Twenty-Six Years of HIV science: an overview of anti-HIV drugs metabolism

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From the identification of HIV as the agent causing AIDS, to the development of effective antiretroviral drugs, the scientific achievements in HIV research over the past twenty-six years have been formidable. Currently, there are twenty-five anti-HIV compounds which have been formally approved for clinical use in the treatment of AIDS. These compounds fall into six categories: nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), cell entry inhibitors or fusion inhibitors (FIs), co-receptor inhibitors (CRIs), and integrase inhibitors (INIs). Metabolism by the host organism is one of the most important determinants of the pharmacokinetic profile of a drug. Formation of active or toxic metabolites will also have an impact on the pharmacological and toxicological outcomes. Therefore, it is widely recognized that metabolism studies of a new chemical entity need to be addressed early in the drug discovery process. This paper describes an overview of the metabolism of currently available anti-HIV drugs.

Uniterms: AIDS/treatment. Drugs/anti-HIV. Drugs/metabolism. Antiretroviral drugs. Biotransformation.

Da identificação do HIV como o agente causador da AIDS, ao desenvolvimento de fármacos antirretrovirais eficazes, os avanços científicos na pesquisa sobre o HIV nos últimos vinte e seis anos foram marcantes. Atualmente, existem vinte e cinco fármacos anti-HIV formalmente aprovados pelo FDA para utilização clínica no tratamento da AIDS. Estes compostos são divididos em seis classes: inibidores nucleosídeos de transcriptase reversa (INTR), inibidores nucleotídeos de transcriptase reversa (INtTR), inibidores não-nucleosídeos de transcriptase reversa (INNTR), inibidores de protease (IP), inibidores da entrada celular ou inibidores de fusão (IF), inibidores de co-receptores (ICR) e inibidores de integrase (INI). O metabolismo consiste em um dos maiores determinantes do perfil farmacocinético de um fármaco. A formação de metabólitos ativos ou tóxicos terá impacto nas respostas farmacológicas ou toxicológicas do fármaco. Portanto, é amplamente reconhecido que estudos do metabolismo de uma nova entidade química devem ser realizados durante as fases iniciais do processo de desenvolvimento de fármacos. Este artigo descreve uma abordagem do metabolismo dos fármacos anti-HIV atualmente disponíveis na terapêutica.

Unitermos: AIDS/tratamento. Fármacos/anti-HIV. Fármacos/antirretrovirais. Fármacos/metabolismo. Biotransformação.

INTRODUCTION

The human immunodeficiency virus (HIV) has been established as the causative agent of the acquired immunodeficiency syndrome (AIDS) for over twenty-six years now (Barré-Sinoussi *et al*., 1983). During this time, an unprecedented success has been achieved in discovering anti-HIV drugs as reflected by the fact that there are now more drugs approved for the treatment of HIV than for all other viral infections put together (Mehellou, De Clercq, 2009). Despite this progress, the HIV pandemic remains one of the most serious challenges to global health and is set to continue to be one of the leading causes of death and disability in the world for decades to come (Vitoria *et al*., 2009). Since 1983,

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more than 60 million people have been infected with the virus, and more than 25 million people have died as a result (Cohen *et al.*, 2008).

According to the World Health Organization (WHO), globally there were an estimated 33 million [30 million–36 million] people living with HIV in 2007. Sub-Saharan Africa remains the region most heavily affected by HIV, accounting for 67% of all people living with HIV and for 75% of AIDS deaths in 2007 (UNAIDS/WHO, 2008). In addition, according to the same report, it was estimated that almost 3 million HIV-infected people were receiving antiretroviral treatment in low- and middle-income countries, representing approximately 31% of the estimated 9.7 million people currently in need of treatment (UNAIDS/WHO, 2008).

Currently, there are twenty-five anti-HIV compounds that have been formally approved for clinical use in the treatment of AIDS. These compounds fall into six categories: nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), cell entry inhibitors or fusion inhibitors (FIs), co-receptor inhibitors (CRIs), and integrase inhibitors (INIs). Metabolism by the host organism is one of the most important determinants of the pharmacokinetic profile of a drug. Formation of active or toxic metabolites will have an impact on the pharmacological and toxicological outcomes. Therefore, it is widely recognized that metabolism studies of a new chemical entity need to be addressed early in the drug discovery process. This paper describes an overview of the metabolism of currently available anti-HIV drugs.

ANTI-HIV DRUGS

The antiretroviral therapy advanced with the elucidation of the HIV cycle of life. Zidovudine (3'-azido-3'-deoxythymidine or AZT) (Retrovir®), a nucleoside analogue, was the first anti-HIV drug. AZT was originally synthesized as a potential anticancer drug by Horwitz in 1964 (Horwitz, Chua, Noel, 1964). In 1985, its anti-HIV activity was confirmed *in vitro* by a screening process using compounds that had previously been produced for others purposes (Fauci, 2003). AZT triphosphate acts by competitively inhibiting the utilization of thymidine triphosphate by reverse transcriptase (RT) of HIV-1 (Mitsuya *et al.*, 1985). In 1987, AZT was approved by the United States Food and Drug Administration (FDA) and supplied by Burroughs-Wellcome Laboratories.

In 2009, twenty-six years after the discovery of HIV as causative agent of AIDS, no less than twenty-five anti-HIV compounds have been formally approved for clinical use in the treatment of AIDS (Table I) (Adamson, Freed, 2009). These compounds fall into different categories, depending on the target within the HIV replicative cycle they interact with. In all, there are six categories: nucleoside reverse transcriptase inhibitors (NRTIs: zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, emtricitabine), nucleotide reverse transcriptase inhibitors (NtRTIs: tenofovir), non-nucleoside reverse transcriptase inhibitors (NNRTIs: nevirapine, delavirdine, efavirenz, etravirine), protease inhibitors (PIs: saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir, fosamprenavir, tipranavir, darunavir), cell entry inhibitors or fusion inhibitors (FIs: enfuvirtide), co-receptor inhibitors (CRIs: maraviroc), and integrase inhibitors (INIs: raltegravir) (Mehellou, De Clercq, 2009).

Although treatment with antiviral agents has proven to be a highly effective way to improve the health and survival of infected individuals, the epidemic will continue to grow and there is an urgent need to develop new anti-HIV drugs.

METABOLISM STUDIES

Metabolism of a drug is one of the most important determinants of its pharmacokinetic properties. In most cases, metabolism leads to the inactivation of drugs. However, the metabolic transformation of a xenobiotic can also sometimes lead to the generation of an active metabolite, which can be solely or partially responsible for the pharmacological response. In some instances, metabolic transformation can also produce reactive or toxic intermediates or metabolites, with potential toxicological implications (Kumar, Surapaneni, 2001). Hence, a good understanding of the metabolism of a new chemical entity is needed early in the drug discovery process.

Mammalian metabolism of xenobiotics is traditionally subdivided into Phase I and Phase II processes. In Phase I, oxidation, reduction, or hydrolysis results in the introduction of new functional groups. Phase II involves conjugation reactions, where a highly hydrophilic moiety such as sulfate or glucuronide is attached to make the compounds more water-soluble and to prepare for excretion through urine or bile (Kumar, Surapaneni, 2001; Lee, Obach, Fisher, 2003).

The most important group of Phase I enzymes is the cytochrome P450 (CYP) superfamily. About 90% of catalysis mediated by P450 is performed by the human isoforms: CYPs 1A2, 2C9, 2C19, 2D6, 2E1 and 3A4 (Guengerich, 2006). They include oxidation reactions, such as aromatic hydroxylation, aliphatic hydroxylation,

N-oxidation, oxidative desalkylation and epoxide formation (Omura, 1999).

Key Phase II enzymes include, for instance, uridine diphosphate-dependent glucuronosyltransferase (UGT), sulfotransferases and glutathione- *S* -transferase. Glucuronidation of small lipophilic molecules by UGTs is probably the most important Phase II process for the clearance of drugs. Consistent with its broad substrate profile, UGT exists as an enzyme superfamily. So far, 18 UGT proteins, which can be divided into two families, UGT1 and UGT2, have been identified (Mackenzie *et al*., 1997).

Prediction of drug metabolism in humans

In vitro and in vivo extrapolation

The ability to predict human pharmacokinetic from *in vitro* and *in vivo* models with a reasonable degree of accuracy (2.0-fold variation) is an ongoing challenge with many different approaches yielding varying degrees of success (Prakash, Vaz, 2009). Traditionally, *in vivo* drug metabolism studies have relied on the use of model systems to produce the expected human metabolites of drugs. Usually whole animal systems are used, especially

TABLE I - Approved antiretroviral drugs in the US and Europe

Generic Name	Brand Name	Manufacturer	Date of FDA approval
Zidovudine	Retrovir	GlaxoSmithKline	19 March 1987
Didanosine	Videx (tablet)	Bristol Myers-Squibb	9 October 1991
	Videx EC (capsule)	Bristol Myers-Squibb	31 October 2000
Zalcitabine	Hivid	Hoffmann-La Roche	19 June 1992
Stavudine	Zerit	Bristol Myers-Squibb	24 June 1994
Lamivudine	Epivir	GlaxoSmithKline	17 November 1995
Saquinavir	Invirase (hard gel capsule)	Hoffmann-La Roche	6 December 1995
	Fortovase (soft gel capsule)	Hoffmann-La Roche	7 November 1997
Ritonavir	Norvir	Abbott Laboratories	1 March 1996
Indinavir	Crixivan	Merck	13 March 1996
Nevirapine	Viramune	Boehringer Ingelheim	21 June 1996
Nelfinavir	Viracept	Agouron Pharmaceuticals	14 March 1997
Delavirdine	Rescriptor	Pfizer	4 April 1997
Efavirenz	Sustiva (US)	Bristol Myers-	17 September 1998
	Stocrin (Europe)	Squibb Merck	17 September 1998
Abacavir	Ziagen	GlaxoSmithKline	17 December 1998
Amprenavir	Agenerase	GlaxoSmithKline	15 April 1999
Lopinavir + ritonavir	Kaletra	Abbott Laboratories	15 September 2000
	Aluvia (developing world)	Abbott Laboratories	15 September 2000
Tenofovir	Viread	Gilead Sciences	26 October 2001
Enfuvirtide	Fuzeon	Hoffmann-La Roche & Trimeris	13 March 2003
Atazanavir	Reyataz	Bristol-Myers Squibb	20 June 2003
Emtricitabine	Emtriva	Gilead Sciences	2 July 2003
Fosamprenavir	Lexiva (US)	GlaxoSmithKline	20 October 2003
	Telzir (Europe)	GlaxoSmithKline	20 October 2003
Tipranavir	Aptivus	Boehringer Ingelheim	22 June 2005
Darunavir	Prezista	Tibotec, Inc.	23 June 2006
Maraviroc	Celsentri (Europe)	Pfizer	18 September 2007
	Selzentry (US)	Pfizer	18 September 2007
Raltegravir	Isentress	Merck & Co., Inc.	12 October 2007
Etravirine	Intelence	Tibotec Therapeutics	18 January 2008

small laboratory animal models like rat, dog, cat, guinea pig, rabbit etc. However, interspecies differences must be considered since there is no "perfect" animal model destined for extrapolation to the human model. *In vitro* studies are generally used to complement and specify the data obtained using perfused organs, tissue or cell cultures and microsomal preparations. Such methods suffer from a number of limitations, such as the cost of experimental animals, the ethical concerns and species variations (Asha, Vidyavathi, 2009; Collins, 2001).

Isolated perfused rat liver has the advantages of controlling blood and bile flows, ease of manipulation of the perfusion medium and the large number of perfusate samples that may be collected (Meijer, Swart, 1997). Instead, microsomal preparations are derived from endoplasmic reticulum after homogenization and centrifugation and present CYPs and UGTs. They are developed to predict metabolic clearance of the compound and drug-drug interactions, and are widely used for drug metabolism studies (Pelkonen *et al.*, 2005).

Another important tool employed in drug metabolism is the application of human hepatocyte cultures, that present both Phase I and Phase II enzymes, representing a great model for biotransformation study mediated by P450, and allowing a good correlation with *in vivo* studies. On the other hand, the use of hepatocyte cultures is limited by the restricted availability of liver tissue (Li *et al.*, 1997; Gomez-Lechon *et al.*, 2004). Liver-derived HepG-2 and BC2 cell lines and lung-derived line A549 have the disadvantage of expressing few enzymes, restricting their metabolic capacity (Yoshitomi *et al.*, 2001; Le Vee *et al.*, 2006). However, the recent human hepatoma-derived cell line HepaRG has been shown to possess functions and morphological resemblance to normal human hepatocytes (Aninat *et al.*, 2006).

The use of microorganisms as models of mammalian metabolism was introduced in the early 1970s by Smith and Rosazza (Smith, Rosazza, 1974, 1975). This method uses eukaryotic microorganisms to produce metabolites similar to mammals, since microbial transformation systems could closely mimic most of the Phase I transformations of drugs observed in mammals, mainly those catalyzed by cytochrome P450 (Azerad, 1999).

Compared with traditional methods, there are clearly a number of practical advantages in using microbial transformation as a model for drug metabolism, such as its chemo-, regio-, and stereoselectivity, impressive catalytic efficiency, and capability of accepting a wide range of complex molecules as substrates. In addition, it is conducive to easy scale up to produce sufficient amounts of metabolites for unambiguous structural determination,

and to provide such metabolites as authentic standards (Zmijewski *et al.*, 2006; Asha, Vidyavathi, 2009).

Therefore, it is a valuable tool in the production of molecules with improved, different or toxic activity originated by the fungal enzymatic biodiversity (Azerard, 1999; Asha, Vidyavathi, 2009). Recently, our laboratory has carried out a number of studies applying microbial models to study mammalian metabolism (Gomes *et al.,* 2006; Pazzini *et al.,* 2005, 2010; Carneiro *et al.,* 2005, 2010; Dias *et al.,* 2005; Costa *et al.*, 2008; Braga *et al.,* 2011).

In silico Computational Tools

In recent years, several *in silico* computational methods have become available for prediction of metabolism and pharmacokinetics of the NCEs and are increasingly becoming a part of the drug discovery process to select candidates with desirable ADME properties (Canavan, 2007; Afzelius *et al.,* 2007; De Graff, Vermeulen, Feenstra, 2005). Mechanism-based quantum chemical calculations on substrates and the enzyme, pharmacophore modeling of ligands, and protein homology modeling in combination with automated docking and molecular dynamics simulations have been used to rationalize and predict ligand binding and formation of metabolites (Prakash, Vaz, 2009).

Moreover, the prediction of metabolites can be performed by means of large collections of transformation rules. A given compound is fragmented and then passed through all rules to identify putative metabolically labile sites. Expert systems and their databases, such as MetabolExpert (Darvas, 1988), META (Klopman, Dimayuga, Talafous, 1994), METEOR (Testa *et al.*, 2005), MetaDrug (Ekins *et al.*, 2006), and PK/DB (Moda *et al.*, 2008), are examples of *in silico* methods used to predict drug biotransformation pathways and possible metabolites, that provide a ranked list of most likely metabolites.

Metasite (Cruciani *et al.*, 2005) is a computational program for metabolite formation prediction, which combines structural information, by matching the structural complementarity of the substrate and the protein, and rule-based and reactivity methods.

The availability of more and more 3D protein structures of P450 paves the way for molecular modeling approaches that zoom in on the atomic details of enzyme-ligand interactions. Docking is the most commonly used structure-based method, able to predict the site of metabolism of the substrate based on which atoms are exposed or close to the catalytic centre (the heme iron) in order for metabolism to take place (Stjernschantz, Vermeulen, Oostenbrink, 2008).

ANTI-HIV DRUGS METABOLISM

Since the discovery of antiretrovirals, metabolism studies of these drugs have been increasingly carried out, aiming to ensure their efficacy and safety and to explain their toxic effects (Li, Chan, 1999). This trend is graphically illustrated in Figure 1, which presents the number of publications about antiretrovirals metabolism, between 1987 and 2009, retrieved upon searching with the *SciFinder* engine.
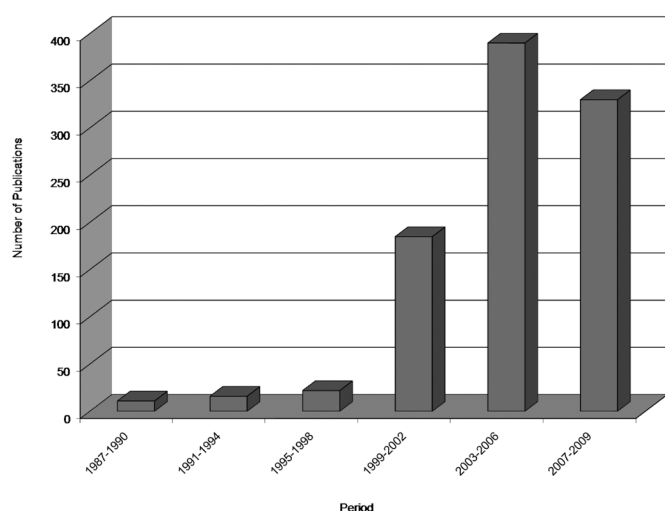

FIGURE 1 - Graphical representation of the increasing trend for number of anti-HIV drugs metabolism publications, from 1987 to October 2009 (data presented are those listed by *SciFinder*).

Nucleoside Analogue Reverse Transcriptase Inhibitors (NRTIs)

The reverse transcriptase (RT) of HIV is actually the target for three classes of inhibitors: nucleoside RT inhibitors (NRTIs), nucleotide RT inhibitors (NtRTIs) and non-nucleoside RT inhibitors (NNRTIs). The NRTIs and NtRTIs interact with the catalytic site (that is the substrate-binding site) of the enzyme, whereas the NNRTIs interact with an allosteric site located a short distance from the catalytic site (De Clercq, 2007).

For the NRTIs and NtRTIs to interact with the substrate-binding site they need to be phosphorylated to the triphosphate and diphosphate forms, respectively. There are at present, seven NRTIs that have been formally approved for the treatment of HIV infections: zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC) and emtricitabine [(-)FTC]. All the NRTIs can be considered 2',3'-dideoxynucleoside (ddN) analogues and act in a similar fashion. After they have been taken up as such by the cells, they are phos-

phorylated to their 5'-mono-, 5'-di- and 5'-triphosphate (MP, DP and TP, respectively) form following the same mechanism: $ddN \rightarrow ddNMP \rightarrow ddNDP \rightarrow ddNTP$, before the latter will then act as a competitive inhibitor/alternate substrate of the normal substrate dNTP (either dATP, dTTP, dGTP or dCTP) (Furman *et al.*, 1986; De Clercq, 2007). As competitive inhibitor of the normal substrate, the ddNTP will inhibit incorporation of this substrate into the growing DNA chain; as alternate substrate it will be incorporated into this chain (as ddNMP), thereby acting as a chain terminator (since the ddNMP is missing the 3'-hydroxyl group required for further chain elongation) (De Clercq, 2007).

Among the NRTIs, metabolism studies have been focused on zidovudine (AZT). There are three main metabolic pathways of AZT: intracellular phosphorylation, glucuronidation and reduction (Figure 2). The intracellular metabolism, which involves the formation of zidovudine-5'-triphosphate (AZT-TP, **1**), is limited in the last two steps of phosphorylation due to an inefficiency of the enzyme thymidine kinase to form zidovudine-5'-diphosphate (AZT-DP), leading to accumulation of zidovudine-5'-monophosfate (AZT-MP) within the cell, and serving as a deposit for the formation of AZT-TP. High concentrations of AZT-MP inhibit the thymidylate kinase, decreasing the intracellular concentration of deoxythymidine-5'-triphosphate and increasing the activity of antiretroviral. Furthermore, the accumulation of intracellular AZT-MP may be related to the toxicity observed during the use of AZT, mainly represented by hematological toxicity, likely caused by mitochondrial DNA polymerase γ inhibition (Veal, Back, 1995).

AZT undergoes fast first-pass hepatic metabolism, producing zidovudine-5'-glucuronide (GAZT, **2**), an inactive metabolite (Li, Chan, 1999). In studies using human microsomal preparations, it was observed that AZT's glucuronidation is independent of sex or age, but the polymorphism displayed by UGT2B7 isoform may contribute to the interindividual variations observed in plasma concentration of AZT and its conjugate metabolite (Court *et al.*, 2003). However, these variations may be involved in the conjugation catalyzed by other isoforms, as well as the stage of intracellular AZT's phosphorylation, which implies a greater or lesser availability of substrate for hepatic metabolism. Also, according to studies on liver microsomes, the extent of metabolism of AZT by UGT can range from 60 to 75% in humans, while in rats it is only 10% (Naritomi *et al.*, 2003) due to higher catalytic efficiency of these enzymes in the former (Trapnell *et al.*, 1998).

The third metabolic pathway of AZT consists of its azido group reduction, probably mediated by both
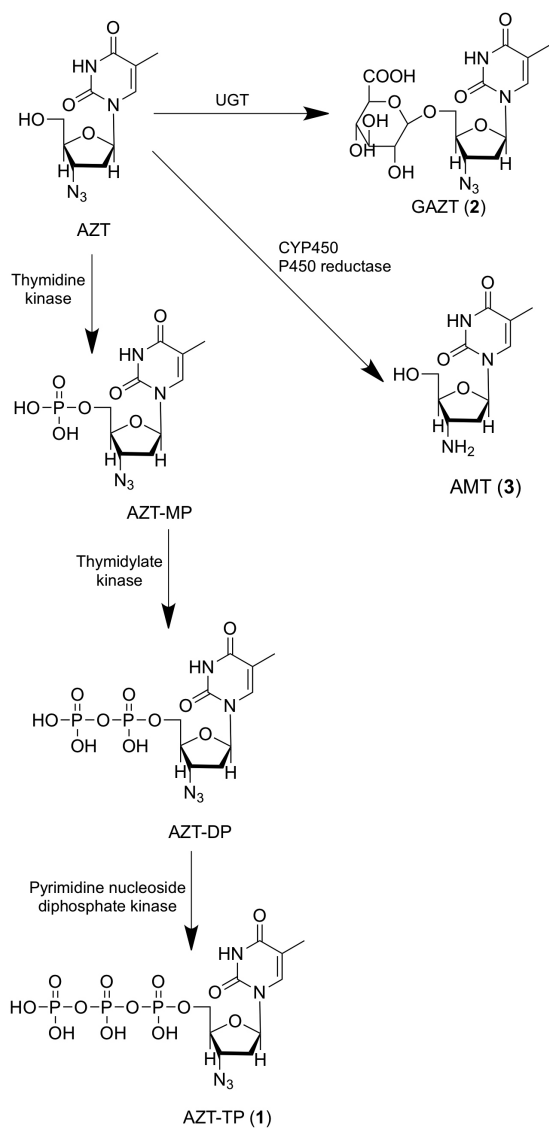
FIGURE 2 – Main metabolic routes of zidovudine in humans.

cytochrome P450 isoenzymes, NADPH-cytochrome P450 reductase and cytochrome b5 reductase, resulting in 3'-amino-3'-deoxythymidine (AMT, **3**). In studies using rat hepatocytes and liver microsomes of rats and humans, the participation of the subfamilies CYP2B, CYP3A and CYP4A was observed in this reaction, evidenced by an increased formation of AMT through the use of inducers of these enzymes. The CYP2C9 isoform also appears to be involved in this process. These studies have also indicated that the AMT is five to seven times more toxic to the hematopoietic cells than AZT, confirming its role in the cytotoxicity of antiretroviral therapy observed in patients. In another similar study, the AMT glucuronide conjugate (GAMT), produced from the reduction of zidovudine-5'-glucuronide, was found to discard the AMT as a substrate for UGT (Cretton *et al.*, 1991).

AZT metabolism studies were also performed using microbial models obtaining derivatives not previously reported in the literature (Figure 3). Kruszewska and co-workers (2003) studied the bioconversion of AZT with an environmental bacterium, *Stenotrophomonas maltophilia* PCM 1942. After ten days of incubation, a hydroxylated metabolite at the C-2' deoxyribose ring (**4**) was produced with a yield of about 97% (Kruszewska *et al.*, 2003). In our laboratory, biotransformation of AZT was performed using *Cunninghamella echinulata* ATCC 9244 as a catalyst (Nunes, 2008). This filamentous fungus strain is widely known for performing biotransformation reactions of a variety of compounds, similar to mammals, and is frequently employed in bioconversion processes. In our experiment, *C. echinulata* produced a glycosylated metabolite of AZT (**5**) (Figure 3) after seven days of incubation, under mild conditions of temperature and pH, with a yield of about 46% (Nunes, 2008).
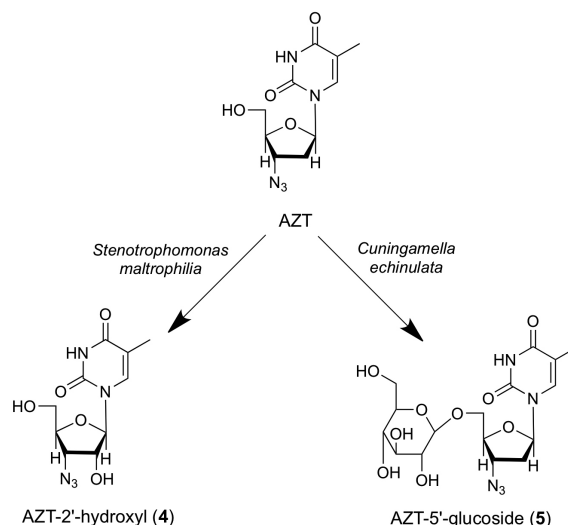

FIGURE 3 - Microbial metabolites of AZT: AZT-2'-hydroxyl (**4**) (Kruszewska *et al.*, 2003) and AZT-5'-glucoside (**5**) (Nunes, 2008).

Didanosine (2',3'-dideoxyinosine or ddI) (Figure 4) is first phosphorylated by 5'-nucleotidase to form didanosine-5'-monophosfate (ddI-MP) in human lymphoid cells. Then, ddI-MP is converted to dideoxyadenosine-5'-monophosfate (ddA-MP) by adenylosuccinate synthetase and lyase, with subsequent phosphorylation to dideoxyadenosine-5'-triphosphate (ddA-TP) by adenylate kinase (miokinase), producing active metabolite (**6**) responsible for inhibiting viral reverse transcriptase. In another metabolic pathway, ddI is hydrolyzed by purine nucleoside phosphorylase, to release the sugar deoxyribose (**7**) and hypoxanthine (**8**), which is degraded into

uric acid (**9**) and eliminated (Navé *et al.,* 1994). In a study using beagle dogs, the following possible metabolites of didanosine were found in plasma and urine: allantoin, uric acid, hypoxanthine and xanthine (Kaul *et al.*, 1993). Probably ddI is not metabolized by CYP450 isoforms, since studies using inducers of these enzymes showed no changed pharmacokinetic profile (Bruzzese *et al.*, 1995).

Zalcitabine (2',3'-dideoxycytidine or ddC), as well as other nucleoside analogues, must be phosphorylated to dideoxycitidine-5'-triphosphate (ddC-TP) in the intracellular environment t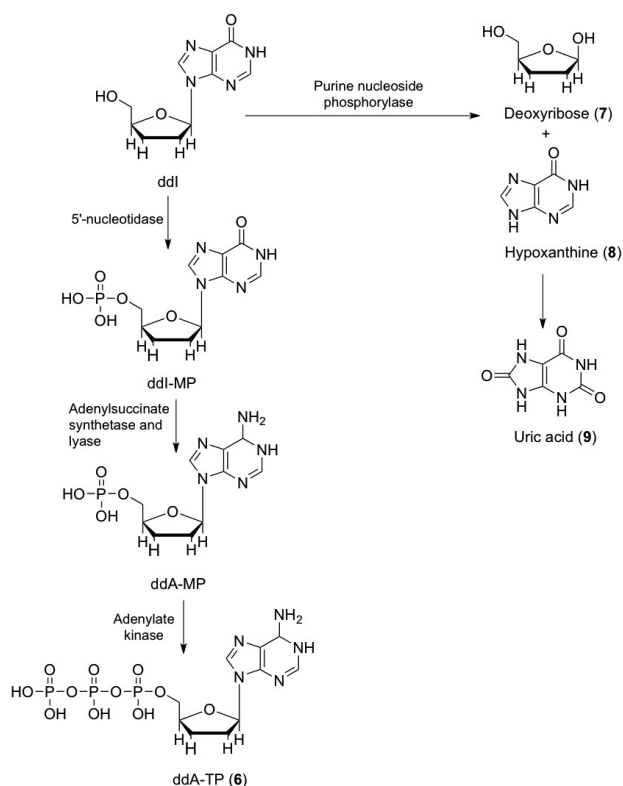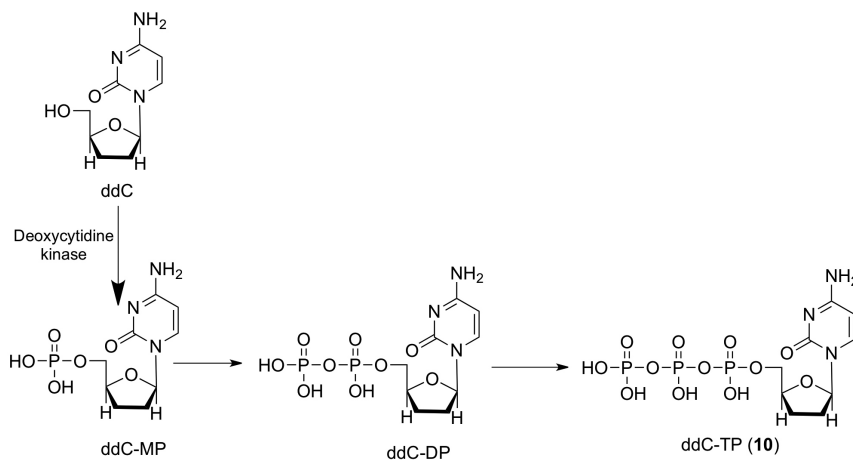o become active (**10**) (Figure 5). The first stage of phosphorylation, with production of ddC-MP, seems to be inefficient due to a low affinity between ddC and the enzyme deoxycytidine kinase. In addition to the mono-, di- and triphosphate, the only reported metabolite of zalcitabine is dideoxyuridine, found in urine (Li, Chan, 1999).

Human metabolism of stavudine (2',3'-didehydro-2',3'-dideoxythymidine or d4T) (Figure 6) has yet to be completely elucidated. Intracellularly, it is sequentially phosphorylated to produce its active form, stavudine-5'-triphosphate (d4T-TP) (**11**). In a study using monkeys and isolated rat hepatocytes, the degradation of stavudine was detected in thymine (**12**) and the d4T-TP derivative, with subsequent production of beta aminoisobutyric acid (BAIBA) (**13**) (Cretton *et al.,* 1993). The glucuronide metabolite (**14**) of stavudine was found in the urine of monkeys but has remained controversial (Schinazi, *et al.*, 1990).

A recent study reported by our group (Freitas, 2009) described the metabolic profile of stavudine, applying *in vivo, in vitro* and *in silico* approaches. The bioconversion experiments with nine strains of filamentous fungi, detected one major metabolite, which was further identified as thymine (**12**). The *in vivo* study with Balb/c mice detected the same metabolite (thymine, **12**), highlighting the strains used in this study as microbial models of stavudine metabolism (Figure 7). Unlike *in vivo* and *in vitro* experiments, the *in silico* study based upon the docking and molecular dynamics simulations of stavudine in complex with the active site of cytochrome P450 CYP3A4 structure, identified that the methyl group is the most probable site for the oxidation reaction mediated by CYP450 enzymes. It is likely that the *in vivo* and *in vitro* results described in this study must be mediated by enzymes other than CYP-450.

Lamivudine (3TC) (Figure 8) is another NRTI, whose intracellular phosphorylation occurs efficiently to produce its active metabolite, lamivudine-5'-triphosphate (3TC-TP) (**15**). There was no difference in the concen-
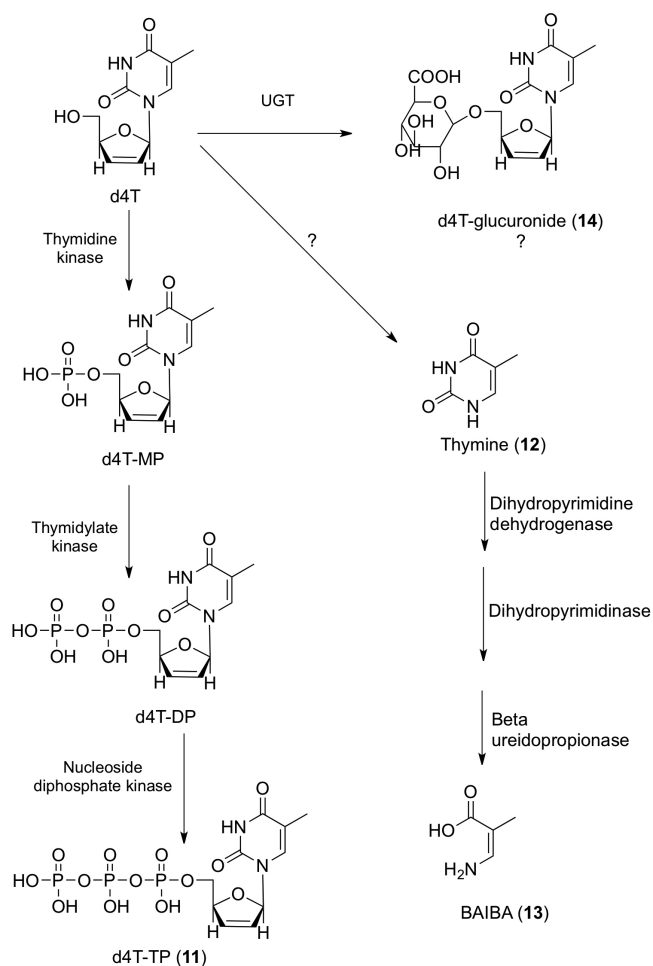

FIGURE 4 – Proposed metabolic pathways of didanosine.

FIGURE 5 – Metabolic scheme for zalcitabine.

FIGURE 6 – Proposed metabolic routes of stavudine.

FIGURE 7 – Metabolism studies of stavudine by *C. elegans* and mice, producing the metabolite thymine (Freitas, 2009).

catalyzed by the enzyme adenosine phosphotransferase, producing its monophosphate form, which is then converted into the monophosphate form of carbovir (CBV-MP) by a cytosolic deaminase. Subsequently, CBV-MP is converted into the di- and triphosphate form (**16**), the latter is another analogue of deoxyguanosine with potent inhibitory activity of viral reverse transcriptase (Faletto *et al.,* 1997).

In addition, it also undergoes hepatic biotransformation in humans, mice and monkeys, producing two major metabolites, identified as 5'-carboxylate (**17**) and 5'-glucuronide derivatives (**18**), formed by cytosolic alcohol dehydrogenase (ADH) and UGT, respectively (Good *et al.,* 1995; McDowell *et al.*, 1999). In *in vitro* experiments with human liver microsomes, it was demonstrated that CYP450 enzymes appear to be involved in the metabolism of abacavir (Ravitch *et al.,* 1998).

Nucleotide Reverse Transcriptase Inhibitors (NtRTIS)

NtRTIs should be clearly distinguished from the NRTIs as they are nucleotide analogues (not nucleoside analogues), which means they only need two (not three) phosphorylation steps to be converted to their active form. Most importantly, they contain a phosphonate group that

tration of 3TC and its pharmacokinetic profile in healthy individuals and patients with moderate to severe hepatic dysfunction, suggesting that lamivudine metabolism by liver enzymes is minimal (Johnson, Horak, Breuel, 1998).

Abacavir (ABC) (Figure 9), an analogue of 2'-deoxyguanosine, undergoes fast intracellular phosphorylation
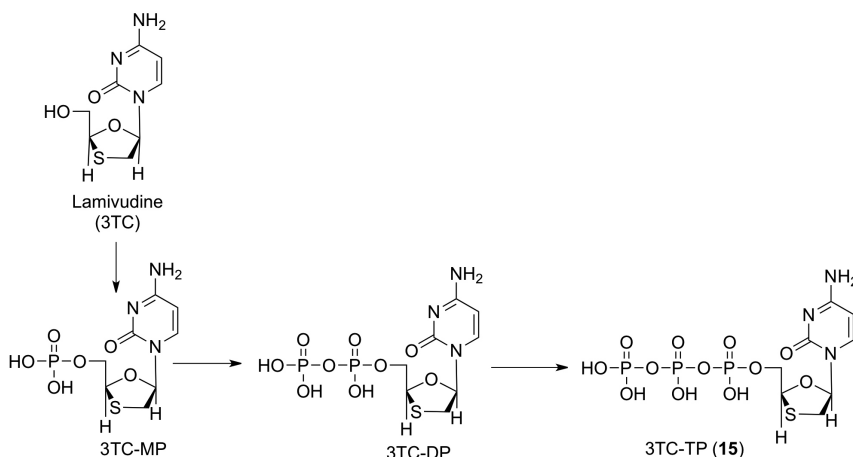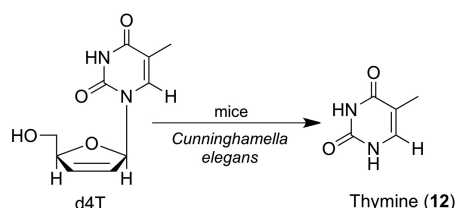

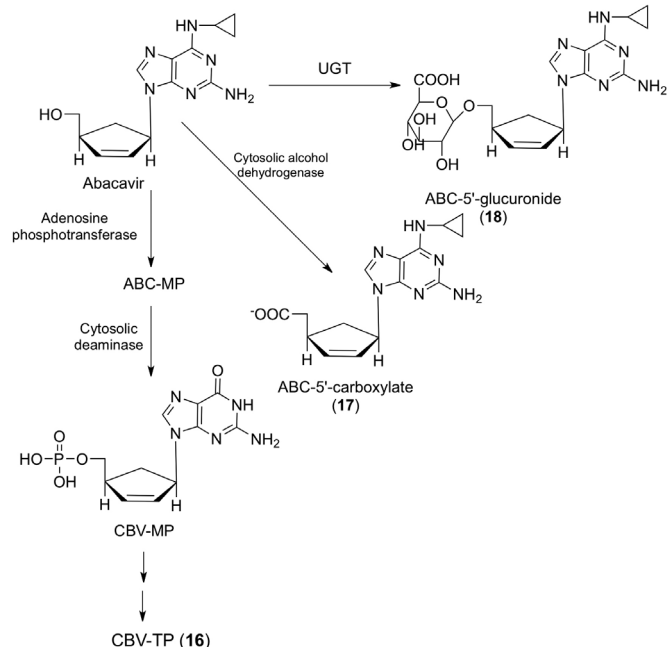FIGURE 8 – Intracelular metabolism of lamivudine.

FIGURE 9 – Main metabolic pathways of abacavir in humans.

cannot be cleaved by hydrolases (esterases), which would make it more difficult to cleave off these compounds once incorporated at the 3'-terminal end, as compared to their regular nucleotide counterparts (i.e. AZTMP, ddAMP, ddCMP, etc.). The prototype of the NtRTIs, (R)-9-(2- phos-phonomethoxypropyl) adenine (tenofovir) (Figure 10) was first described in 1993 (Balzarini et al., 1993) and is the only NtRTIs currently approved for use in patients suffering from HIV. Due to its limited oral bioavailability, tenofovir has been converted to its oral prodrug form, tenofovir disoproxil fumarate (TDF, Viread®) and has become one of the most frequently prescribed drugs for the treatment of HIV infections (De Clercq, 2003). Since 2008, it has also been approved for the treatment of chronic hepatitis B virus (HBV) infections. Tenofovir is metabolized intracellularly to its active metabolite, tenofovir diphosphate (**19**), which is a competitive inhibitor of HIV-1 RT that

terminates the growing DNA chain. Phosphorilation to its diphosphate form can be achieved in one or two steps with the aid of 5-phosphoribosyl-1-pyrophosphonate (PRPP) synthetase (Balzarini et al., 1995) or AMP kinase (Merta et al., 1992), respectively (Figure 10).

Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

These compounds are noncompetitive inhibitors of HIV-1 RT, because they act differently compared with nucleoside analogues. They are mainly metabolized by the CYP3A subfamily of cytochrome P450 enzymes. Nevirapine (NVP) (Figure 11), for example, is capable of inducing its own metabolism mediated by CYP450 with production of several hydroxylated metabolites, with subsequent glucuronidation. These derivatives were found in urine and feces of human and animals (dog, mouse, rat, chimpanzee, rabbit and monkey), after oral administration of nevirapine, and identified as 2-hydroxy-nevirepine (**20**), 3-hydroxy-nevirapine (**21**), 8-hydroxy-nevirapine (**22**), 12-hydroxy-nevirapine (**23**), their respective glucuronide conjugates (**24-27**), and 4-carboxy-nevirapine (**28**), formed by secondary oxidation of **23** (Riska et al., 1999a, b). In experiments with human liver microsomes, it was observed that CYP3A and CYP2B6 are responsible for 2- and 3-hydroxy-nevirapine production, respectively, while the formation of 8- and 12-hydroxi-nevirapine is mediated by several enzymes, primarily CYP3A4 and CYP2D6 (Erickson et al., 1999).

Delavirdine (Figure 12) is another NNRTI, which produces various metabolites in rats (Chang et al., 1997a), mice (Chang et al., 1997b) and monkeys (Chang et al., 1997c) through oxidation reactions catalyzed mainly by CYP450. An N-desalkylation reaction produces the desalkyl delavirdine metabolite (**29**), with subsequent sulfation (**30**), while hydroxylation at C-6' position of the pyrimidinic ring produces 6'-hydroxy-delavirdine (**31**), followed by glucuronidation to form the
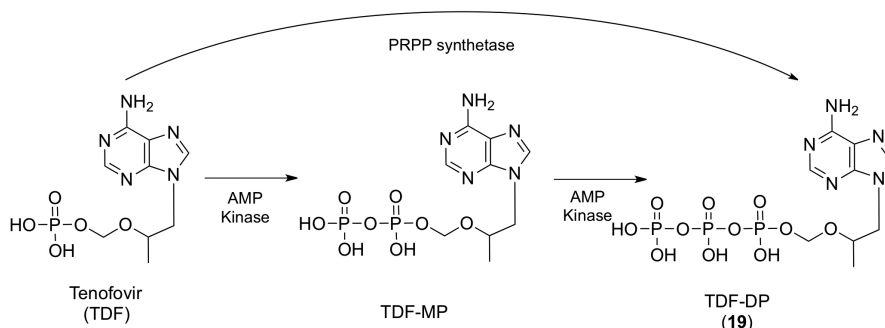

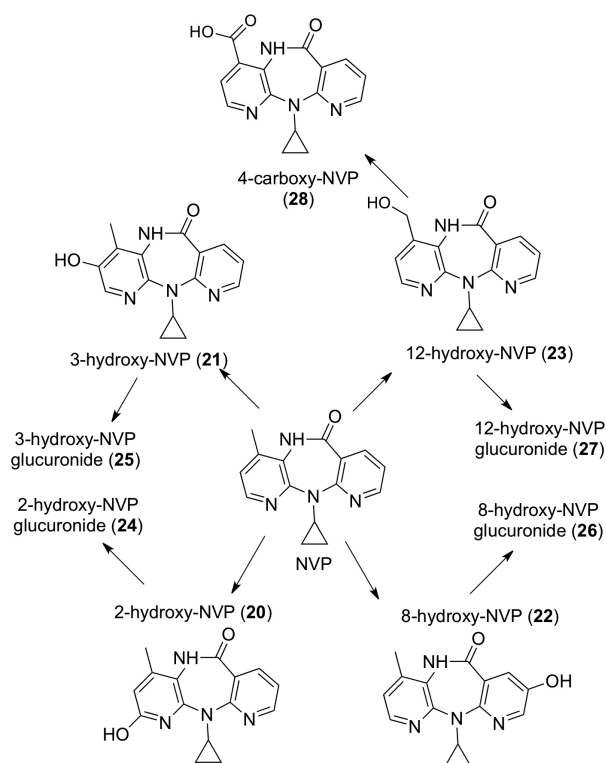
FIGURE 10 – Metabolism of tenofovir.

FIGURE 11 – Main metabolic routes of nevirapine.

FIGURE 12 – Main metabolic routes of delavirdine.

6'-*O*-glucuronide conjugate (**32**). The cleavage of pyrimidinic ring of 6'-hydroxy-delavirdine was also observed, resulting in depyridinyl delavirdine, and cleavage of the amide bond forming indole carboxylic acid (**33**) and *N*-isopropylpyridinepiperazine (**34**). Moreover, 6'-hydroxy delavirdine is sulfated (**35**) in rats, depyridinyl delavirdine is conjugated in rats and mice, and desalkyl delavirdine is hydroxylated at positions C-4' (**36**) and C-6' (**37**) of the pyrimidinic ring in rats and monkeys, with subsequent sulfation (**38** and **39**).

In mice, *N*-desalkylation of *N*-isopropylpyridine-piperazine produces *N*-deisopropylpyridinepiperazine, while the indole carboxylic acid undergoes conjugation. In monkeys, three additional metabolites were observed, identified as conjugation of desalkyl delavirdine with *N*-acetylglucosamine, hydroxylation of delavirdine at the C-4 position of the indole ring to form 4-hydroxy delavirdine, followed by glucuronidation of 4-*O*-glucuronide, and *N*-glucuronidation of the indole ring of delavirdine. The *N*-desalkylation was the major metabolic fate in rats and monkeys, while in mice it was the cleavage of the amide bond (Chang *et al.,* 1997a, b, c).

Experiments with human liver microsomes showed that delavirdine is an inhibitor of the isoforms CYP2C9, CYP2C19, CYP2D6 and CYP3A4, and that the *N*-desalkylation reaction is mediated by CYP2D6 and CYP3A4, while hydroxylation at position C-6' of pyrimi-
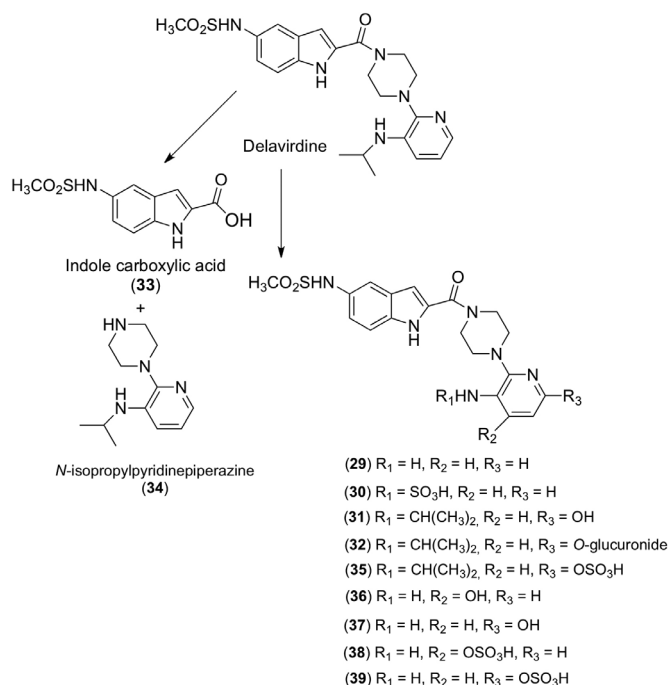
dinic ring is catalyzed mainly by CYP3A4 (Voorman *et al*., 1998a, b; Voorman *et al.,* 2001).

Efavirenz (EFV) (Figure 13) is extensively metabolized and small or insignificant amounts of the unchanged drug were found in urine of several species, including humans (Mutlib *et al.,* 1999). The major metabolite isolated is the *O*-glucuronide (**40**) conjugate of the metabolite 8-hydroxy-efavirenz (**41**). EFV also undergoes glucuronidation to form the *N*-glucuronide conjugate (**42**). The sulfation of 8-hydroxy-efavirenz (**43**) occurs in all species, except humans, while EFV hydroxylation to form 7-hydroxy-efavirenz (**44**), with subsequent glucuronidation (**45**) or sulfation (**46**), is not only observed in rats. Other reactions include the hydroxylation of the cyclopropane ring (C-14) of 8-hydroxy-efavirenz, producing 8,14-dihydroxy-efavirenz (**47**), and hydroxylation of its sulfated conjugate (**48**), which can also be formed from the sulfation of **47**, followed by glucuronidation (**49**) or conjugation with glutathione (GSH), with subsequent degradation, forming the adducts cysteinylglicine and cysteine. The hydroxylation of **44** is also observed, followed by glucuronidation (**50**) or sulfation (**51**) in position C-7 whereas **57** can still undergo glucuronidation (**52**) in position C-8 (Mutlib *et al.,* 1999).

In vitro experiments using human liver microsomes have shown that 8-hydroxy-efavirenz was obtained as the major metabolite, as well as during in *in vivo* studies, while the metabolites 8,14-dihydroxy-efavirenz and 7-hydroxy-efavirenz were also isolated. It was further demonstrated

that CYP2B6 is the major isoform responsible for the production of 8-hydroxy-efavirenz and 8,14-dihydroxy-efavirenz, and that other isoforms (CYPs 1A2, 3A4 and 3A5) seem to participate in a minority of this process (Ward *et al.,* 2003). Rodriguez-Novoa and co-workers (2005) reported that CYP2B6 genetic polymorphisms contribute to variations in plasma concentration of EFV, proving the involvement of this isoform in its metabolism. In addition to being metabolized by CYP2B6, efavirenz is also its inhibitor, as well as its major metabolite, 8-hydroxy-efavirenz (Bumpus *et al.*, 2006), which is also an inducer of the isoform CYP3A4 (Mouly *et al.,* 2002).
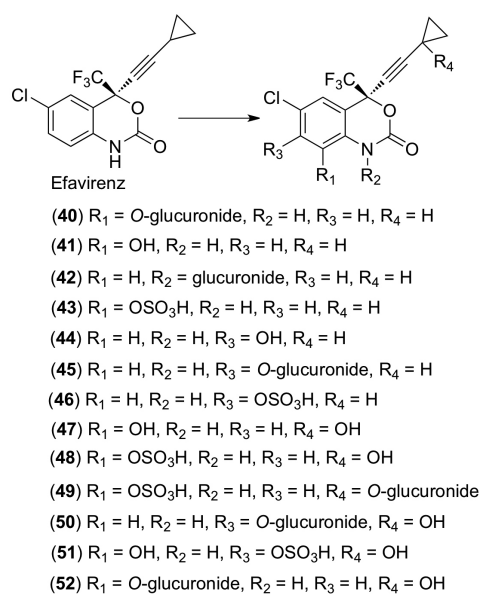

(**40**) R_1 = *O*-glucuronide, R_2 = H, R_3 = H, R_4 = H

(**41**) R_1 = OH, R_2 = H, R_3 = H, R_4 = H

(**42**) R_1 = H, R_2 = glucuronide, R_3 = H, R_4 = H

(**43**) R_1 = OSO_3H, R_2 = H, R_3 = H, R_4 = H

(**44**) R_1 = H, R_2 = H, R_3 = OH, R_4 = H

(**45**) R_1 = H, R_2 = H, R_3 = *O*-glucuronide, R_4 = H

(**46**) R_1 = H, R_2 = H, R_3 = OSO_3H, R_4 = H

(**47**) R_1 = OH, R_2 = H, R_3 = H, R_4 = OH

(**48**) R_1 = OSO_3H, R_2 = H, R_3 = H, R_4 = OH

(**49**) R_1 = OSO_3H, R_2 = H, R_3 = H, R_4 = *O*-glucuronide

(**50**) R_1 = H, R_2 = H, R_3 = *O*-glucuronide, R_4 = OH

(**51**) R_1 = OH, R_2 = H, R_3 = OSO_3H, R_4 = OH

(**52**) R_1 = *O*-glucuronide, R_2 = H, R_3 = H, R_4 = OH

FIGURE 13 – Main metabolites of efavirenz.

Protease Inhibitors (PIs)

At present, there are ten protease inhibitors (PIs) licensed for clinical use in the treatment of HIV infections. With the exception of tipranavir (which is based on a coumarine scaffold), all these PIs are based on the "peptidomimetic" principle, that is they contain an hydroxyethylene scaffold which mimics the normal peptide linkage (cleaved by the HIV protease) but which can itself not be cleaved. They thus prevent the HIV protease from carrying out its normal function, which is the proteolytic processing of precursor viral proteins into mature viral proteins. The ten PIs presently available for the treatment of HIV infections are saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir, fosamprenavir, tipranavir and darunavir (Madruga *et al.,* 2007; Lazzarin *et al*., 2007).

Akin to NNRTIs, PIs are usually metabolized by oxidation mediated by CYP450. Saquinavir (SQV) (Fi-

gure 14) has low oral bioavailability due to its extensive first-pass metabolism, which occurs mainly by CYP3A4 in small intestine, but also in liver, producing the same metabolites mono- and dihydroxy. In incubations of saquinavir with human small intestine and liver microsomes, two major metabolites (**53** and **54**) were isolated, produced from mono-hydroxylations of octahydro-2-(1*H*)-isoquinolynil and (1,1-dimethylethyl)amino, respectively. Another five minority metabolites were found (Flitzsimmons, Collins, 1997). One of the major metabolites was subsequently identified as 6-equatorial-hydroxy-saquinavir (Eaglings *et al*., 2002).

Biotransformation of indinavir (IDV) (Figure 14) takes place primarily in liver by CYP3A4 (Chiba *et al.,* 1996; Lin *et al.,* 1996), and can also occur in the small intestine. Oxidative metabolites were found in both *in vivo* and *in vitro* experiments using liver and intestinal microsomes of humans and rats (Chiba, Hensleigh, Lin, 1997). In a study with healthy volunteers, seven major metabolites were found in urine, whose metabolic pathways were identified as: nitrogen pyridine glucuronidation (**55**); *N*-oxidation of pyridine (**56**); *p*-hydroxylation of the phenylmethyl radical (**57**); 3'-hydroxylation of indan (**58** and **59**); and *N*-depyrido methylation (Balani *et al.,* 1995). In experiments with liver microsomes, the production of the metabolite *cis*-(indan) hydroxylated by isoforms of the CYP2D subfamily was observed in Rhesus and Cynomolgus monkeys, while *trans*-(indan) hydroxylation and *N*-desalkylation were also observed in chimpanzees and humans, mediated by CYP3A4. The extent of metabolism in humans was the lowest among the four species studied (Chiba *et al*., 2000).

Anari and co-workers (2004) described the metabolic profile of indinavir, combining data obtained after analysis of human liver post-mitochondrial preparations in liquid chromatography coupled to mass spectroscopy (LC-MS/MS), and metabolic predictions generated by research based on structural similarity using the MDL Metabolite Database software. Two desalkylated, eleven monoxydated, three deoxygenated and two desalkylated/monoxydated metabolites were identified, and the major derivatives resulted from the oxidation of pyridine, indanyl and fenylmethyl groups, and from *N*-desalkylation of piperazinil (Anari *et al.,* 2004). Similar metabolites were found previously in another experiment employing LC-MS/MS analysis of the metabolites obtained from *in vitro* study (Yu, Cui, Davis, 1999).

Ritonavir (Figure 14) is extensively metabolized by rat, dog and human livers, through a variety of oxidative pathways and conjugation with glucuronic acid (**60**). The latter was only observed in dogs. The major metabolites identified in urine and feces of these three species were
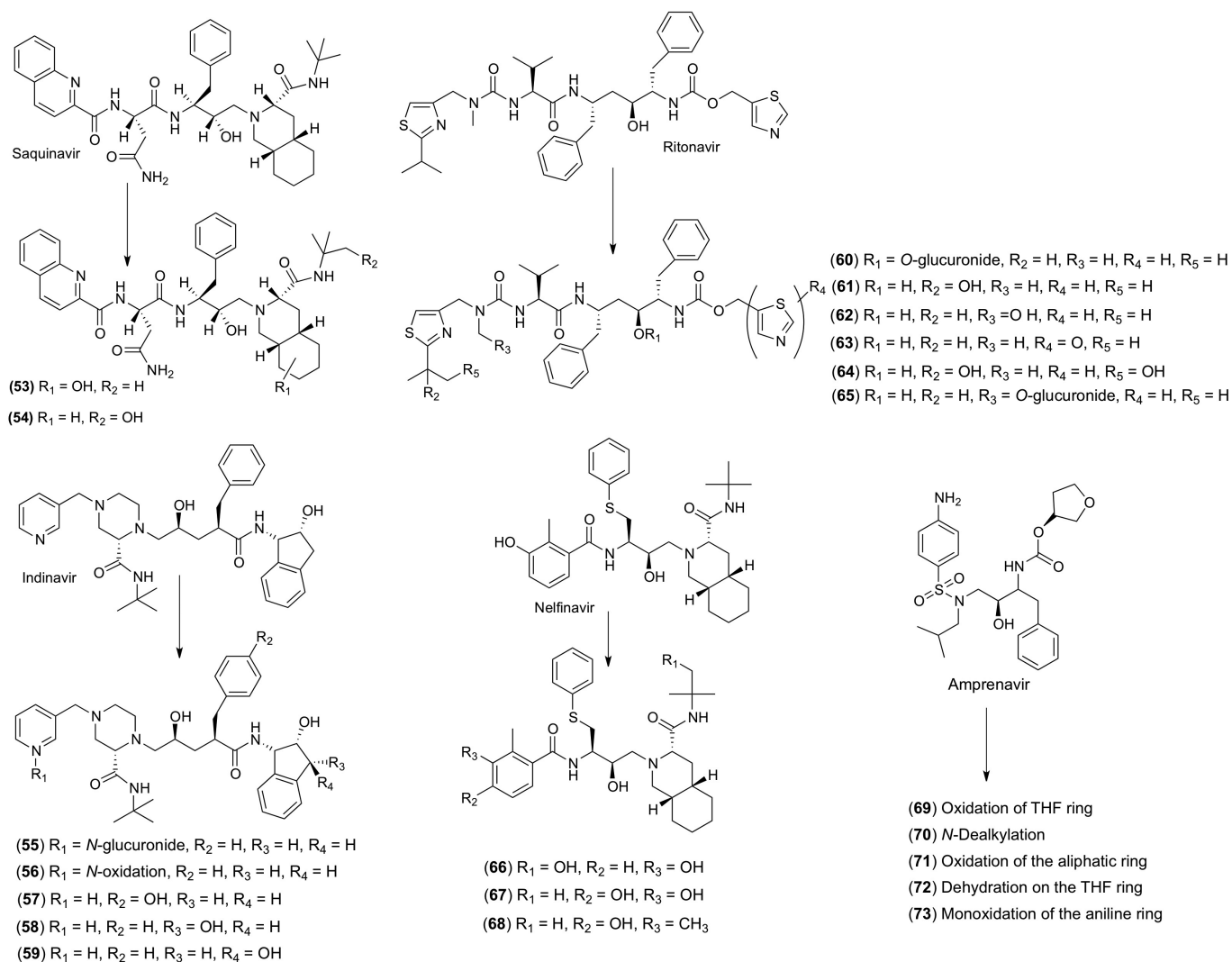
(60) R₁ = O-glucuronide, R₂ = H, R₃ = H, R₄ = H, R₅ = H
(61) R₁ = H, R₂ = OH, R₃ = H, R₄ = H, R₅ = H
(62) R₁ = H, R₂ = H, R₃ = O H, R₄ = H, R₅ = H
(63) R₁ = H, R₂ = H, R₃ = H, R₄ = O, R₅ = H
(64) R₁ = H, R₂ = OH, R₃ = H, R₄ = H, R₅ = OH
(65) R₁ = H, R₂ = H, R₃ = O-glucuronide, R₄ = H, R₅ = H

(53) R₁ = OH, R₂ = H
(54) R₁ = H, R₂ = OH

(55) R₁ = N-glucuronide, R₂ = H, R₃ = H, R₄ = H
(56) R₁ = N-oxidation, R₂ = H, R₃ = H, R₄ = H
(57) R₁ = H, R₂ = OH, R₃ = H, R₄ = H
(58) R₁ = H, R₂ = H, R₃ = OH, R₄ = H
(59) R₁ = H, R₂ = H, R₃ = H, R₄ = OH

(66) R₁ = OH, R₂ = H, R₃ = OH
(67) R₁ = H, R₂ = OH, R₃ = OH
(68) R₁ = H, R₂ = OH, R₃ = CH₃

(69) Oxidation of THF ring
(70) N-Dealkylation
(71) Oxidation of the aliphatic ring
(72) Dehydration on the THF ring
(73) Monoxidation of the aniline ring

FIGURE 14 – Main metabolites of protease inhibitors, saquinavir, indinavir, ritonavir, nelfinavir and amprenavir.

produced by loss of the thiazolyl carbamate, oxidation and loss of isopropyl-thiazolyl (**61**). Moreover, there was formation of *N*-hydroxymethyl urea (**62**), oxidation of the thiazolyl heteroatom (**63**) and a second oxidation of isopropyl of **61** (**64**), in rats only, as well as oxidation of the methyl thiazolyl from the metabolite that lost the carbamate thiazolyl, in dogs, loss of formaldehyde from **62** in rats and dogs, producing *N*-demethyl urea followed by oxidation to produce *N*-hydroxy urea. Glucuronidation of **62** was also observed in dogs (**65**). *In vitro* experiments demonstrated that the metabolite **61** and the metabolite that lost the isopropyl-thiazolyl group have antiretroviral activity (Denissen *et al.*, 1997). A study using human liver microsomes showed that the production of **61** is mediated by enzymes of the CYP3A subfamily and by the isoform CYP2D6 (Kumar *et al.,* 1996). In addition to being metabolized by CYP3A, ritonavir is also a potent inhibitor of this subfamily (Kumar *et al.,* 1999).

Nelfinavir (NFV) (Figure 14) is biotransformed in the liver into three major metabolites, identified as nelfinavir-hydroxy-*t*-butyl-amide (**66**), 3,4-dihydroxy-nelfinavir (**67**) and 3-methoxy-4-hydroxy-nelfinavir (**68**) (Zhang *et al.,* 1998). Compound **66** shows antiretroviral activity *in vitro* comparable to that of NFV, contributing to its oral activity in anti-HIV therapy, while **68** showed low activity (Zhang *et al.,* 2001; Hirani, Raucy, Lasker, 2004). It has been shown that CYP3A4 is involved in the formation of metabolites **67** and **68**, while **66** is produced by CYP2C19 (Wu *et al.,* 1998; Lillibridge *et al.,* 1998; Khaliq *et al.*, 2000). **66** can also be metabolized by CYP3A4. Nelfinavir is also an inhibitor of CYP3A4 (Lillibridge *et al.*, 1998) and CYP2B6 (Hesse *et al.,* 2001) isoforms.

Amprenavir (Figure 14) is primarily oxidized in the liver by enzymes CYP450 (Decker *et al.*, 1998). Experiments with human liver microsomes showed the production of five metabolites, the majority obtained by

oxidation of the tetrahydrofuran ring (THF) (**69**). The others were formed by *N*-desalkylation (**70**), oxidation of the aliphatic chain (**71**), dehydration of the THF ring (**72**) and mono-oxidation of the aniline ring (**73**). Enzymes of CYP3A subfamily appear to be involved in the formation of **69**, **71** and **73**, while CYP2C9 contributes to the achievement of **71**. It was also observed that the metabolism of amprenavir is reduced in neonates due to non-maturation of CYPs 3A4 and 3A5 (Tréluyer *et al.,* 2003).

Fusion Inhibitors (FIs)

Enfuvirtide is the only fusion inhibitor (FI) approved (March 2003) by the US FDA, and to be licensed for the treatment of HIV-1 infection in the US, Australia and Europe (Fletcher, 2003). This compound is a synthetic polypeptide of 36 amino acids that is homologous to, and engages in a coil-coil interaction, with the heptad repeat (HR) regions of the viral glycoprotein gp41 (Matthews *et al.,* 2004). As a consequence of this interaction, the fusion of the virus particle with the outer cell membrane is blocked. The FI enfuvirtide is the only anti-HIV compound that has a polymeric (i.e. polypeptidic) structure (structure not disclosed), and hence, is not orally bioavailable: it has to be injected parenterally (subcutaneously) twice daily. This makes the long-term use of enfuvirtide cumbersome and problematic. Enfuvirtide is primarily used in salvage therapy as part of drug combination regimens. Because enfuvirtide is a peptide, it is expected to undergo catabolism rather than metabolism by cytochrome P450 enzymes (Ferraiolo, Mohler, Gloff, 1992). Enfuvirtide did not inhibit the activities of CYP1A2, CYP2C19, CYP2D6, CYP2E1 or CYP3A4 in an open label, crossover trial in 12 HIV-infected adults (Hammer *et al.,* 2006).

Co-Receptor Inhibitors (CRIs)

Co-receptor inhibitors (CRIs) interact with the co-receptor CCR5 or CXCR4 used by M (macrophage)-tropic and T (lymphocyte)-tropic HIV strains, respectively (now generally termed R5 and X4 strains, respectively), to enter the target cells. Within the whole virus-cell entry process, the interaction of the viral glycoprotein gp120 and the co-receptor falls between the interaction of the viral glycoprotein gp120 and the CD4 receptor, and the fusion of the viral glycoprotein gp41 with the outer cell membrane (Westby, van der Ryst, 2005). There is, at present, only one CRI available (licensed in 2007 for clinical use), namely, the CCR5 antagonist maraviroc (Perros, 2007). The major problem with CCR5 antagonists is that they are only active against R5 HIV strains, and that from a mixed population of X4/R5 HIV strains, they stimulate the selection of X4 strains. Ideally, a CCR5 antagonist should be combined with a CXCR4 antagonist so as to block both X4 and R5 HIV strains (Door *et al.*, 2005).

Maraviroc is rapidly absorbed and extensively metabolized, although unchanged drug is the major circulating component in plasma and is the major excreted component after oral dosing. The metabolite profiling of plasma, urine and fecal samples showed that maraviroc is extensively metabolized with the major pathways involving oxidation and *N*-dealkylation reactions (Figure 15) (Walker *et al.,* 2005; Abel *et al.,* 2008). The most abundant metabolite detected in human plasma was a secondary amine product of *N*-dealkylation (**74**, Figure 15). Moreover, Hyland and co-workers (2008) have demonstrated that maraviroc is predominantly metabolized by CYP3A4, and is not itself an inhibitor of CYP enzymes.

Integrase Inhibitors (INIs)

Although the integrase has been pursued for many years as a potential target for the development of new anti-HIV compounds, the first integrase inhibitor (INI) licensed to treat HIV-1 infection, raltegravir, has only recently (in late 2007) been approved. The HIV integrase has essentially two important catalytic functions (3'-processing and strand transfer) (Chiu, Davies, 2004). Raltegravir (Figure 16) is targeted at the strand transfer reaction, as is elvitegravir (Figure 16), which is now entering phase III clinical trials. Elvitegravir is intended for once daily dosing (orally), while raltegravir has to be administered twice daily. It has proven highly effective in reducing viral loads in HIV-infected patients (Grinsztejn *et al.,* 2007; Steigbigel *et al.,* 2008; Cooper *et al.*, 2008).

In preclinical studies, raltegravir was metabolized predominantly in the liver via the UDP-glucuronosyltransferase (UGT)-mediated pathway. *In vivo* and *in vitro* studies using isoform selective chemical inhibitors and cDNA-expressed UGTs showed that UGT1A1 is the primary enzyme responsible for the formation of raltegravir-glucuronide (**87**) (*Merck & Co,* 2007; Kassahun *et al.,* 2007). *In vitro* studies demonstrate that raltegravir is not a substrate for cytochrome P450 (CYP) enzymes and does not inhibit CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A or induce 3A4 (Iwamoto *et al.,* 2008). Similarly, raltegravir is not an inhibitor of UGTs (UGT1A1 and UGT2B7) or P-glycoprotein (P-gp)-mediated transport (*Merck & Co,* 2007).

In clinical studies, metabolism via UGT1A1-mediated glucuronidation is the major pathway of elimination of raltegravir, with a minor component of elimination via kidney (~9% of total dose excreted unchanged
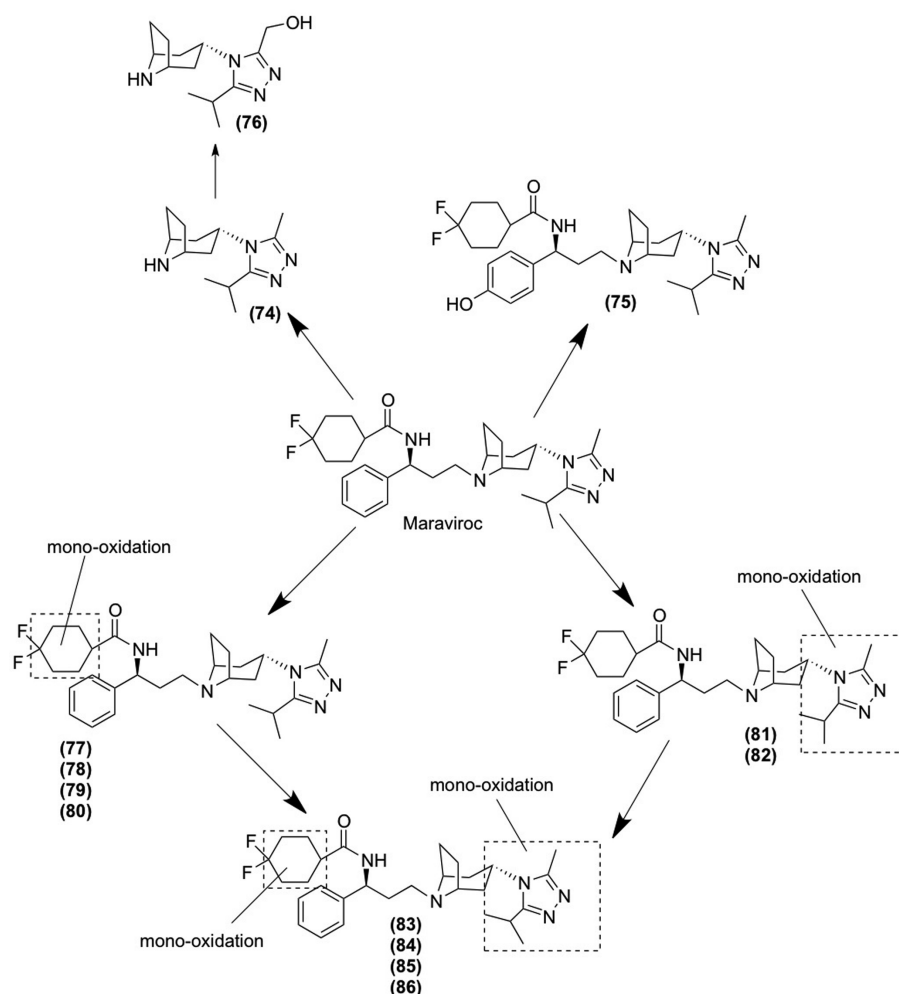
FIGURE 15 – Proposed metabolic pathways for maraviroc.

into urine). The only metabolite detected in humans was the phenolic hydroxyl glucuronide (**87**), and this metabolite does not have antiviral activity (Kassahun *et al.,* 2007) (Figure 16). In a human ADME study, ~51 and 32% of a radiolabeled raltegravir dose (parent and metabolite) was excreted in the feces and urine, respectively. The major circulating entity in plasma was the parent compound (69% of the total drug-related material in plasma), whereas most of the drug-related material in urine was accounted for by the glucuronide derivative (72% of the drug-related material in urine). In feces, only parent compound was detected, but it is likely that a good proportion of the raltegravir detected in feces is derived from hydrolysis of the glucuronide derivative secreted in bile as observed in preclinical species (Kassahun *et al.,* 2007).

Elvitegravir undergoes metabolism primarily by CYP3A and also by glucuronidation via UGT1A1/3, which is the major pathway of elimination. Metabolites formed by these pathways are markedly less potent (5- to
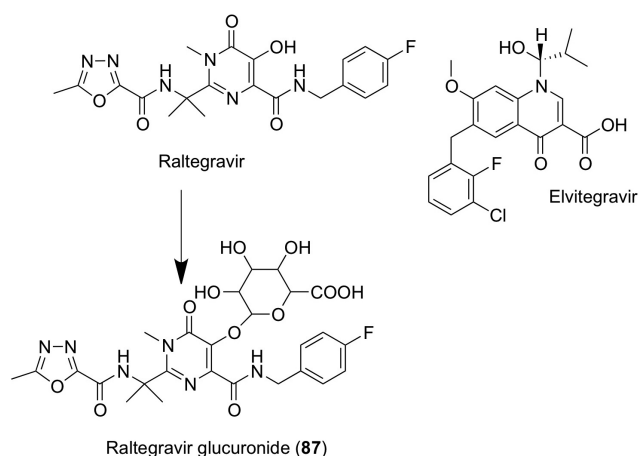

FIGURE 16 – Structures of raltegravir, its glucuronide metabolite and elvitegravir.

38-fold) than the parent drug and are present in plasma at lower concentrations; thus, the metabolites do not contribute to the antiviral activity of elvitegravir (Ramanathan *et al*., 2007a).

In a human ADME study with radiolabeled elvitegravir and boosting doses of ritonavir, >90% of the circulating plasma was present as the parent compound. Radiolabeled compound was excreted primarily in the feces (94.8%), with a minor amount in the urine (6.7%). Fecal radioactivity consisted primarily of parent compound (36%) and an oxidative metabolite (46%). Urine contained low levels of various metabolites (Ramanathan *et al.,* 2007b).

FINAL CONSIDERATIONS

Despite the significant advances made in understanding the mechanism of HIV infection and in identifying effective treatment approaches, the search for optimum treatment strategies for AIDS remains a major challenge.

This review has demonstrated that the cytochrome P450 family of enzymes is responsible for the metabolism of the majority of antiretroviral drugs, but for some of the nucleoside reverse transcriptase inhibitors (NRTIs), hepatic conjugation via UDP-glucuronosyltransferases as well as via alcohol dehydrogenase is important. NRTIs are the most studied class of anti-HIV agents in terms of metabolism. In contrast, limited published data are currently available on the other classes of anti-HIV agents and much of the available information is obtained from manufacturers and the clinical studies sponsored by the manufacturers. More systematic studies on metabolism, such as metabolite formation, the presence of active metabolites and mechanisms of chemical toxicity, are needed regarding antiretroviral therapy.

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