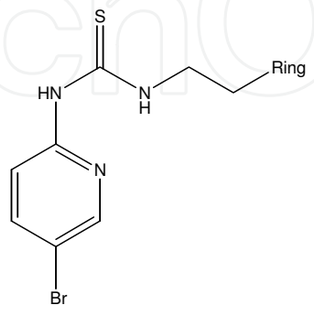
Compound	Ring	X	CC_{50} MTA (μM)	IC_{50} (μM)			
				p24	RT-MDR	A17	A17 variant
HI-346	Cyclo-hexenyl	Br	>100	0.003	0.020	N.D.	18.7
HI-445	Cyclo-hexenyl	Br	>100	0.003	0.001	0.068	30
HI-347	Cyclo-hexenyl	Cl	>100	0.079	0.038	0.300	>100
HI-504	Adamantyl*	CF_3	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]
HI-444	Myrtanyl*	Br	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]
TRV	Pyridyl	Br	>100	0.007	0.020	0.500	>100
AZT	-----	----	>100	0.004	0.2	0.006	0.004

* methylene group, instead of ethyl linker

[a] N.D.= not determined, because IC_{50} rRT (μM) > 100

Table 7. CC_{50} and IC_{50} values for series of PETT derivatives.

Uckun and collaborators (Uckun et al., 1999b) carried on performing their PETT derivatives SAR study, and decided to promote new replacements of the pyridyl ring of **TRV** with eight different heterocyclic substituents, including heterocyclic amines, heteroaromatic rings furan and thiophene, as well as aromatic acetal piperonyl. After evaluation of HIV-RT inhibitory activity, the authors concluded that these proposed modifications were critical for the biological activity of said series of compounds, as only the thiophene-substituted derivative **HI-443** inhibited recombinant RT *in vitro* in more than 90% (Table **8**).



Compound	Ring	IC_{50} rRT (μM)	IC_{90} rRT (μM)
HI-443	Thiophene	0.8	15.0
HI-230	Pyrrolidine	4.9	>100
HI-436	Imidazole	>100	>100
HI-442	Indole	0.9	>100
HI-206	1-methyl-pyrrolidine	>100	>100
HI-276	Morpholine	>100	>100
HI-257	Piperonyl*	0.7	>100
HI-503	Furan*	1.2	>100
TRV	Pyridine	0.6	12.0

* Methylene linker group, instead of an ethyl linker.

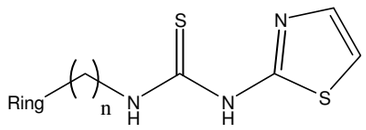
Table 8. RT inhibitory activity of PETT derivatives, expressed as IC_{50} and IC_{90} values.

After proceeding with docking studies, it was observed that the thiophene group of **HI-443** occupies the same Wing 2 region of the NNI binding pocket of RT as **TRV**, although with a smaller molecular volume. Moreover, the geometry of hydrogen bond between 2´-NH atom and the amide carbonyl or TR residue 101 deviates from the optimum geometry that authors had observed in relation to **TRV** and other PETT derivatives, such as **HI-172** (Table **6**). Said remarks could justify the lower inhibitory activity of **HI-443** against $HTLV_{IIIB}$ RT, in comparison with **TRV** (Table **9**). Surprisingly, when **HI-443** was assayed against NNI-resistant and MDR-HIV strains, it showed excellent results (Table **9**). This finding is in perfect accordance with the docking analysis, as this latter revealed that thiophene group is located very close to the Y181 residue. Therefore, in the Y181C mutant strains, the sulfur atom from its thiophene group may be more compatible with the sulfur-containing cysteine 181 residue than the pyridyl group of **TRV**.

Compound	CC_{50} MTA (μM)	IC_{50} (μM)			
		p24	RT-MDR	A17	A17 variant
HI-443	>100	0.030	0.004	0.048	3.3
HI-172	>100	<0.001	>100	>100	>100
HI-240	>100	<0.001	0.005	0.200	41
TRV	>100	0.007	0.020	0.500	>100
AZT	>100	0.004	0.2	0.006	0.004

Table 9. Anti-HIV activity of PETT derivatives.

In their next work, Venkatachalam and collaborators (Venkatachalam et al., 2001) designed, synthesized and evaluated 13 aromatic/heterocyclic thiazolyl thiourea compounds, since thiazolyl group was expected to favorably bind to Wing 1 region of the binding pocket of HIV-1 RT (Table **10**).



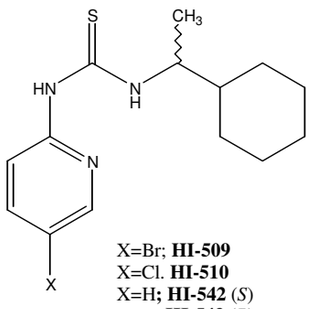
2-14

Compound	n	Ring	IC_{50} (μM)			CC_{50} (μM)	SI
			HTL_{VIIIB}	A17	A17 variant		
2	Et	2-Thiophene	<0.001	>100	N.D.	71	71,000
3	Et	2-Cyclo-hexenyl	0.007	0.9	>100	4	571
4	Et	1-phenoxy	<0.001	4.4	>100	>100	>100,000
5	Et	4-methyl-phenyl	0.07	3.9	>100	>100	1459
6	Me	1-Adamantyl	<0.001	0.6	1.3	40	40,000
7	Pr	2-Furan	<0.001	2.0	0.6	>100	>100,000
8	Et	1-Imidazole	<0.001	>100	>100	35	35,000
9	Et	3-indole	<0.001	2.2	3.7	28	28,000
10	Et	1-pyperidine	>100	N.D.	N.D.	N.D.	N.D.
11	Et	4-hydroxy-phenyl	>100	N.D.	N.D.	N.D.	N.D.
12	Et	2-pyridine	1	N.D.	N.D.	100	100
13	Pr	1-pyrrolidinone	9	N.D.	N.D.	18	2
14	2-Bu	Phenyl	0.009	2.1	1.5	10	1111
NVP	----	----	0.034	>100	>100	N.D.	N.D.
DLV	----	----	0.009	50	>100	N.D.	N.D.

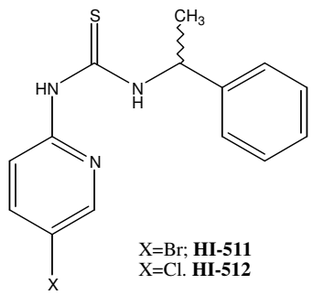
Table 10. Anti-HIV activity of thiazolyl thiourea derivatives.

Among 13 compounds, six lead ones were detected (**2**, **4** and **6-9**). They were 9-34 times more active than the standard NNRTI nevirapine and delavirdine. The compounds **2-9** and **14** were also tested against NNRTI-resistant strains A17 with Y181C mutation and A17 variant with a Y181C plus K103N mutations in RT. The most promising compounds were **6**, **7**, **9** and **14**, as they were effective against both NNRTI-resistant HIV-1 isolates and showed much greater potency against both wild-type and NNRTI-resistant HIV-1 than nevirapine and delavirdine. Among these compounds, the most promising was the **7**, due to its minimal cytotoxic effects on PBMC, as well as to its selectivity index > 100,000.

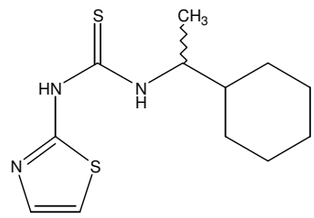
Subsequently, Venkatachalam and collaborators (Venkatachalam et al., 2000) proposed to study the influence of stereochemistry of Halopyridyl and Thiazolidyl thiourea compounds on their potency as NNRTI. For this purpose, they synthesized and measured anti-HIV activity of *R* and *S* stereoisomers of two cyclohexyl methyl haloperidyl thiourea compounds (**HI-509** and **HI-510**), of two α-methyl benzylhalopyridyl thiourea compounds (**HI-511** and **HI-512**) and of one cyclohexyl ethyl thiazolyl thiourea compound (**HI-513**) (Table **11**).



X=Br; **HI-509**
X=Cl. **HI-510**
X=H; **HI-542** (*S*)
HI-543 (*R*)



X=Br; **HI-511**
X=Cl. **HI-512**



HI-513

Compound	IC_{50} (μM)				
	rRT	**$HTLV_{IIIB}$**	**RT-MDR**	**A17**	**A17 variant**
HI-509(*R*)	1.2	0.001	0.2	0.4	10.0
HI-509(*S*)	>100	>1	N.D.	N.D.	N.D.
HI-510(*R*)	1.4	0.025	0.06	0.07	8.2
HI-510(*S*)	>100	>1	N.D.	N.D.	N.D.
HI-511(*R*)	1.6	0.01	0.005	0.01	2.7
HI-511(*S*)	>100	>1	N.D.	N.D.	N.D.
HI-512(*R*)	1.2	0.010	0.010	0.2	10.2
HI-512(*S*)	>100	>1	N.D.	N.D.	N.D.
HI-513(*R*)	13.0	0.001	5.6	0.9	5.8
HI-513 (*S*)	>100	>1	N.D.	N.D.	N.D.
HI-542 (*S*)	>100	>1	N.D.	N.D.	N.D.
HI-543 (*R*)	>100	>1	N.D.	N.D.	N.D.
TRV	0.8	0.007	0.02	0.5	>100

Table 11. Effects of stereochemistry on anti-HIV activity of thiourea compounds.

The results showed that *R* stereoisomers of all five compounds inhibited the recombinant RT *in vitro* with IC_{50} values that were 100-fold lower. Each one of these five compounds was also active against NNI-resistant HIV-1 strains. Among them, **HI-511(*R*)** (see Table **11**) presented a potent antiviral activity against NNI-resistant and multidrug resistant strains of HIV-1.

Molecular modeling studies indicated that the *R* steroisomer [**HI-509(*R*)**] would fit the target NNI binding pocket on HIV-RT much better than its enantiomer [**HI-509(*S*)**]. Due to the presence of unfavorable steric interactions with the NNI binding pocket residues near the Y181 side chain, **HI-509(*S*)** adopts an energetically unfavorable eclipsed conformation, which reflects in its higher IC_{50} value. These assumptions could also apply in favor of **HI-510(*R*)**. As a relevant data concerning this study, it is worthy to mention that the methyl group on chiral carbon of **HI-509(*R*)** and **HI-510(*R*)** probably promotes the strong binding to the NNI binding pocket via van der Waals with residue V179.

The substitution of the pyridyl ring of **HI-509(*R*)** and **HI-510(*R*)** by a thiazolyl ring (compound **HI-513(*R*)**) resulted in 10-fold higher IC_{50} values in cell free RT inhibition assays, as the unsubstituted thiazole could be better accommodated by the binding site than the unsubstituted pyridine (in combination with the bulky cyclohexylethyl group) as a whole molecule. Nevertheless, halogen substitution on pyridine adds a considerable number of favorable interactions at Wing 1 region, which improves the final interaction score for the substituted pyridine thiourea compounds.

While studying the effect of stereochemistry on anti-HIV activity of chiral thiourea compounds, Venkatachalam and collaborators (Venkatachalam et al., 2004) synthesized two new series of PETT compounds: β-methyl phenylethyl thiourea (β-MPT), and α-methyl benzyl thioureas (α-MBT) (Fig. 5).

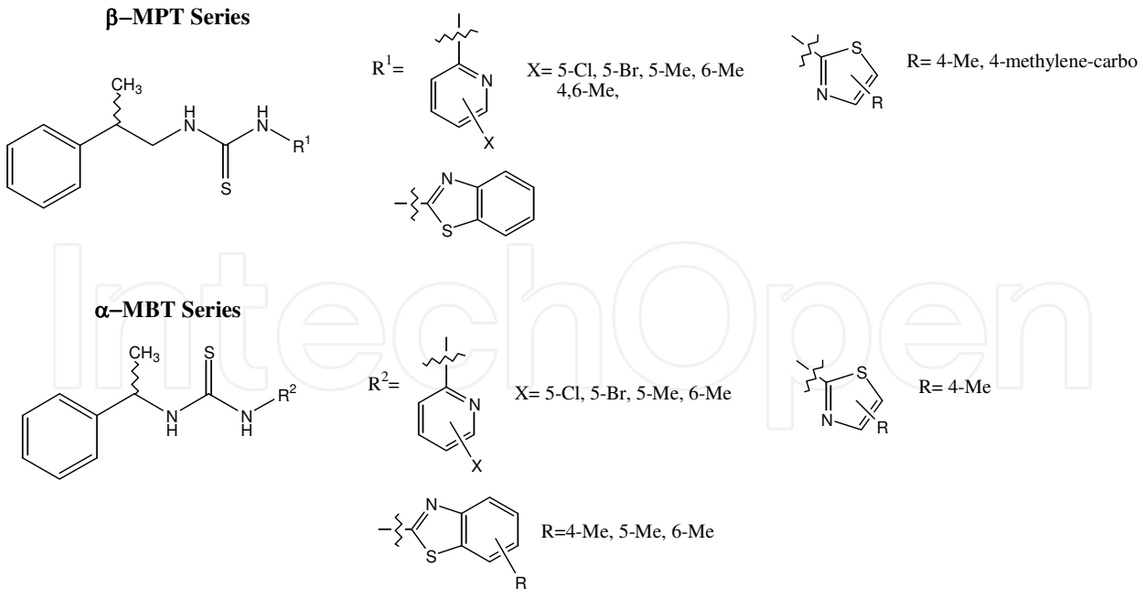


Fig. 5. β-MPT and α-MBT series.

These derivatives were evaluated against HIV-1 strain $HTLV_{IIIB}$, and also against NNRTI-resistant strains. Upon analysis of these results, the authors expressed critical positions regarding SAR of this class of compounds (Fig. 6).

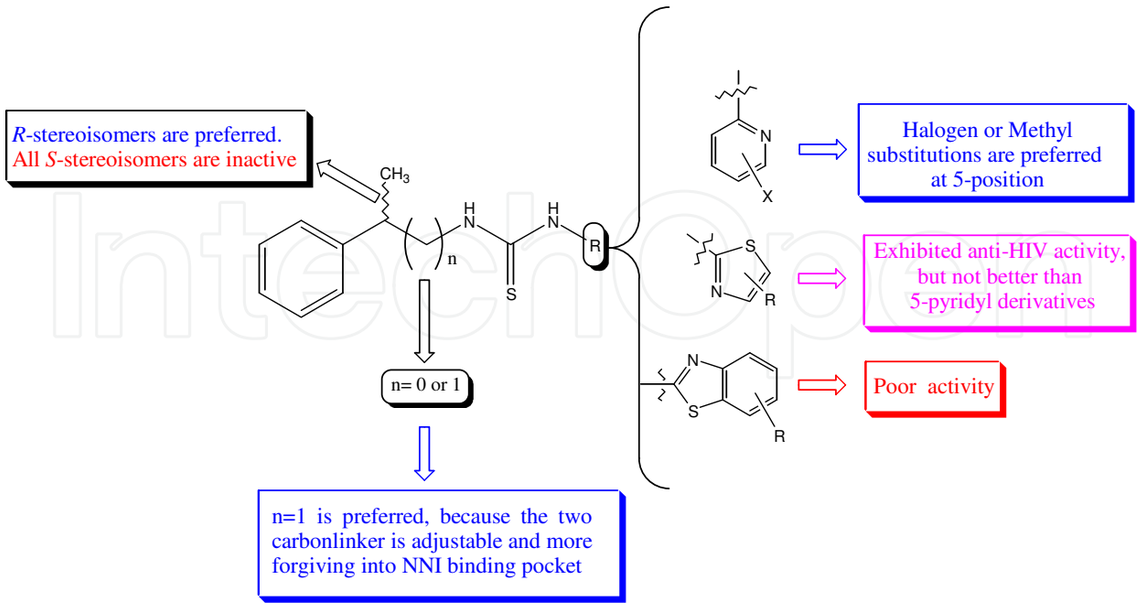
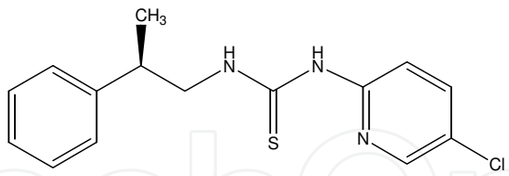


Fig. 6. Complementary SAR study of chiral thiourea compounds.

Among all synthesized compounds, the derivative **15** (Table **12**), namely the *R* isomer 5-chloropyridyl (β-MPT Series), was 380-fold more active than nevirapine, 2-fold more active than trovirdine and 190-fold more potent against A17 than delavirdine. The compound **15** was also >200-fold more potent against A17V than nevirapine, trovirdine or delavirdine. In view of these results, the authors postulated that β-MPT compounds may be useful candidates to further development as anti-HIV agents, especially due to their remarkable activity against mutant strains of HIV-1.

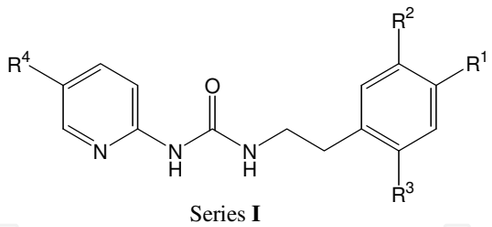


15

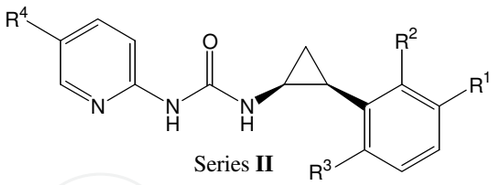
Compound	IC_{50} (μM)				
	rRT	$HTLV_{IIIB}$	RT-MDR	A17	A17 variant
15	0.1	<0.001	0.02	0.3	0.5
TRV	0.8	0.007	0.02	0.5	>100

Table 12. Anti-HIV activity of thiourea derivative 15.

Besides the modifications on the pyridyl ring, Sahlberg and collaborators (Sahlberg et al., 1998) decided to investigate the replacement of thiourea by urea moiety in PETT derivatives. Furthermore, they have prepared compounds with an ethyl linker (Series **I**) and other conformationally restricted cyclopropyl analogues (Series **II**, Table **13)**.



Series **I**
16-21



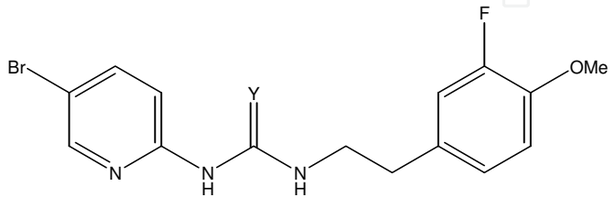
Series **II**
22-28

Compound	R^1	R^2	R^3	R^4	ED_{50} (μM)[a]			
					wt	wt[b]	T181C	L100I
16	H	F	F	Cl	0.03	0.85	>32	32
17	NMe_2	F	F	Br	0.5	N.D.	>25	>25
18	OMe	F	OMe	Cl	0.011	0.09	3	17
19	OEt	F	OMe	Cl	0.13	N.D.	>27	14
20	COMe	F	OMe	Cl	0.2	N.D.	>27	>27
21	OEt	Cl	F	Br	0.07	N.D.	8	15
22	H	F	F	Cl	0.006	0.01	N.D.	N.D.
23	OEt	Cl	F	Cl	0.016	0.06	N.D.	0.1
24	OEt	Cl	F	CN	0.01	0.02	0.53	0.27
25	OEt	F	F	Cl	0.008	0.03	0.27	0.75
26	OMe	F	OMe	Cl	0.012	0.1	2.7	1.6
27	OEt	F	Cl	Cl	0.008	N.D.	0.1	0.1
28	OEt	F	Br	Br	0.025	0.8	N.D.	N.D.
TRV	----	----	----	----	0.02	5	>5	0.8

[a] The cell culture assay used MT4 cells infected with HIV-1_{IIIB}.
[b] The assay contains 15% human AB serum.

Table 13. Inhibition of HIV-1 by urea-PETT compounds.

Cyclopropyl compounds use to be more potent than the ethyl linked ones, especially on mutants (Table **13**). The authors hypothesized that, in comparison with the most restricted cyclopropyl analogues, the most flexible ethyl derivatives might present inconvenient in adopting conformations that fit in the mutant enzymes.
When compared with thiourea analogues, urea derivatives are less active (Fig. 7), although these compounds might have better toxicological and pharmacokinetic properties. In addition, it was verified later that, in cell culture and in the presence of added human serum, urea-PETT compounds keep their antiviral activity in standards that are much higher than those shown by the respective thiourea compounds.



Y= S : IC_{50}= 1.3 nM
Y= O: IC_{50}= 50 nM
TRV: IC_{50}= 15nM

Fig. 7. Anti-HIV activities of thiourea and urea derivatives.

Following this study, Högberg and collaborators (Högberg et al., 2000) have proposed several bioisosteric substitutions of both thiourea and urea moieties of PETT compounds by sulfamide, cyanoguanidine and guanidine groups (Series **III**, Fig.8). Furthermore, they have promoted the replacement of the phenetyl group by benzophenethyl group, in an attempt to evaluate the influence of said modifications on the antiviral activities of this class of compounds (Series **IV**, Fig.8).

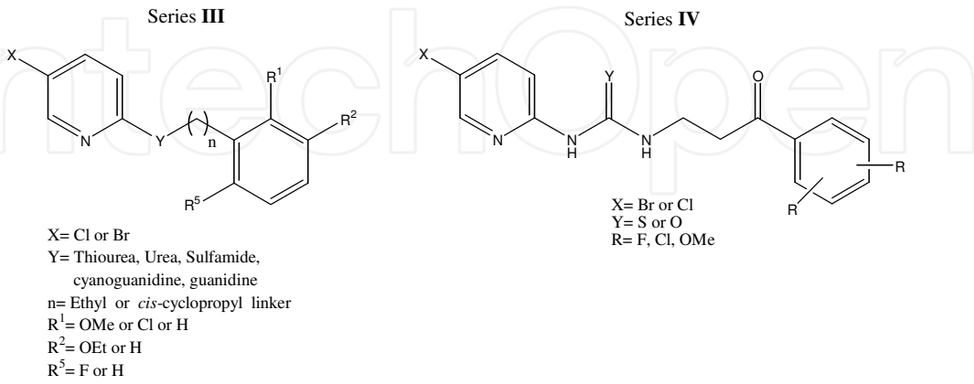
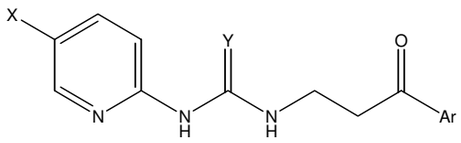


Fig. 8. Structures of two series of PETT analogues, obtained through bioisosteric modifications.

The biological results showed that thiourea and urea moieties play an essential role in the optimal activity of PETT compounds, as all the proposed bioisosteric replacements lead to a reduction (compounds with guanidine group) or to the complete loss of activity (sulfamide and cianoguanidine derivatives). Moreover, the benzoylethyl derivatives were reasonably potent inhibitors of wild-type HIV-1 RT and HIV-1 virus in cell culture. However, these derivatives were less active than the phenethyl compounds (see compound **39**, Table **14**).

Series **IV**



Compound	Ar	Y	X	IC_{50} HIV-1 RT (μM)[a]	ED_{50} (μM)[b]
29	phenyl	S	Br	<0.027	0.036
30	2-F- phenyl	S	Br	0.004	0.009
31	2-Cl- phenyl	S	Br	0.010	0.100
32	2-OMe- phenyl	S	Br	0.003	0.076
33	2-F- phenyl	O	Br	0.054	0.201
34	3-OMe- phenyl	S	Br	0.080	0.911
35	4-F- phenyl	S	Br	0.047	0.300
36	2,5-F- phenyl	S	Br	<0.025	0.067
37	2,6-F- phenyl	S	Br	0.006	0.028
38	2,6-F- phenyl	S	Cl	0.003	0.050
39	2,6-F- phenyl*	S	Br	0.001	0.010

[a] HIV-1 RT assay which used (poly)rC.(oligo)dG as the templae/primer.
[b] Anti-HIV activity assay, using MT4 cells (human T cell line) infected with $HIV\text{-}1_{IIIB}$
*Phenylethyl, instead of benzoylethyl linker.

Table 14. IC_{50} and ED_{50} values for Series **IV**.

3.1.2.3 PETT derivatives as anti-HIV microbicides

D´Cruz and collaborators started to develop a vaginal microbicidal contraceptive, which was potentially able to prevent HIV transmission. Thus, they decided to evaluate two potent anti-HIV PETT derivatives (**HI-240** and **HI-236**, Table **5**), that had been previously identified by Venkatachalam and collaborators (Vig et al, 1998; Mao et al., 1998), in an attempt to verify a possible dual-function, namely its anti-HIV and spermicidal activity. After proceeding with the analysis of the results, they observed that only the derivative **HI-240** showed a spermicidal activity, although the underlying mechanism involved in such a function remains unknown. Moreover, **HI-240** inhibited the sperm motility, in a concentration-and time-dependent way (D´Cruz & Venkatachalam et al., 1999).
Subsequently, the authors investigated the cytotoxic characteristics and selectivity of this compound, and, then, compared their results to nonoxynol-9 (**N-9**), which is the most widely used vaginal spermicide. This substance immobilizes sperm, as a result from a detergent-type action on the sperm plasma membrane. Due to its membrane-disruptive properties, a continued use of **N-9** has been shown to be likely to damage the cervicovaginal epithelium, causing an acute tissue inflammatory response, and thus enhancing the probability of HIV infection by heterosexual transmission. When compared with **N-9**, **HI-240** was selectively spermicidal, without detergent-type toxicity against membrane of the female genital tract, which may present significant clinical advantages. Another important feature of **HI-240** may still be pointed out, namely the possibility for spermicidal NNI to remain stable as protonated species, even within the acidic environment of the vagina, due to the presence of pyridine ring, containing a basic nitrogen atom. Furthermore, the vaginal concentrations of this substance required for dual-action anti-HIV and spermicidal activity are well below the systemic concentrations achieved by the oral dosages that are generally prescribed for NNIs.
In view of these promising results, D´Cruz and collaborators (D´Cruz et al., 2000) decided to evaluate other 31 PETT derivatives for anti-HIV and sperm inhibitory activity (SIA) (Fig.9).

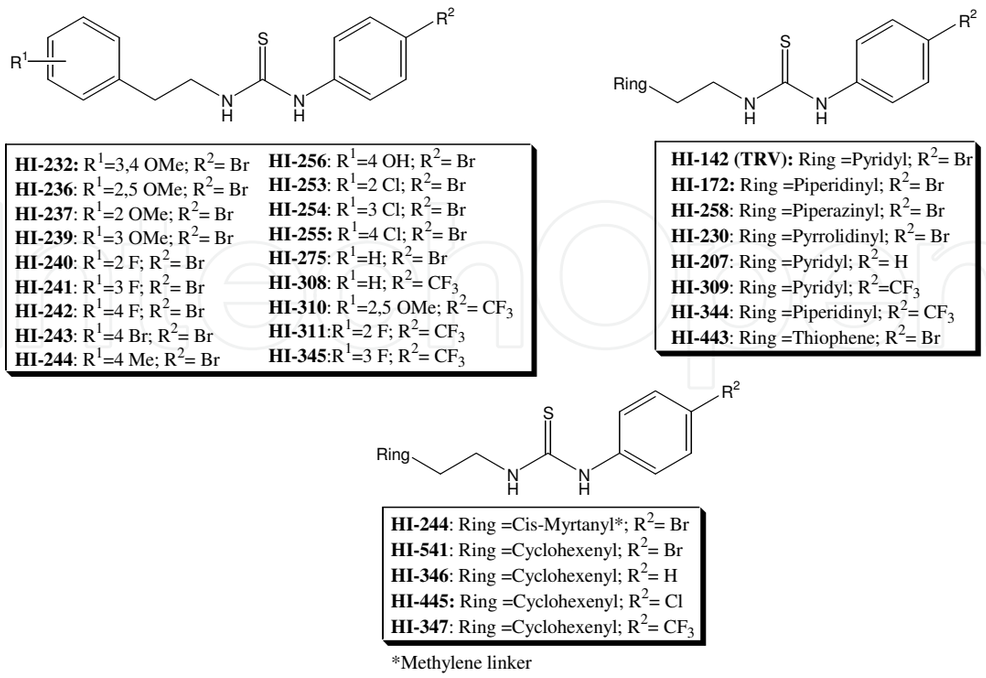


Fig. 9. Structures of thiourea derivatives, evaluated as spermicidal microbicides.

Among all these substances, they identified several dual-function of thiourea compounds, such as the phenyl ring-containing derivatives **HI-240** (2-fluoro), **HI-253** (2-chloro) and **HI-255** (4-chloro) and the cyclohexenyl ring-containing derivatives **HI-346** and **HI-445**, which were identified as potential lead compounds for the development of clinically useful dual-function anti-HIV spermicides (Table **15**). In this study, the authors also demonstrated that, at a spermicidal concentration, the dual-function thiourea derivatives were selectively spermicidal, without cytotoxic effects against human vaginal, ectocervical and endocervical epithelial cells .

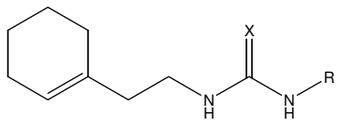
Compound	IC_{50} (μM)		EC_{50} (μM) SIA
	p24	rRT	
HI-240	<0.001	0.60	147 ± 18
HI-253	<0.001	0.70	70 ± 8
HI-255	0.001	2.50	160 ± 16
HI-346	0.003	0.6	42 ± 9
HI-445	0.003	0.5	57 ± 5
HI-142 (TRV)	0.007	0.8	>500

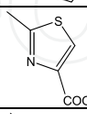
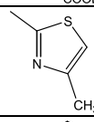
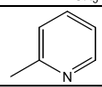
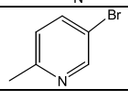
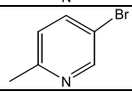
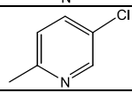
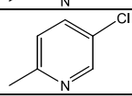
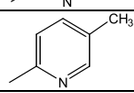
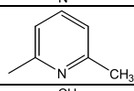
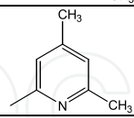
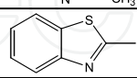
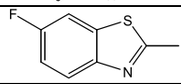
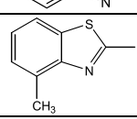
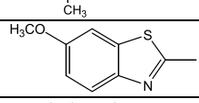
Table 15. Effect of PETT derivatives on p24 antigen production in HIV-infected PBMC, as well as enzymatic activity of HIV-1 RT and human sperm mobility.

Considering that the most promising dual-function microbicides PETTs were **HI-346** and **HI-445**, which are cyclohexenyl ring-containing derivatives, D´Cruz and collaborators decided to investigate other cyclohexenyl thiourea (CHET) compounds, in order to examine the way how heterocyclic rings and their functionalization affect the anti-HIV and SIA potency of these substances (D´Cruz et al., 2002a). Furthermore, some urea compounds were also evaluated, in an attempt to determine the role of thiourea moiety in the biological activity of these series (Table **16**).

In view of the data obtained, the authors concluded that, among the thiazolyl-ring containing CHET compounds, only the 4-methyl-substituted derivative (**42**) showed a significant anti-HIV activity. As a matter of fact, compounds containing benzothiazole-ring generally present poor anti-HIV activities. Among the compounds containing *N*-pyridyl nucleus, the functionalization at 5-position with bromine, chlorine and methyl groups led to a potent anti-HIV activity of CHET compounds. The thiourea moiety was essential to trigger said anti-HIV activity, as its replacement by urea group either abolished (**44**) or reduced (**45**) the biological activity.

Among 15 derivative compounds that were evaluated for spermicidal potential, 12 showed an improvement of spermicidal function, when analyzed at micromolar concentrations. The authors observed that compounds with methyl group at 4- or 6-postion were non-spermicidal, even at concentrations >500μM. The pyridyl-ring containing CHET compounds with bromine or chlorine attached to 5-position showed an excellent SIA activity, in addition to their remarkable anti-HIV activity. Interestingly, the urea compounds, although having retained SIA, presented a poor anti-HIV activity, . Another curious data arises from the comparison between the values of $T_{1/2}$ relating to thiourea and urea compounds, showing that urea analogues immobilized sperm 38-fold faster than thiourea derivatives.



Compound	X	R	HIV		Sperm	
			IC_{50} [rRT] (μM)	IC_{50} [p24] (μM)	EC_{50} [SIA] (μM)	$T_{1/2}$* (min)
40	S		>100	0.03	80 ± 3	1.0 ± 0.2
41	S		>100	N.D.	132 ± 22	77 ± 3
42	S		3.0	0.008	>500	>180
43	S		22.2	0.03	144 ± 18	37 ± 5
HI-346	S		0.6	0.003	45 ± 9	65 ± 6
44	O		>100	N.D.	100 ± 4	1.5 ± 0.1
HI-445	S		0.5	0.003	60 ± 5	34 ± 2
45	O		12.3	N.D.	113 ± 9	0.9 ± 0.1
46	S		1.2	0.005	96 ± 7	34 ± 2
47	S		5.6	0.017	>500	>180
48	S		>100	N.D.	45 ± 7	80 ± 2
49	S		2.0	0.02	149 ±16	70 ± 6
50	S		>100	N.D.	194 ± 20	63 ± 2
51	S		>100	N.D.	>500	>180
52	S		>100	N.D.	261 ± 12	>180

* Time required for 50% sperm mobility loss.

Table 16. Anti-HIV and sperm immobilization activity of a serie of PETT derivatives.

Furthermore, the lead compounds **HI-346** and **HI-445** were evaluated in their activity against NNI-resistant strains (RT-MDR, A17 variant), and were found to be more effective than **TRV**, delavirdine and nevirapine. These findings established the dual-function compounds **HI-346** and **HI-445** as potent NNI of drug-sensitive, as well as multidrug-resistant strains of HIV-1.

Due to the fact that the compound **HI-346** is a broad-spectrum anti-HIV agent with SIA, and also to the characteristic of its urea analogue **44** as an extremely rapid spermicidal, the authors decided to evaluate the *in vivo* fertilizing ability of sperm exposed to the lead dual-function CHET compound **HI-346,** either alone or in combination with its structural analogue (compound **44)**, in the rabbit model. They observed that the conception was completely inhibited after insemination with semen treated with compound **44,** or **HI-346** plus **44**.

As to the cytotoxic profile, CHET-NNIs showed high selectivity indexes against these genital tract epithelial cells *in vitro*. Moreover, in rabbits, the lead thiourea/urea compounds are not harmful to vaginal mucosa, in spite of their potent spermicidal properties, when added either to human or to rabbit semen.

These results indicated that the extremely rapid SIA of the urea analogue, as well as the broad-spectrum anti-HIV activity of spermicidal CHET-NNIs, together with their lack of mucosal toxicity and the remarkable ability to reduce *in vivo* fertility, appear as features that are particularly attractive to encourage the clinical development of a dual-function spermicidal microbicide (D´Cruz et al., 2002a).

The next step performed by D´Cruz and collaborators was the investigation on subchronic intravaginal toxicity of **HI-346** in mice. Thus, **HI-346** was formulated via lipophilic gel-microemulsion for intravaginal use as a potential dual-function microbicide. In order to evaluate the potential toxicity of short-term intravaginal exposure to **HI-346**, groups of 15 female B6C3F1 and CD-1 mice underwent an intravaginal exposure to a gel-microemulsion containing 0, 0.5, 1.0, or 2.0% **HI-346**, 5 days per week, for 13 consecutive weeks. Subsequently, the authors concluded that repetitive intravaginal administration of **HI-346** to yield effective spermicidal and antiviral concentrations is not expected to lead to local, systemic, or reproductive toxicity (D´Cruz et al., 2002b).

In another study, D´Cruz and collaborators decided to evaluate the PETT derivative **HI-236** as microbicide, as, in accordance with a previous work, this compound had already presented a broad-spectrum of anti-HIV activity (Mao et al., 1999). In view of the above the authors demonstrated that **HI-236** showed a high-selectivity index against both human vaginal and cervical epithelial cells, without affecting the human sperm functions. Furthermore, **HI-236** was able to prevent the HIV systemic infection via vaginal route. Therefore, **HI-236** presented a clinical utility as non-spermicidal microbicide to curb the transmission of HIV via semen: (a) in sexually active women, to allow pregnancy, while protecting both mother and her fetus or infant from HIV-1 and (b) as a prophylactic antiviral agent for HIV-1 serodiscordant couples, or for use before assisted reproductive technology procedures (D´Cruz & Uckun, 2005).

3.2 Thiourea derivatives with potential activity against TB

3.2.1 Isoxyl

The thiocarbanilide Isoxyl (**ISO**, thiocarlide, 4,4'-diisoamythio-carbanilide) (Fig.1) was synthesized in 1953 by Buu-Hoi an Xuong. In this work, the authors described the antimicobacterial activity of several thioureas, among which some are more active than **ISO;** however, this substance was chosen for subsequent assays, due to its better absorption,

and also to its good tolerance even at the highest therapeutic doses (König, 1970). Pharmacological data demonstrated that, upon this drug ingestion, the human serum presented fluctuating levels of **ISO,** that exceeded its minimum inhibitory concentration (Tousek, 1970). Besides, given its remarkable characteristics, this substance has been used at the clinical treatment of TB since the 1960's. **ISO** was employed both: in monotherapy and in combined therapy, such as: ISO-isoniazid (I), ISO-streptomycin (S), ISO-I-S and ISO-*para*-aminosalicylic acid (PAS). These studies reached good results (some of which are shown at Table **17**), especially in patients with pulmonary TB resistant to isoniazid, streptomycin and PAS, as well as in patients with hyper allergic reactions against others drugs or hepatitis as a result from I (Tousek, 1970; Urbancik, 1970). However, when compared with other combinatory regimes, such as I-PAS and S-PAS, the results achieved with **ISO** appeared to be quite mediocre, thus culminating with the introduction of more powerful antituberculosis drugs, such as ethambutol, and with the consequent discontinuation of **ISO** administration. Nevertheless, in view of the worldwide dissemination of MDR-TB and XDR-TB (World Health Organization, 2010a), a re-evaluation of drugs which were formerly deemed to be effective against TB is a promising strategy for the development of new treatments, so that, in this context, **ISO** may be considered as a strong candidate.

Combination	Months of therapy	Negative on culture (%)[a]
ISO (6 g)	1.5 – 4.5	47
ISO – I (6 g/600 mg)	4	89
ISO – I (6 g/10 mg/Kg)	6	75
ISO – I (6 g/5 mg/Kg)	6	63
ISO – S – I (4 g/1 g/200 mg)	6	83
ISO – PAS (6 g/12 g)	6	50

[a]percentage of patients who had presented negative cultures.

Table 17. Examples of results achieved in clinical trials with ISO (Tousek, 1970)

In a recent report, Phetsuksiri and collaborators (Phetsuksiri et al., 1999) evaluated the minimal inhibitory concentration (MIC) of **ISO** against various clinical isolates of susceptible and MDR-TB strains (Table **18**). The growing of all tested strains was inhibited at low concentrations, with a MIC ranging between 1-10µg/mL, which are smaller than the maximum serum levels observed in humans for **ISO** (10-13.2µg/mL) (König, 1970). The authors also verified the effect of **ISO** on viable *M. tuberculosis in vitro* bone marrow macrophage assay, where this substance showed an ability to inhibit bacterial growth inside the macrophage, as well as a bactericidal activity, by reducing the initial inoculum of virulent *M. tuberculosis*. Another interesting result from this assay disclosed that **ISO** showed no acute level of toxicity for mouse macrophages.

Together with the early studies performed with **ISO** in the 1960s, the recent results corroborate the capacity of this substance to be used as an anti-TB drug, particularly in the development of new regimes to MDR and XDR-TB treatment. Such a characteristic is a direct result from **ISO** mechanism of action, which considerably differs from those presented by other known anti-TB drugs.

Strain Designation	Drug Resistance	MIC (μg/mL)
CSU 15	I	5.0
CSU 21	I, R, E, S, Rfb	5.0
CSU 22	I, R, E, S, Kan, Cap, AMK	10.0
CSU 31	I, R, E	10.0
CSU 32	I, R, E, Cyc, ETH, Z	10.0
CSU 37	----------	5.0
CSU 39	I, R, S, Kan, AMK, ETH	10.0
CSU 44	I, R, E, S, Kan, Z	10.0
W 670	I, R, E, S, Kan	5.0
W 3432	I, R, E, S, Kan, AMK, ETH, Cip	5.0
BB	----------	1.0
LL	I, R, S, Kan, AMK, Z	1.0

kanamycin (Kan), cycloserine (Cyc), rifabutin (Rfb), ethionamide (ETH), amikacin (AMK), capreomycin (Cap).

Table 18. Minimal inhibitory concentration of **ISO** against susceptible and MDR-TB strains.

3.2.2 Mechanism of action

Similarly to what happens with Isoniazid, a substance used in the first line treatment of TB, and with ethionamide, a second line drug, **ISO** strongly inhibits the synthesis of mycolic acids. However, **ISO** can also inhibit shorter chain fatty acid synthesis, thus producing an effect that had never been observed in other drugs showing antimycobacterial properties (Phetsuksiri et al., 2003).

ISO interferes in the fatty acid metabolism, through inhibition of the Δ9 desaturase DesA3. This mechanism leads to the inhibition of the of oleic acid synthesis, which is the most abundant unsaturated fatty acid in *Mycobacterium* spp. and a constituent of mycobacterial membrane phospholipids. Due to the vital functions of oleic acid, the inhibition of its synthesis leads to cell death. Another membrane phospholipid constituent which is indirectly affected during this pathway is the tuberculostearic acid, given that this substance is synthesized through direct methylation of oleic acid by S-adenosylmethionine.

ISO inhibitory mechanism in mycolic acids synthesis has not been described. Nevertheless, it was already demonstrated that there is no relationship between oleic acid and mycolic acids synthesis inhibition (Phetsuksiri et al., 2003).

Another relevant aspect involved in the **ISO** mechanism of action concerns the fact that its activation by the flavin-containing monooxygenase EthA is mandatory to trigger an activity against *M. tuberculosis* (Korduláková, 2007). Based on the LC/MS analyses of the compounds formed after **ISO** treatment with the partially purified recombinant EthA (compounds **53**, **55** and **57**, Fig. 10), the following activation pathway was proposed: Initially, oxidation reactions with sulfur atom lead to the formation of intermediary **54**, which undergoes an elimination reaction, yielding the formimidamide **55**. Further reactions lead to the formation of carbodiimide **56**, which can be hydrolyzed, yielding the urea derivative **57** (Fig. 10). The data obtained from previous studies with ETH and thiacetazone could suggest that **ISO** would be a prodrug, activated by oxidations reactions catalyzed by EthA, and that carbodiimide **56** would appear as its active form. However, the real function of this process and its role in the inhibition of oleic acid and/or mycolic acids synthesis are not well understood. Besides, it is worthy to consider that EthA could only serve to retain **ISO** or its metabolites inside the bacterial cell.

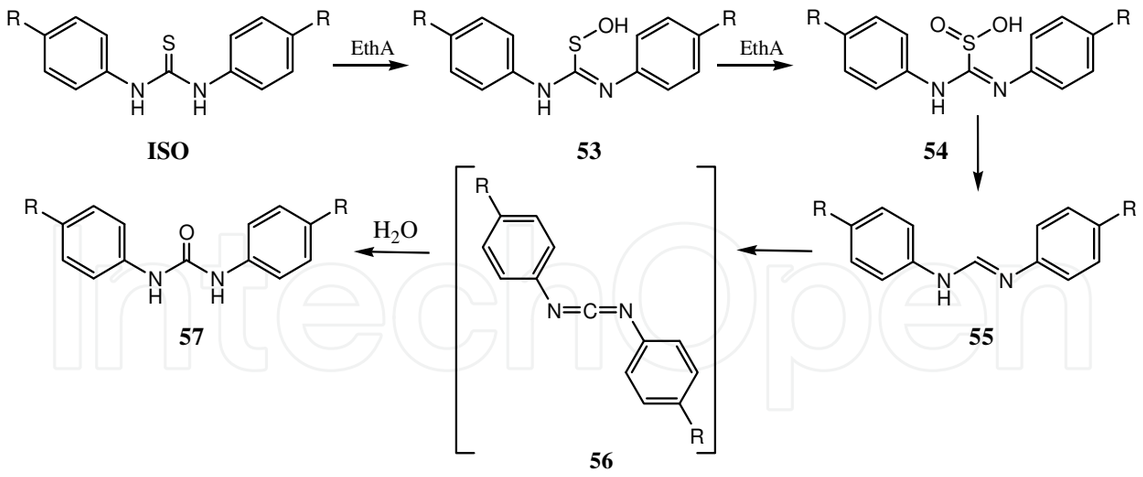


Fig. 10. Proposed pathway for **ISO** activation by EthA.

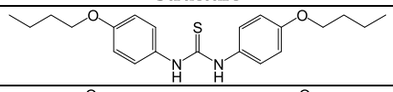
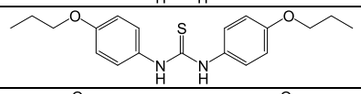
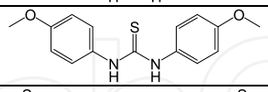
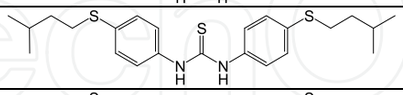
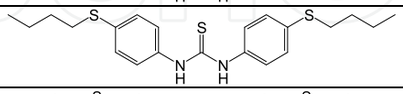
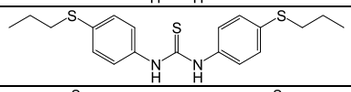
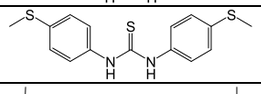
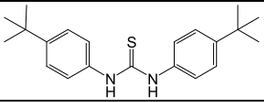
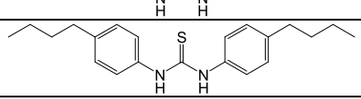
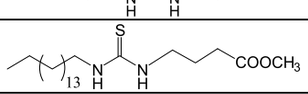
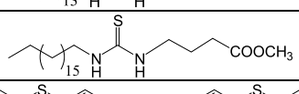
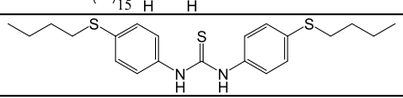
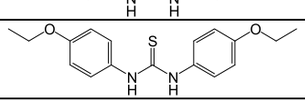
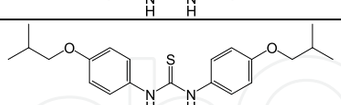
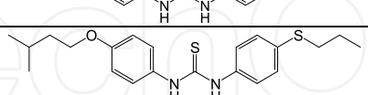
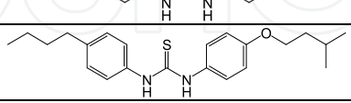
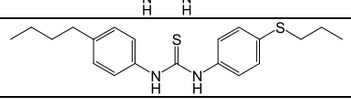
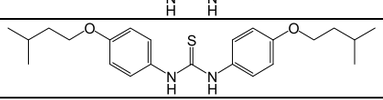
3.2.3 ISO analogues

In spite of the interesting results shown by the prior clinical trials with **ISO** , this substance was found to present some pharmacokinetic disadvantages, which limited its clinical use as an antimycobacterial agent (Wang & Hickey, 2010). In view of the above, and in an attempt to overcome this kind of inconvenient, several **ISO** derivatives have been synthesized throughout the last years, as may be illustrated by the following examples.

Phetsuksiri and collaborators described the antimycobacterial evaluation of series of **ISO** analogues (Phetsuksiri et al., 1999). These compounds were prepared through random substitutions in the side chains attached to the thiourea nucleus, which it is required for animycobacterial activity (Phetsuksiri et al., 2003; Kordulákova, 2007). This strategy led to the formation of a pool of new **ISO** derivatives, which present variations both in the symmetric and asymmetric side chains with alkyl, alkoxy or sulfur fuctional groups substituted in *para* and *para´* positions (Table **19**).

Said results show that the most part of derivatives presented a similar or a better activity (MIC <0.1 to 2.5μg/mL), when compared to **ISO** (MIC= 2.0μg/mL). Among some relevant aspects concerning SAR, it may be noted that the replacement of oxygen (**58-60**) by sulfur (**62-64**) in the side chain provides a considerable improvement in the antimycobacterial activity. Moreover, the introduction of a long alkyl chain plus an ester group leads to the formation of inactive compounds (**67**,**68**). Bulky groups, such as *t*-butyl, attached to *para* and *para´* position of phenyl ring, also gave rise to an inactive derivative (**65**). These results suggest that chemical modifications on thiourea nucleus basis could lead to the constitution of an inhibitor, that would be even more powerful against *M. tuberculosis*.

In another study, Bhowruth and collaborators (Bhowruth et al., 2006) synthesized and evaluated series of symmetrical and unsymmetrical **ISO** analogues against *M. tuberculosis* (Table **20**). Several compounds disclosed by this study present similar or better activities than **ISO** (MIC <0.1 to 1.56μg/mL). It is worthy to mention that the introduction of an aliphatic C_4 chain to either or both R^1 and R^2-positions increased the potency of the inhibitor (**76-79**, **81**). Among them, the derivatives **78** and **79** were the most active, with a significant 10-fold increase in potency against *M. tuberculosis,* when compared to **ISO**.

Compound	Structure	MIC_{99} (μg/mL)*
58		0.1-0.5
59		0.1-0.5
60		1.0
61		2.5
62		<0.1
63		<0.1
64		0.5
65		>20.0
66		0.1-0.5
67		>20.0
68		>20.0
69		N.D.
70		1.0
71		0.5
72		0.1
73		0.5
74		0.5
ISO		2.0

* MIC_{99} is defined as the lowest concentration of compound that reduced 99% of the number of *M. tuberculosis* colonies on the plates, in comparison with those at the control plate free of compound.

Table 19. **ISO** analogues prepared by Phetsuksiri and collaborators, as well as their respective MIC values.

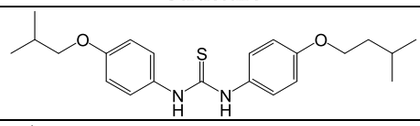
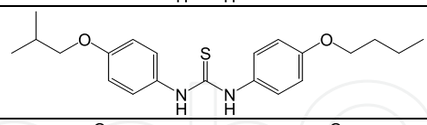
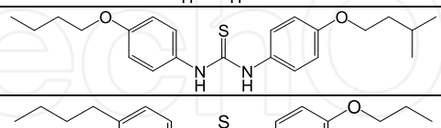
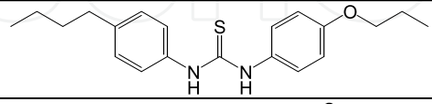
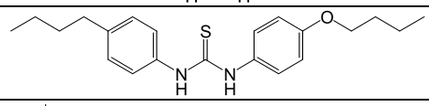
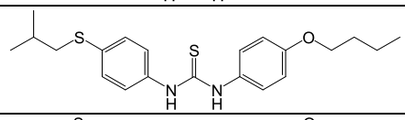
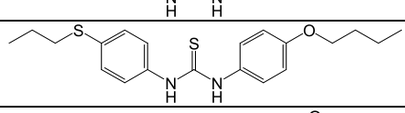
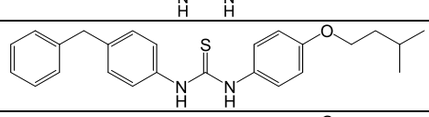
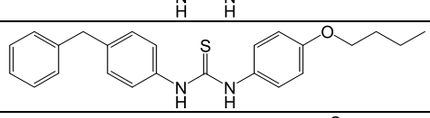
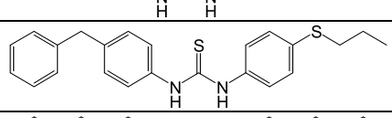
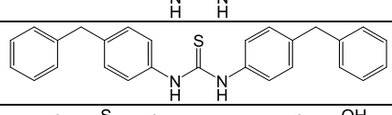
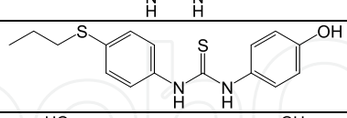
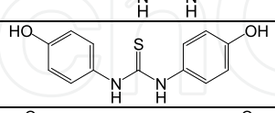
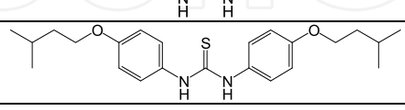
Compound	Structure	MIC_{99} (μg/mL)*
75		0.9
76		0.6
77		0.4
78		<0.1
79		0.7
80		>6.25
81		0.2
82		0.2
83		0.39
84		1.56
85		3.13
86		0.39
87		5.0
ISO		2.0

Table 20. Structures and antimycobacterial activity of ISO derivatives.

Liav and collaborators investigated the effect of replacement of one of the isoamyloxyphenyl **ISO** moieties by a carbohydrate (Liav et al., 2008a). Said modification aimed at the production of **ISO** analogues with better hydrophilic properties, which could be useful to mitigate the inconvenient represented by the poor bioavailability of this drug (Fig.11). Among these compounds, only the arabinfuranosyl derivative **90** has a MIC value of 2.5μg/mL, which is almost more potent than **ISO**.

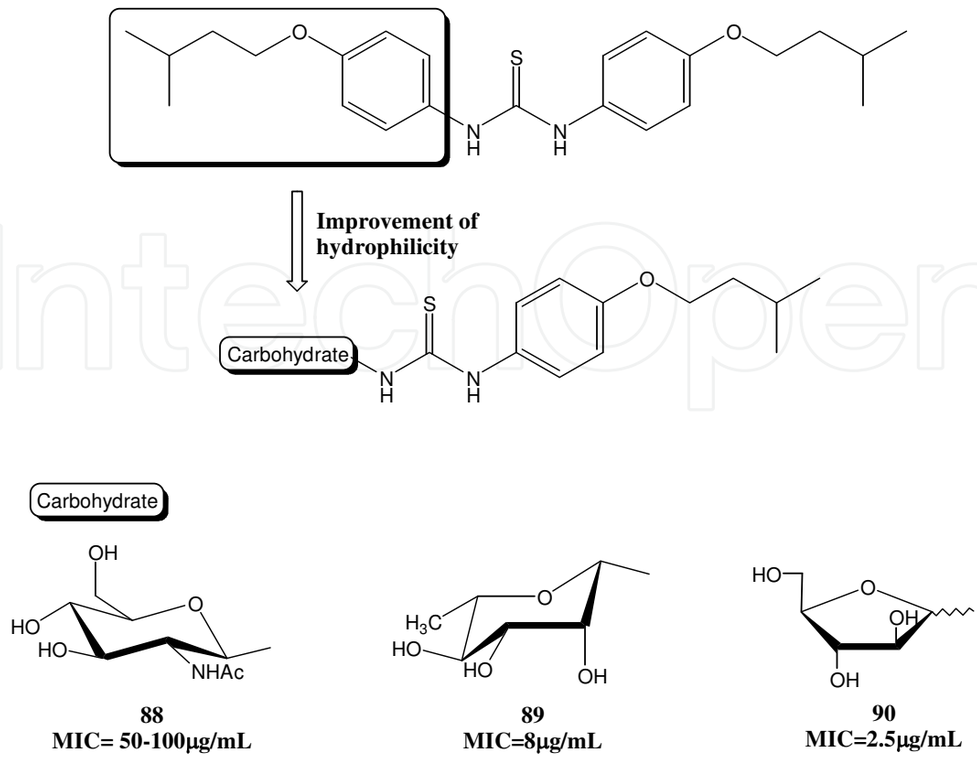


Fig. 11. **ISO** derivatives containing carbohydrates moieties.

This promising result encouraged Liav and collaborators (Liav et al., 2008b) to synthesize and evaluate the D-aldopentofuranosyl derivatives (**90-93**, Fig.12). Arabino analogue (**90**) was re-tested, and found to be more potent than **ISO**, while the ribo analogue (**91**) presented a MIC value in the same range of **ISO**. The D-xylo analogue (**92**) was rather less active, and the D-lyxo product (**93**) only acted at a concentration of 50μg/mL. These results could suggest that the products are very sensitive to stereochemical configuration, thus indicating that the carbohydrate group does not only add the required hydrophilicity to the molecule, being also responsible for adding specificity to it.

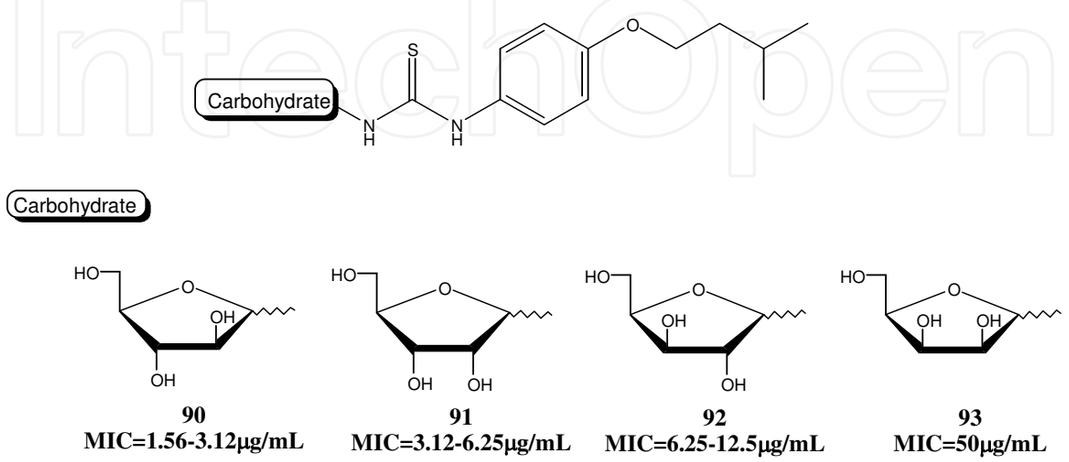


Fig. 12. Other **ISO** derivatives containing carbohydrates moieties.

4. Conclusion

Thiourea is a very important functional group for anti-HIV and anti-TB drug discovery, and, as seen above, literature has already described the promising biological activities of several derivatives. In the context of AIDS drug discovery, this scaffold is found in two remarkably important classes of compounds, namely among PETT and TIBO derivatives. The PETT compound **TRV** has been exploited as an excellent lead compound, whose several derivatives have been described. TIBO derivatives were essential for the development of NNRTI, a class of antiretroviral agents that currently present a relevant role in AIDS treatment. In parallel, as refers to TB drug discovery, **ISO** was found to present promising activities against resistant strains, especially due to its unique mechanism of action. In view of said data, the development of substances containing thiourea moiety and showing a potential activity against both diseases could be considered an important research field, which should be particularly orientated towards the improvement of therapeutic options for HIV-TB co-infected patients.

5. References

Abdool, K. S. S., Naidoo, K., Grobler, A. et al. (2010). Timing of initiation of antiretroviral drugs during tuberculosis therapy. *The New England Journal of Medicine*, vol. 362, no. 8, pp. 697–706, ISSN 0028-4793.

Ahgren, C., Backro, K., Bell, F.W. et al. (1995). The PETT Series, a New Class of Potent Nonnunleoside Inhibitors of Human Immunodeficiency Virus Type 1 Reverse Transcriptase. *Antimicrobial Agents and Chemotherapy*, vol. 39, No.6, pp.1329-1335, ISSN 0066-4804.

Antonelli, L.R.V., Mahnke, Y., Hodge, J.N. et al. (2010). Elevated frequencies of highly activated CD4+ T cells in HIV+ patients developing immune reconstitution inflammatory syndrome. *Blood*, vol. 116, No.19, pp. 3818-3827,ISSN 0006-4971.

Bell, F.W., Cantrell, A.S., Högberg, M. et al. (1995). Phenethylthiazolethiourea (PETT) Compounds, a New Class of HIV-1 Reverse Transcriptase Inhibitors. 1. Synthesis and Basic Structure-Activity Relationship Studies of PETT Analogs. *Journal of Medicinal Chemistry*, vol. 38, pp.4929-4936, ISSN 0022-2623.

Bhowruth, V., Brown, A.K., Reynolds, R.C. et al. (2006). Symmetrical and unsymmetrical analogues of isoxyl; active agents against Mycobacterium tuberculosis. *Bioorganic & Medicinal Chemistry Letters*, vol.16, No.18, pp. 4743–4747, ISSN 0960-894X.

Blanc, F. X., Sok, T. & Laureillard, D. Significant enhancement in survival with early (2 weeks) vs. late (8 weeks) initiation of highly active antiretroviral treatment (HAART) in severely immunosuppressed HIV-infected adults with newly diagnosed tuberculosis, *Proceedings of the 18th International AIDS Society Conference*, Vienna, Austria, July, 2010.

Breslin, H.J., Kukla, M.J., Ludovici, D.W. et al. (1995). Synthesis and Anti-HIV-1 Activity of 4,5,6,7-Tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepine-2(1*H*)-one (TIBO) Derivatives 3. *Journal of Medicinal Chemistry*, vol. 38, pp.771-793, ISSN 0022-2623.

Burman, W.J. & Jones, B.E. (2001). Treatment of HIV-related tuberculosis in the era of effective antiretroviral therapy. *American Journal of Respiratory and Critical Care Medicine*, vol.164, No.1, (January 2001), pp. 7–12, ISSN 1073-449X.

Buu-Hoi, N.P., Xuong, N.D. (1953). The Thiourea Type of Tuberculostatic Compounds and Their Mechanism of Action. *Comptes Rendus Chimie*, vol. 237, pp.498-500, ISSN 1631-0748.

Centers for Disease Control and Prevention. (2007). Managing Drug Interactions in the Treatment of HIV-Related Tuberculosis, In: *Centers for Disease Control and Prevention*, 29.01.2011, Available from: <http://www.cdc.gov/tb/TB_HIV_Drugs/default.htm>

Chaisson, R.E., Schechter, G.F., Theuer, C.P. et al. (1987). Tuberculosis in patients with the acquired immunodeficiency syndrome: clinical features, response to therapy, and survival. *American Review of Respiratory Disease*, vol.136, pp.570–574, ISSN 0003-0805.

D'Cruz, O.J. & Uckun. F.M. (1999). Novel derivatives of phenethyl-5-bromopyridylthiourea and dihydroalkoxybenzyloxopyrimidine are dual-function spermicides with potent anti-human immunodeficiency virus activity. *Biology of reproduction*, vol.60, No.6, pp. 1419–1428, ISSN 0006-3363.

D'Cruz, O.J., Venkatachalam, T. K. & Uckun. F.M. (2000). Novel thiourea compounds as dual-function microbicides. *Biology of reproduction*, vol.63, No.1, pp. 196–205, ISSN 0006-3363.

D'Cruz, O.J., Venkatachalam, T. K. Mao, C. et al. (2002a). Structural requirements for potent anti-human immunodeficiency virus (HIV) and sperm-immobilizing activities of cyclohexenyl thiourea and urea non-nucleoside inhibitors of HIV-1 reverse transcriptase. *Biology of reproduction*, vol.67, No.6, pp. 1959–1974, ISSN 0006-3363.

D'Cruz, O.J., Waurzyniak, B. & Uckun. F.M. (2002b). A 13-week subchronic intravaginal toxicity study of the novel broad-spectrum anti-HIV and spermicidal agent, *N*-[2-(1-cyclohexenyl)ethyl]-*N* -[2-(5-bromopyridyl)]-thiourea (PHI-346) in Mice. *Toxicologic Pathology*, vol.30, No.6, pp. 687–695, ISSN 0192-6233.

D'Cruz, O.J. & Uckun. F.M. (2005). Discovery of 2,5-dimethoxy-substituted 5-bromopyridyl thiourea (PHI-236) as a potent broad-spectrum anti-human immunodeficiency virus microbicide. *Molecular Human Reproduction*, Vol.11, No.10, pp. 767–777, ISSN 1360-9947.

De Clercq, E. (2004). Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs): Past, Present, and Future. *Chemistry & Biodiversity*, vol. 1, pp.44-64, ISSN 1612-1872.

Fitzgerald, D.W., Desvarieux, M., Severe, P. et al. (2000). Effect of post-treatment isoniazid on prevention of recurrent tuberculosis in HIV-1-infected individuals: a randomized trial. *The Lancet*, vol.356, No.9240, (October 2000), pp.1470–1474, ISSN 0140-6736.

Ho, W., Kukla, M.J., Breslin, H.J. et al. (1995). Synthesis and Anti-HIV-1 Activity of 4,5,6,7-Tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepine-2(1*H*)-one (TIBO) Derivatives 4. *Journal of Medicinal Chemistry*, vol. 38, pp.794-802, ISSN 0022-2623.

Högberg, M., Engelhardt, P., Vrang, L. et al. (2000). Bioisosteric modification of PETT-HIV-1 RT-inhibitors: synthesis and biological evaluation.*Bioorganic & Medicinal Chemistry Letters*, vol.10, No.3, pp. 265-268, ISSN 0960-894X.

Khan, F.A., Minion, J., Pai, M. et al. (2010).Treatment of active tuberculosis in HIV-coinfected patients: a systematic review and meta-analysis. *Clinical Infectious Diseases*, vol. 50, No.9, pp.1288–1299, ISSN 1058-4838

Kim, R.B., Fromm, M.F., Wandel, C. et al. (1998). The drug transporter Pglycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *Journal of Clinical Investigation*, vol.101, No.2, pp.289–294, ISSN 0021-9738.

König, A. (1970). Discussion on Isoxyl. *Antibiotica et Chemotherapia*, vol. 16, pp.187-202, ISSN 0376-0227.

Korduláková, J., Janin, Y.L., Liav, A. et al. (2007). Isoxyl Activation is Required for Bacteriostatic Activity against *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy*, vol. 51, No.11, pp.3824-3829, ISSN 0066-4804.

Kukla, M.J., Breslin, H.J., Pauwels, R. et al. (1991a). Synthesis and Anti-HIV-1 Activity of 4,5,6,7-Tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepine-2(1*H*)-one (TIBO) Derivatives. *Journal of Medicinal Chemistry*, vol. 34, pp.746-751, ISSN 0022-2623.

Kukla, M.J., Breslin, H.J., Diamond, C.J. et al. (1991b). Synthesis and Anti-HIV-1 Activity of 4,5,6,7-Tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepine-2(1*H*)-one (TIBO) Derivatives 2. *Journal of Medicinal Chemistry*, vol. 34, pp.3187-3197, ISSN 0022-2623.

Liav, A., Angala, S.K. & Brennan, P.J. (2008a). N-Glycosyl-N0-[p-(isoamyloxy)phenyl]-thiourea Derivatives: Potential Anti-TB Therapeutic Agents. *Synthetic Communications*, vol.38, No.8, pp. 1176–1183, ISSN 0039-7911.

Liav, A., Angala, S.K., Brennan, P.J. et al. (2008b). N-D-Aldopentofuranosyl-N´-[*p*-(isoamyloxy)phenyl]-thiourea derivatives: Potential anti-TB therapeutic agents. *Bioorganic & Medicinal Chemistry Letters*, vol.18, No.8, pp. 2649–2651, ISSN 0960-894X.

Mao, C., Vig, R., Venkatachalam, T. K. et al. (1998). Structure-based design of N-[2-(1-piperidinylethyl)]-N´-[2-(5-bromopyridyl)]-thiourea and N-[2-(1-piperazinylethyl)]-N´-[2-(5-bromopyridyl)]-thiourea a potent non-nucleoside inhibitors of HIV-1 reverse transcriptase. *Bioorganic & Medicinal Chemistry Letters*, vol.8, No.16, pp. 2213-2218, ISSN 0960-894X.

Mao, C., Sudbeck, E.A., Venkatachalam, T. K. et al. (1999). Rational design of N-[2-(2,5-dimethoxyphenylethyl)]-N´-[2-(5-bromopyridyl)]-thiourea (HI-236) as a potent non-nucleoside inhibitor of drug-resistant human immunodeficiency virus. *Bioorganic & Medicinal Chemistry Letters,* vol.9, No.11, pp. 1593-1598, ISSN 0960-894X.

Pauwels, R., Andries, K., Desmyter, J. et al. (1990). Potent and Selective Inhibition of HIV-1 replication *in vitro* by a Novel Series of TIBO Derivatives. *Nature*, vol. 343, pp.470-474, ISSN 0028-0836.

Perriens, J.H., St Louis, M,E., Mukadi, Y.B. et al. (1995). Pulmonary tuberculosis in HIV-infected patients in Zaire: a controlled trial of treatment for either 6 or 12 months. *The New England Journal of Medicine*, vol.332, No.12, (March 1995), pp.779–784, ISSN 0028-4793.

Phetsuksiri, B., Baulard, A.R., Cooper, A.M. et al. (1999). Antimycobacterial activities of isoxyl and new derivatives through the inhibition of mycolic acid synthesis. *Antimicrobial Agents and Chemotherapy*, vol.43, No.5, (May 1999), p. 1042–1051, ISSN 0066-4804.

Phetsuksiri, B., Jackson, M., Sherman, H. et al. (2003). Unique Mechanism of Action of the Thiourea Drug Isoxyl on *Mycobacterium tuberculosis*. *The Journal of Biological Chemistry*, vol. 278, No.52, pp.53123-53130, ISSN 0021-9258.

Pialoux, G., Youle, M., Dupont, B. et al. (1991). Pharmacokinetics of R 82913 in Patients with AIDS or AIDS-related Complex. *The Lancet*, vol. 338, pp.140-143, ISSN 0140-6736.

Piggott, D.A. & Karakousis, P.C. (2011). Timing of Antiretroviral Therapy for HIV in the Setting of TB Treatment. *Clinical and Developmental Immunology*, Vol.2011, pp.1-10, ISSN 1740-2522.

Sahlberg, C., Norren, R., Engelhardt, P. et al. (1998). Synthesis and anti-HIV activities of urea-PETT analogs belonging to a new class of potent nonnucleoside HIV-1 reverse transcriptase inhibitors. *Bioorganic & Medicinal Chemistry Letters*, vol.8, No.12, pp. 1511-1516, ISSN 0960-894X.

Schuetz, E.G., Schinke, A. H., Relling, M.V.,et al. (1996). P-glycoprotein: a major determinant of rifampicin–inducible expression of cytochrome P4503A in mice and humans. *Proceedings of the National Academy of Sciences*, vol.93, No.9, pp. 4001–4005, ISSN 0027-8424.

Sterling, T.R., Pham, P.A. & Chaisson, R.E. (2010). HIV Infection–Related Tuberculosis: Clinical Manifestations and Treatment. *Clinical Infectious Diseases*, vol.50, No.S3, pp.S223–S230, ISSN 1058-4838.

Sudbeck, E.A., Mao, C., Vig, R. et al. (1998). Structure-based design of novel dihydroalkoxybenzyloxopyrimidine derivatives as potent nonnucleoside inhibitors of the human immunodeficiency virus reverse transcriptase. *Antimicrobial Agents and Chemotherapy*, Vol. 42, No. 12, (December 1998), pp. 3225–3233, ISSN 0066-4804.

Tousek, J. (1970). On the Clinical Effectiveness of Isoxyl. *Antibiotica et Chemotherapia*, vol. 16, pp.149-155, ISSN 0376-0227.

Uckun, F.M., Mao, C., Pendergrass, S. et al. (1999a). N-[2-(1-cyclohexenyl)ethyl)]-N´-[2-(5-bromopyridyl)]-thiourea and N`-[2-(1-cyclohexenyl)ethyl)]-N´-[2-(5-chloropyridyl)]-thiourea as potent inhibitors of multidrug-resistant human immunodeficiency virus-1. *Bioorganic & Medicinal Chemistry Letters*, vol.9, No.18, pp. 2721-2726, ISSN 0960-894X.

Uckun, F.M., Pendergrass, S., Maher, D. et al. (1999b). N´-[2-(2-thiophene)ethyl)]-N´-[2-(5-bromopyridyl)]-thiourea a potent inhibitor of NNI-resistant and multidrug-resistant human immunodeficiency virus-1.*Bioorganic & Medicinal Chemistry Letters*, vol.9, No.24, pp. 3411-3416, ISSN 0960-894X.

United Nations Programme on HIV/AIDS. (2010). *Global report: UNAIDS report on the global AIDS epidemic 2010*, WHO press, ISBN 978-92-9173-871-7, Geneva, Switzerland.

Urbancik, B. (1970). Clinical Experience with Thiocarlide (Isoxyl). *Antibiotica et Chemotherapia*, vol. 16, pp.117-123, ISSN 0376-0227.

Velasco, M., Castilla, V., Sanz, J. et al. (2009). Effect of simultaneous use of highly active antiretroviral therapy on survival of HIV patients with tuberculosis. *Journal of Acquired Immune Deficiency Syndromes*, vol. 50, no. 2, pp. 148–152, ISSN 1525-4135.

Venkatachalam, T. K., Sudbeck, E.A., Mao, C. et al. (2000). Stereochemistry of halopyridyl and thiazolyl thiourea compounds is a major determinant of their potency as nonnucleoside inhibitors of HIV-1 reverse transcriptase. *Bioorganic & Medicinal Chemistry Letters*, vol.10, No.18, pp. 2071-2074, ISSN 0960-894X.

Venkatachalam, T. K., Sudbeck, E.A., Mao, C. et al. (2001). Anti-HIV activity of aromatic and heterocyclic thiazolyl thiourea compounds. *Bioorganic & Medicinal Chemistry Letters*, vol.11, No.4, pp. 523-528, ISSN 0960-894X.

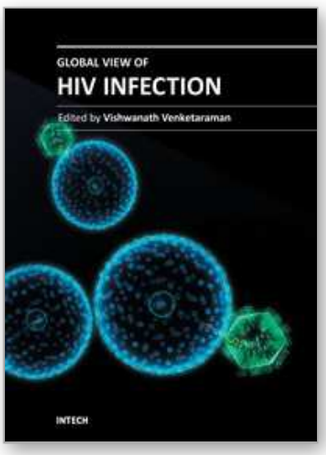
Venkatachalam, Mao, C., Uckun, F.M. et al. (2004).Effect of stereochemistry on the anti-HIV activity of chiral thiourea compounds. *Bioorganic & Medicinal Chemistry,* vol.12, No.12, pp.4275–4284, ISSN 0968-0896.

Vig, R., Mao, C., Venkatachalam, T. K. et al. (1998). Rational design and synthesis of phenethyl-5-bromopyridyl thiourea derivatives as potent non-nucleoside inhibitors of HIV reverse transcriptase. *Bioorganic & Medicinal Chemistry*, vol.6, No.10, pp.1789-1797, ISSN 0968-0896.

Wang, C. & Hickey, A.J. (2010). Isoxyl particles for pulmonary delivery: In vitro cytotoxicity and potency. *International Journal of Pharmaceutics*, vol.396, No.1-2, pp. 99–104, ISSN 0378-5173.

World Health Organization. (2010a). *Global tuberculosis control: WHO Report 2010*, WHO press, ISBN 978 92 4 156406 9, Geneva, Switzerland.

World Health Organization. (2010b). *Treatment of tuberculosis: guidelines* (4th ed.), WHO Press, ISBN 978 92 4 154783 3, Geneva, Switzerland.



Global View of HIV Infection
Edited by Dr. Vishwanath Venketaraman

ISBN 978-953-307-671-3
Hard cover, 176 pages
Publisher InTech
Published online 26, October, 2011
Published in print edition October, 2011

Some of the topics covered in this book are: HIV infection HIV transmission Clinical symptoms of AIDS AIDS and opportunistic infection Prevention and treatment of HV Treatment of HIV infection and immune reconstitution

How to reference
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Marcus Vinicius Nora de Souza, Marcelle de Lima Ferreira Bispo, Raoni Schroeder Borges Gonçalves and Carlos Roland Kaiser (2011). Thiourea Derivatives: A Promising Class Against HIV/TB Co-Infection, Global View of HIV Infection, Dr. Vishwanath Venketaraman (Ed.), ISBN: 978-953-307-671-3, InTech, Available from: http://www.intechopen.com/books/global-view-of-hiv-infection/thiourea-derivatives-a-promising-class-against-hiv-tb-co-infection

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