CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-814

Pharmacology Review(s)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-814
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	12/22/04
PRODUCT:	Aptivus (Tipranavir)
INTENDED CLINICAL POPULATION:	HIV-1 infected patients who are protease inhibitor treatment experienced.
SPONSOR:	Boehringer Ingelheim Pharmaceuticals, Inc. 900 Ridgebury Road P.O. Box 368 Ridgefield, CT 06877
DOCUMENTS REVIEWED:	Modules 1, 2 and 4
REVIEW DIVISION:	Division of Antiviral Drug Products (HFD-530)
PHARM/TOX REVIEWER:	Anita Bigger, Ph.D.
PHARM/TOX SUPERVISOR:	James Farrelly, Ph.D.
DIVISION DIRECTOR:	Debra Birnkrandt, M.D.
PROJECT MANAGER:	Tanima Sinha, M.S.

Date of review submission to Division File System (DFS): June 22, 2005

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The recommendation from the pharmacology/toxicology perspective is to approve.

B. Recommendation for nonclinical studies

Carcinogenicity studies are ongoing and will be completed as a Post-Marketing Commitment.

C. Recommendations on labeling

The wording agreed upon by the NDA team and the sponsor for the pharmacology/toxicology portions of the label can be found at the end of this document (see Overall Conclusions and Recommendations).

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Safety pharmacology assays assessed TPV effects on a number of organ systems, including cardiovascular, central nervous, pulmonary, renal and gastrointestinal (GI) systems. TPV was well-tolerated with some effects in the renal and GI. In renal studies in rats, females exhibited increases in sodium excretion at all doses and decreases in potassium excretion at the high dose. Male rats exhibited decreases in potassium excretion at all doses. Although the changes in urinary electrolyte excretion were considered TPV-related, neither of these findings was correlated with any significant observation in a 4-week oral dose toxicity study of TPV in rats. In GI studies in rats, gastric emptying and GI propulsion were significantly decreased at all doses in males and at middle and high doses in females. Gastric fluid volume was increased at the high dose in males and acid concentration of gastric fluid was decreased at the high dose in males and females. These changes were considered TPV-related. Results of these studies suggest that TPV may elicit some effects on renal and/or GI function at therapeutic doses.

TPV was assessed both in vitro and in vivo for cardiovascular toxicity potential. TPV showed an inhibitory effect in vitro on the HERG-associated potassium channel (IC_{50} = 2.9 μM) but no effect on action potential duration in guinea pig papillary muscle tissue at concentrations up to 10 μM. TPV demonstrated no drug related effects in vivo on mean arterial pressure, heart rate or ECG (including QT interval) in the beagle dog dosed with TPV up to 160 mg/kg. In addition, no evidence of cardiovascular effects was observed in toxicity studies of up to 26 weeks in dogs with TPV/RTV or up to 39 weeks in dogs with TPV alone and no prolongation of QT interval has been observed in multiple clinical studies.

The primary TPV target organs identified through repeat-dose toxicology studies in mice, rats, dogs and/or monkeys are the liver and gastrointestinal tract. Additional organs that were affected included the thyroid gland, testes and to a lesser extent the adrenal gland, kidneys, spleen and heart. Co-administration of TPV and RTV in rats and dogs revealed only target organs or signs of toxicity seen when each compound was administered alone. Neither drug exacerbated the toxicity of the other.

It should be noted that in rats and dogs, TPV exposure in animals at the NOAEL doses is equivalent to or slightly above exposure in humans at the clinical dose of 500/200 TPV/RTV BID. Early toxicity studies were performed with TPV alone. Once the decision was made for co-administration with RTV in the clinic, bridging toxicity studies with both compounds were performed. Co-administration does increase the exposure of TPV, especially at low doses, but the effect is lower at higher doses. In animals, the boosting effect of co-administration is generally lower than in humans (11-fold increase) and in particular much lower at NOAEL doses in repeat-dose toxicology studies: mice (12- to 22-fold), rats (6- to 7-fold), dogs (3- to 13-fold) and monkeys (2-fold). However, toxicities seen in repeat-dose toxicology studies are not considered to preclude chronic administration of TPV to the intended patients, even though plasma levels are equivalent to or below human exposure. These toxicities are reversible, manageable, species specific and/or considered secondary to species-specific hepatic enzyme-inducing effects of TPV in the rodent.

Gastrointestinal effects included emesis, soft stools, diarrhea and/or excessive salivation after dosing and were observed in all species tested. These effects probably effect local actions since no correlative macroscopic or microscopic changes in the GI tract were observed in mice, rats or dogs.

TPV is a microsomal enzyme inducer and has been shown to increase activity of CYP 3A and CYP 3B in rats and dogs. Changes associated with hepatic enzyme induction, increased liver weights, hepatocellular hypertrophy and increases in smooth endoplasmic reticulum have been seen in nonclinical studies in mice, rats and dogs. Additional changes considered secondary to enzyme induction and specific to rodents were seen generally at high doses and included hepatocellular degeneration, vacuolation, necrosis and mineral deposition in mice and multinucleated hepatocytes in rats. Karyomegaly, an effect of RTV in rats, was observed at a low incidence in the 26-week TPV/RTV study. Histological changes specific to dogs included bile duct hyperplasia and gallbladder cystic hyperplasia at a high dose in the 39-week TPV alone study. This effect was not seen in the 26-week TPV/RTV dog study. These effects were reversible and enzyme induction caused by TPV with resultant hepatocellular hypertrophy is considered an adaptive response and not evidence of toxicity.

The rat is the more inducible species, with males showing more activity than females. This is reflected in the fact that females have higher plasma concentrations of TPV than males after repeated doses of TPV. Additional effects seen in the rat could be secondary to hepatic microsomal enzyme induction. These include increased metabolism and clearance of thyroid

hormones, slight increase in plasma proteins and potentialy effects on coagulation parameters. Increases in plasma proteins were seen in rat studies as increases in total protein, albumin (accompanied by a slight increase in plasma calcium in several studies) and/or globulin. Increased thyroid gland weights, thyroid follicular hypertrophy, increases in TSH and decreases in T3 and T4 were seen in rat studies and are considered to reflect a rodent specific increase in thyroid hormone metabolism secondary to induction of hepatic enzymes. Changes in thyroid parameters were monitored in the clinic in early trials. The changes seen in rodents in nonclinical studies were not seen in humans. TPV clearly increases coagulation parameters (prothrombin time and activated partial thromboplastin time) in rodents but the mechanism is unknown. It may be caused by an indirect mechanism related to hepatic enzyme induction in rodents. No similar changes were seen in dog studies. Monitoring of PT was performed in clinical trials and no significant changes in this parameter were observed in humans.

Dogs exposed to TPV or TPV/RTV exhibited mild increases in liver alkaline phosphatase (AP) isoenzymes and this suggests an effect on the liver. Histopathological effects in dogs included gallbladder cystic glandular hyperplasia and bile duct hyperplasia in long term repeat dose studies. These effects are common in older beagle dogs but were increased in TPV treated dogs. In the absence of more severe histopathology, such as biliary stasis or cholestasis, these changes raise little concern for humans. This is also in contrast to the observation that rats exhibited decreased serum AP at higher dose levels in a number of studies.

In mice, enzyme leakage (ALT, AST) at high dose levels was correlated with hepatocellular necrosis. Increases in AST and/or ALT were observed minimally or not at all in toxicology studies on rats and dogs. Based on this difference in species, the importance for humans is not clear. However, liver function can be easily monitored in humans and the nonclinical studies support monitoring as a way of managing this potential human toxicity.

The safety of the self-emulsifying drug delivery system (SEDDS) formulation was explored in a 26-week TPV/RTV dog study to eliminate concerns over the high dose [ ] from 500/200 mg/kg TPV/RTV BID) of Cremophor EL (CrEL) contained in this formulation. Of concern was the possibility that CrEL might pass from the GI tract into systemic circulation and thereby pose a risk of anaphylactoid reactions since CrEL is known to cause these reactions if given in high levels IV. Consequently, exposures to the SEDDS formulation were chosen to achieve 1, 10 and 30-fold exposure to CrEL in humans. CrEL plasma levels were detectable 2 hours after the first or second dose in several animal administered high dose SEDDS and one animal intermediate dose SEDDS. Plasma CrEL levels ranged from [ ] mg/ml. These levels are unlikely to cause anaphylactoid reactions. The NOAEL for SEDDS is considered to be 910 mg/kg/day SEDDS which supports a 10-fold safety factor for the SEDDS vehicle in the recommended human dose.

TPV was tested for the ability to induce point mutations in DNA (mutagenicity) and the ability to damage chromosomes (clastogenicity) in five in vitro and in vivo assays including the battery of assays specificed in the ICH S2B guidance on genotoxicity. These included the in vitro bacterial

reverse mutation assay, unscheduled DNA synthesis (UDS) in rat hepatocytes, induction of gene mutation in Chinese hamster ovary cells and a chromosome aberration assay in human peripheral lymphocytes, as well as an in vivo bone marrow micronucleus assay in mice. TPV was negative in these assays, indicating that TPV has no mutagenic or clastogenic potential.

A male and female fertility and early embryonic development study (oral) in Sprague-Dawley rats demonstrated that TPV did not affect spermatogenesis, estrous cycle, copulation, conception, fertility, implantation or early embryonic development at doses up to 1000 mg/kg/day. This corresponds to a Cmax of 258 μM which is approximately two-fold the human Cmax at the proposed clinical dose of 500/200 TPV/RTV BID.

In an embryo-fetal development studies in Sprague-Dawley rats, there was no evidence of TPV-related embryolethality or teratogenicity at doses of 40 to 1000 mg/kg/day. However, the NOAEL for both maternal and developmental toxicity was 40 mg/kg/day, based on findings of postdose salivation, decreased body weight and food consumption in dams and decreased body weight and sternebrae ossification in fetuses. This NOAEL corresponds to a mean Cmax 30.4 μM and a mean AUC of 340 μM.h, which is 0.2-fold of the expected human exposure at the proposed dose of 500/200 TPV/RTV BID.

In embryo-fetal development studies in pregnant rabbits treated with TPV (on gestation days 6 through 20), maternal toxicity (death of one female, abortions, decreased body weight and food consumption and increased clinical signs) and developmental toxicity (slightly decreased fetal body weights, fetuses with wavy ribs and bent femurs and increased incidence of fetuses with gross malformations) were observed at the high dose. Interpretation of these fetal findings is complicated by maternal toxicity and a litter effect. These gross malformations were not observed in fetuses at 375 and 759 mg/kg/day TPV in the dose range-finding study in rabbits. Therefore, it is unlikely that TPV was teratogenic at 375 mg/kg/day. The NOAEL for maternal toxicity was 75 mg/kg/day while the NOAEL for developmental toxicity was 150 mg/kg/day. The AUCs associated with these NOAEL doses correspond to 0.04-fold and 0.08-fold, respectively, the human exposure at the proposed clinical dose of 500/200 TPV/RTV BID.

In a pre- and post-natal development study in rats, TPV was toxic to dams and suckling pups at 400 and 1000 mg/kg/day with dose-relationship. Maternal toxicity was restricted to adverse effects on body weight and food consumption. Pup toxicity consisted of slight to marked progressive growth inhibition throughout lactation, resulting in persistent adverse influence on the growth of the pups up to maturity. However, none of the postweaning functions examined in F1 offspring, including reproductive ability, were compromised up to the 1000 mg/kg/day dose and there was no evidence of teratogenicity at any dose. The 40 mg/kg/day dose was an NOAEL for both the dams and offspring.

The results from these reproductive toxicology studies indicate that TPV is not teratogenic. However, since human exposure levels are above the rat and rabbit NOAEL exposure levels

derived from these studies, TPV should be given during pregnancy only if the benefit to the mother and the fetus outweighs the risk to the fetus. Distribution studies in rats administered radiolabeled TPV demonstrated that radioactivity is excreted into the milk of rats. Therefore, women should be cautioned to avoid breastfeeding while taking TPV.

The immunotoxic potential of TPV was assessed in a series of immunotoxicology assays. In a mouse immune function study, a single dose of TPV inhibited anti-CD3-dependent T-cell stimulation as measured by IL-2 concentrations. Also a minimal, albeit statistically significant, T cell-driven delayed type hypersensitivity (DTH) was observed as measured in [redacted]. Although there were no additional appropriate assays for further investigation of immunostimulatory potential of TPV, an additional assay, the T-dependent antigen response to sheep red blood cells was performed to confirm or negate TPV immunosuppressive potential. The results of this study demonstrated lack of immunosuppressive potential for TPV and/or RTV under the conditions of the study.

B. Pharmacologic activity

See review by Microbiology Reviewer.

C. Nonclinical safety issues relevant to clinical use

Major targets organs for TPV in nonclinical studies are the gastrointestinal tract and the liver. The nonclinical studies support monitoring of GI and liver function in the clinic.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

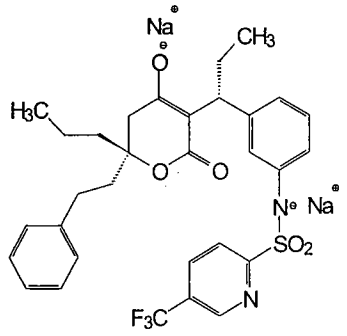
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-814
Review number:
Sequence number/date/type of submission: 000/12-22-04/IT
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Road
P.O. Box 368
Ridgefield, CT 06877

Reviewer name: Anita Bigger, PhD
Division name: Division of Antiviral Drug Products
HFD #: 530
Review completion date: June 22, 2005.

Drug:

Trade name: Aptivus (tipranavir)
Generic name:
Code name: TPV; PNU-140690
Chemical name: N-[3-[(1R)-1-[5,6-dihydro-4-hydroxy-2-oxo-6(R)-(2-phenylethyl)-6-propyl-2H-pyran-3-yl]propyl]phenyl]-5-(trifluoromethyl)-2-pyridinesulfonamide
CAS registry number: 174484-41-4
Molecular formula/molecular weight: $C_{31}H_{33}F_3N_2O_5S/602.7$
Structure:



Relevant INDs/NDAs/DMFs: IND 51,979

Drug class: Protease Inhibitor

Intended clinical population: Treatment of HIV-1 infection in patients who are protease inhibitor treatment experienced.

Clinical formulation: Self Emulsifying Drug Delivery System (SEDDS)

Route of administration: Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

TPV was investigated in safety pharmacology assays, a series of secondary pharmacodynamic immune function tests and a biochemical receptor assay screen.

Safety pharmacology assays assessed TPV effects on a number of organ systems, including cardiovascular, central nervous, pulmonary, renal and gastrointestinal (GI) systems. TPV was well-tolerated with some effects in the renal and GI. In renal studies in rats, females exhibited increases in sodium excretion at all doses and decreases in potassium excretion at the high dose. Male rats exhibited decreases in potassium excretion at all doses. Although the changes in urinary electrolyte excretion were considered TPV-related, neither of these findings was correlated with any significant observation in a 4-week oral dose toxicity study of TPV in rats. In GI studies in rats, gastric emptying and GI propulsion were significantly decreased at all doses in males and at middle and high doses in females. Gastric fluid volume was increased at the high dose in males and acid concentration of gastric fluid was decreased at the high dose in males and females. These changes were considered TPV-related. Results of these studies suggest that TPV may elicit some effects on renal and/or GI function at therapeutic doses.

TPV was assessed both in vitro and in vivo for cardiovascular toxicity potential. TPV showed an inhibitory effect in vitro on the HERG-associated potassium channel (IC_{50} = 2.9 μM) but no effect on action potential duration in guinea pig papillary muscle tissue at concentrations up to 10 μM. TPV demonstrated no drug related effects in vivo on mean arterial pressure, heart rate or ECG (including QT interval) in the beagle dog dosed with TPV up to 160 mg/kg. In addition, no evidence of cardiovascular effects was observed in toxicity studies of up to 26 weeks in dogs with TPV/RTV or up to 39 weeks in dogs with TPV alone and no prolongation of QT interval has been observed in multiple clinical studies.

TPV was evaluated for effects on immune function to determine if it possessed immunomodulatory potential. Evidence from repeat-dose studies in mice, rats and dogs suggested that TPV might have immunostimulatory and/or immunosuppressive potential. In a mouse immune function study (185) a single dose of TPV inhibited anti-CD3-dependent T-cell stimulation as measured by IL-2 concentrations. Also a minimal, albeit statistically significant, T cell-driven delayed type hypersensitivity (DTH) was observed as measured in [redacted] Although there were no additional appropriate assays for further investigation of immunostimulatory potential of TPV, an additional assay, the T-dependent antigen response to sheep red blood cells was performed to confirm or negate TPV immunosuppressive potential. The results of this study showed that treatment with TPV alone or TPV/RTV did not adversely affect the functional ability of the humoral component of the immune system in female CD-1 mice, as evaluated in the IgM antibody-forming cell response to the T-dependent antigen, sheep red blood cells. Thus, the potential for TPV to be immunosuppressive was negated.

TPV was also evaluated in a biochemical receptor screen [redacted] and in general showed a low inhibitory profile against a variety of receptor targets at concentrations up to 10μM. The only exception was the cholecystokinin-A (CCK-A) receptor binding assay where TPV was shown to bind with some affinity (82%) at 10μM. The relevance of this finding is unknown.

2.6.2.2 Primary pharmacodynamics
(Reviewed by Microbiology Reviewer)

Mechanism of action:

Drug activity related to proposed indication:

2.6.2.3 Secondary pharmacodynamics

See IND 51,979 (474) Summary of Immunotoxicity Findings for Tipranavir in Appendix.

Study title: In vivo assessment of Tipranavir (EXRS 1406 XX) effects on murine immune function.

Key study findings: Tipranavir showed modest to marginal effects at oral doses up to 300 mg/kg on assays designed to examine T cell, B cell and complex inflammatory responses.

Study no.: U02-3010
Volume # and page #: Module 4, M-002, vol. 1.2, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc.

Pharmacology Department
900 Ridgebury Road
Ridgefield CT 06877

Date of study initiation: Unknown. Report Date 2-11-02
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: Not reported.

Methods and Results

1. In Vivo Anti-CD3 Assay

A single dose (30, 100 or 300 mg/kg) of tipranavir was administered orally to female BALB/c mice prior to injection with a monoclonal antibody to mouse CD3 to measure the direct effects of the compound on T cell activation as assessed by plasma IL-2 levels.

In the first assay IL-2 was inhibited 46% by tipranavir at 300 mg/kg. These mice had been fasted overnight and exhibited marked diarrhea at the high dose. Since diarrhea can impair immune responses in this model, the assay was repeated using unfasted mice. In the repeat assay, IL-2 was inhibited 39%, 25% and 27% by 300, 100 and 30 mg/kg of tipranavir, respectively.

2. In Vivo Delayed Type Hypersensitivity (DTH)

Male BALB/c mice were immunized intradermally at the tail base with [illegible] Seven days later, DTH responses were elicited by subcutaneous injection of [illegible] into the dorsal surfaces of each ear, eliciting the maximal swelling response at 24 hours post-injection. In the treatment groups, a single dose (30, 100 or 300 mg/kg) was administered 1 hour prior to ear injection. Ear thickness is before and 24 hours after the injection. The change in ear thickness is calculated as a measure of the DTH response.

There was a statistically significant effect at the high dose. The sponsor notes that the mice were still able to mount a robust T cell and inflammatory response even at the high dose.

3. In Vivo T Cell Independent B Cell Activation

Trinitrophenyl (TNP)-conjugated lipopolysaccharide (LPS) was injected into mice to stimulate the polyclonal activation of B cells. Upon activation, these B cells produce TNP-specific IgM at levels which can be detected in the plasma by day 3. Tipranavir (30, 100 or 300 mg/kg) was administered orally 1 hour prior to TNP-LPS injection and 24 hours later. Tipranavir showed no detectable effects on B cell activation.

4. Efforts to address the ability of tipranavir to induce compound-specific immune response (i.e. allergic response to the compound itself) were hindered due to solubility and irritation. In the popliteal lymph node assay, five mg of soluble compounds are injected into the footpads of mice and seven days later the increase in draining lymph node size is measured as an indication of compound-triggered immune responses. Only 3 mg of tipranavir could be injected per paw. Even at this level, the paws were extremely swollen and showed evidence of scabbing, suggesting an irritant response. Thus, immunogenicity could not be adequately assessed for tipranavir.

Study title: T-Cell dependent antigen response to Tipranavir and Ritonavir in female CD-1 mice (BIPI Toxicology Study No. 04R103).

Key study findings: The results of this immunotoxicological evaluation demonstrate that treatment with TPV alone or co-administered with RTV did not adversely affect the functional ability of the humoral component of the immune system in female CD-1 mice. Therefore, the study demonstrated lack of immunosuppressive potential for TPV and/or RTV under the conditions of the study.

Study no.: 04R103

Volume # and page #: Submitted separately to NDA 21-814 on March 3, 2005. Also, submitted to IND 51,979 under submission number N-621.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877-0368

Date of report: January 4, 2005

Objective: The study was performed to assess the potential effects of TPV alone and when co-administered with RTV on the humoral immune component of the immune system, when evaluated in the antibody-forming cell response to the T-dependent antigen, sheep red blood cells. This study was initiated in response to a FDA request on August 30, 2004.

Materials and methods: Female CD-1 mice were administered TPV and/or RTV by oral gavage for 28 days at dose levels of 30/8, 100/26.7 and 300/80 mg/kg/day TPV/RTV, 300 mg/kg/day TPV or 80 mg/kg/day RTV. RTV was administered in propylene glycol at 5 ml/kg, followed approximately 1 hour later by TPV in an aqueous solution, pH 10.5, at 10 mg/kg. The study included a sham-dosed group as well as a vehicle group to control for stress induced by handling and vehicle. Sham treated Controls received deionized water and vehicle Controls received vehicles corresponding to the two drugs. A positive control group received 50 mg/kg/day cyclophosphamide IP for 4 days prior to terminal sacrifice. Clinical signs, mortality, body weights food consumption were monitored during the study. Four days prior to sacrifice, all animals received an IV injection of sheep red blood cells. At termination of the study, each animal was weighed, then sacrificed and the spleen was removed Blood was collected from the

vena cava and then the liver and thymus were removed. These organs were weighed. Spleens were processed for immunological evaluation, while livers and thymuses were retained in formalin for potential histopathologic evaluation.

Results and Discussion: Clinical signs included rough and stained hair. No effects were observed on body weight or food consumption during the course of the study. Terminal body weight measurements showed an increase (+8%) in mean body weights of sham control animals and animals treated with RTV alone, as compared to vehicle control mean body weights. No statistically significant effects were observed in either spleen or thymus weight in TPV and/or RTV treated groups but weights of both organs were decreased significantly with administration of the positive control. Liver weights were increased in animals treated with TPV or RTV.

Treatment with TPV/RTV, TPV or RTV did not result in significant changes in spleen cell number or in the IgM antibody-forming cell (AFC) response to the T-dependent antigen, sheep red blood cells, when evaluated as either specific activity (AFC/10^6 spleen cells) or as total spleen activity (AFC/spleen). The positive control decreased spleen cell number as well as specific activity and total spleen activity.

Dr. Steve Kunder reviewed report and found the study valid. Dr. Kunder agreed with the conclusion of the draft report that the study demonstrated lack of immunosuppressive potential for TPV and/or RTV.

Study title: HIV-1 and HIV-1 protease inhibitor U-140690 tested in 32 assays

Key study findings: TPV was tested in 31 receptor binding assay and 1 phosphoinositide turnover assay as an assessment of its broader selectivity. TPV was inactive in the assays used with the possible exception of the CCK-A receptor binding assay were TPV produced an apparent dose-related inhibition suggestive of a potency (IC_{50}) in the 0.1 to 10 μM range. Quantitative determination of the compound's affinity for the CCK-A site would require a complete competition binding study using a full range of doses. The relevance of this finding is unknown.

Study no.: U01-3033 (Pharmacia & Upjohn Technical Report 7295-96-034).
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI

Date of report: August 13, 1996

Objective: The objective of the study was to test TPV in a broad spectrum of binding and enzyme assays as part of its ongoing development as a potential treatment for AIDS.

Materials and methods: TPV was shipped to [ ] The compound was tested at three concentrations, 10^{-9}, 10^{-7} and 10^{-5} M in duplicate in 31 receptor binding assays and in a phosphoinositide (PI) turnover assay.

Results and Discussion: TPV was inactive in the assays even when tested at the highest concentration, 10^{-5} M, except for the CCK-A receptor. In this case a large degree of inhibition (82%) was observed only at the highest dose, suggesting a potency (IC_{50}) in the 0.1 to 10 μM range. Quantitative determination of the compound's affinity for the CCK-A site would require a complete competition binding study using a full range of doses.

2.6.2.4 Safety pharmacology

Neurological effects:

Study title: U-140690E: Evaluation of locomotor activity following a single oral dose in male and female Sprague-Dawley rats.

Key study findings: Single oral doses of 62.5, 200, 500 (females only) and 625 (males only) mg/kg TPV administered to Spraque-Dawley rats had no significant effect on any parameters of locomotor activity.

Study no.: U00-3102 (Pharmacia & Upjohn Technical Report No. 7228-96-130)
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI
Date of report: October 9, 1996

Objective: The objective of this non-GLP study was to determine the effect of a single oral dose of TPV on parameters of locomotor activity in male and female Sprague-Dawley rats.

Materials and methods: TPV [(A)5075-AS-1720, purity [ ] was administered to male and female — CD[SD]BR Sprague-Dawley rats as single oral doses of 0, 62.5, 200, 500 (females only) and 625 (males only) mg/kg in water adjusted to pH 10.5 with sodium hydroxide at a dose volume of 10 mg/kg. A total of 48 males and 48 females were assigned to the evaluation of locomotor activity with 12 rats/sex/dose. Each group was observed predose and 6 hours post dose.

Results and Discussion: There were no statistically significant changes in any of the parameters of locomotor activity following administration of TPV at all doses.

Study title: U-140690E: Modified Irwin's/Rectal Temperature Test following a single oral dose in male and female Sprague-Dawley rats.

Key study findings: Single oral administration of TPV at doses of 0, 62.5, 200, 500 (female) or 625 (male) mg/kg to rats had no biologically significant effects on any of the parameters of the Modified Irwin's/Rectal Temperature Test.

Study no.: U00-3101 (Pharmacia & Upjohn Technical Report No. 1470-96-016)
Volume # and page #: M002, vol. 1.2, page 1.
Conducting laboratory and location: Pharmacia & Upjohn Ltd., Tsukuba Research Laboratories, 23 Wadai, Tsukuba, Ibaraki, Japan

Date of report: October 14, 1996

Objective: The objective of this GLP-compliant study was to determine the effect of a single oral dose of TPV on parameters of the Modified Irwin's/Rectal Temperature Tests in male and female rats.

Materials and methods: TPV [(A)5075-AS-1768-J395, [ ] purity] was administered to respective groups of rats at doses of 0, 62.5, 200 and 500 (females) or 625 (males) mg/kg in aqueous vehicle adjusted to pH 10.5 using sodium hydroxide or hydrochloric acid at a volume of 10 ml/kg. Twenty-four males and 24 females were assigned to four groups (6/sex/group). Each group of rats was observed predose and six hours postdose.

Results and Discussion: Measurements made in the Modified Irwin's/Rectal Temperature Test were not affected at any dose level of TPV.

Cardiovascular effects:

Study title: Influence of Tipranavir on HERG-mediated potassium current in stably transfected HEK293 cells. Amendment No. 1

Key study findings: The IC_{50} of TPV on the HERG-mediated potassium channel was 2.9 μM. Together with a previous study which had tested the effect of TPV on action potential parameters in guinea pig papillary muscle in vitro, these studies indicate a low proarrhythmic potential.

Study no.: U02-1175

Volume # and page #: Module 4, M002, vol. 1.2, page 1.

Conducting laboratory and location: Boehringer Ingelheim Pharma KG, Birkendorfer Strase 65, 88397 Biberach an der Riss, Germany

Date of report: September 3, 2002

Objective: This non-GLP study was conducted to determine the influence of TPV on HERG (human ether-a-go-go-)-mediated potassium current as a marker for potential effects on myocardial repolarization.

Materials and methods: Experiment on HERG-mediated potassium current were performed using HEK293 (human embryonic kidney) cells stably expressing the HERG-mediated potassium current. Whole-cell experiments were carried out by means of the patch-clamp technique at room temperature. Effects on the HERG-channel current amplitude by TPV were measured for each concentration (0.1, 3.0, 10.0 μM) over 5 minutes. Control experiments were performed on every experimental day to confirm the stability of the preparation and to correct for the current run-down of the test system.

Results and Discussion: The IC_{50} for TPV on the HERG channel was 2.9 μM. Previous results with TPV on action potential configuration in guinea pig papillary muscle had revealed no effects up to 10 μM.

Study title: Effects of tipranavir (0.1 to 10 μM) on action potential configuration in isolated guinea pig papillary muscle.

Key study findings: TPV has no effect on action potential parameters in concentrations up to 10 μM, indicating a lack of effect on cardiac ion channels over a wide range of concentrations.

Study no.: U01-1226
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharma KG, Birkendorfer Strase 65, 88397 Biberach an der Riss, Germany

Date of report: February 13, 2001

Objective: The objective of this non-GLP study was to test the influence of TPV on myocardial action potential configuration.

Materials and methods: Action potentials were measured in isolated guinea pig papillary muscles. Three groups of experiments were performed: The first group (n=5) received TPV in cumulative doses of 0.1, 0.3, 1.0, 3.0 and 10.0 μM, the second group (n=5) received cisapride as a positive control in cumulative concentrations of 0.1, 0.3, 1.0, 3.0 and 10.0 μM and the third group (n=5) served as a control group and received equivalent volumes of the vehicle (DMSO).

Measurements were taken at a stimulation frequency of 0.33 Hz (20 cycles/min) and included action potential duration to 10, 30 and 90% repolarization (APD10, APD30 and APD90, respectively), resting membrane potential (RMP), maximal velocity of phase 0 upstroke (V-max), AP overshoot (OS), AP amplitude (APA) and the force of contraction (FOC).

Results and Discussion: No changes were observed in concentrations up to 10 μM TPV. Cisapride markedly prolonged the action potential by approximately 13.2%. These results indicate that TPV has no effect on cardiac ion channels over the entire concentration range tested.

Study title: U-140690E: Cardiovascular profile following a single oral dose in conscious Sprague-Dawley rats.

Key study findings: TPV administered at single oral doses of 62.5, 200 and 625 (males) or 500 (females) to Sprague-Dawley rats had no effect on mean arterial pressur or heart rate in either sex at doses of 62.5 and 200 mg/kg. There were small drug-related increases in mean arterial pressure in males and females at the high dose but these were not considered toxicologically relevant.

Study no.: U00-3103 (Pharmacia & Upjohn Technical Report 7228-96-143)
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI

Date of report: October 116, 1996

Objective: The objective of this non-GLP study was to determine the effect of a single oral dose of TPV on mean arterial pressure and heart rate in conscious Sprague-Dawley rats.

Materials and methods: The effects of oral administration by gastric intubation of TPV on mean arterial pressure and heart rate were evaluated in male and female Sprague-Dawley rats at dose levels of 0, 62.5, 200 and 625 (males) or 500 (females) mg/kg in aqueous solution, pH 10.5. A total of four males and four females were chronically instrumented with radiotelemetry transmitters to provide measurements of mean arterial pressure and heart rate. Each animal was treated with vehicle and each dose of drug in a Latin Square Cross-Over design. Each day of dosing was separated by a minimum of 72 hours. Heart rate and mean arterial pressure were measured twice prior to dosing and at 15 minute intervals postdose for seven hours.

Results and Discussion: TPV had no effect on mean arterial pressure or heart rate in either sex at doses of 62.5 and 200 mg/kg. A small increase in mean arterial pressure was observed in females dosed at 500 mg/kg. This effect was sustained for six hours. It was considered drug-related but the magnitude was too small to be viewed as toxicologically relevant.

A statistically significant increase in mean arterial pressure was observed in males at 625 mg/kg for the first 45 minutes following dosing. This change was not considered drug-related as it did not coincide with the maximum plasma concentration of TPV. This effect may have been a response to the high dose formulation.

Study title: U-140690E: Cardiovascular profile following a single oral dose in conscious beagle dogs.

Key study findings: Single oral administration of TPV at doses of 37.5, 80 and 160 mg/kg to male and female beagle dogs had no TPV-related effects on heart rate, mean arterial pressure, respiratory rate or on parametersof the electrocardiogram.

Study no.: U00-3104 (Pharmacia & Upjohn Technical Report 7228-96-144)
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI

Date of report: October 14, 1996

Objective: The objective of this non-GLP study was to determine the effect of a single oral dose of TPV on mean arterial pressure, heart rate, electrocardiographic parameters and respiratory rate in conscious beagle dogs.

Materials and methods: Four male and four femal young adult beagle dogs were evaluated with vehicle and three doses of TPV using a Latin Square Cross-Over design. Dose levels were 0, 37.5, 80 and 160 mg/kg of TPV (Lot No. (A)5075-AS-1768, 97.44% purity) in aqueous solution pH 10.5. Each animal received a bolus oral dose, followed by a flush of 15 ml sterile water, once per day on four separate days (different dose levels each day) with a washout period of at least 72 hours between doses. Mean arterial pressure, heart rate, electrocardiogram parameters and respiratory rate data were obtained twice predose and every 30 minutes postdose for nine hours.

Results and Discussion: There were no TPV-related effects on heart rate, mean arterial pressure, respiratory rate or on the parameters of the electrocardiogram at any of the doses tested.

Pulmonary effects:

Study title: Effect of tipranavir on pulmonary function in the conscious Sprague-Dawley rat after single dose administration

Key study findings: TPV had no effect on tidal volume or respiratory frequency at dose up to 500 mg/kg in female rats and 625 mg/kg in male rats.

Study no.: U01-3013
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877

Date of report: January 10, 2001

Objective: The objective of this non-GLP study was to evaluate the effects of a single dose of TPV on pulmonary function (respiratory frequency and tidal volume) in conscious Sprague-Dawley rats.

Materials and methods: Respiratory frequency and tidal volume were monitored using BUXCO whole body unrestrained plethysmograph chambers. TPV in aqueous solution pH 10.5 was studied at oral doses of 0, 62.5, 200 and 500 mg/kg (female rats) and 625 mg/kg (male rats) (n=3/sex/group). Blood samples were collected at 375 minutes postdose for plasma level determinations.

Results and Discussion: TPV exhibited no effects on tidal volume or respiratory frequency. No gross observations were noted for any animals in the study. Analysis of blood samples demonstrated detectable mean plasma levels of TPV.

Renal effects:

Study title: U-140690E: Renal profile following a single oral dose in male and female Sprague-Dawley rats.

Key study findings: Single oral administration of TPV at doses of 62.5, 200, 500 (females) and 625 (males) mg/kg to Sprague-Dawley rats did not affect water consumption or urine volume. Females exhibited increases in sodium excretion at all doses and decreases in potassium excretion at the high dose. Males exhibited decreases in potassium excretion at all doses. Although the changes in urinary electrolyte excretion were considered TPV-related, neither of these findings was correlated with any significant observation in a 4-week oral dose toxicity study of TPV in rats.

Study no.: U00-3098 (Pharmacia & Upjohn Technical Report 1470-96-018)
Volume # and page #: Module 4, M002, vol. 1.2, page 1.
Conducting laboratory and location: Pharmacia & Upjohn Ltd., Tsukuba Research Laboratories, 23 Wadai, Tsukuba, Ibaraki, Japan

Date of report: October 14, 1996

Objective: The objective of this GLP compliant study was to determine the effect of a single oral dose of TPV on water consumption, urine volume and urinary electrolyte excretion in male and female Sprague-Dawley rats.

Materials and methods: TPV was administered at single oral doses of 0, 62.5, 200, 500 (females) and 625 (males) in aqueous solution pH 10.5. A total of 48 rats (24 males and 24 females) were assigned to four groups (6/sex/group). A bolus of 0.9% sodium chloride (20 ml/kg) was given orally approximately two hours after dosing. Animals were placed in metabolic cages immediately after administration of sodium chloride and urine was collected over a period of five hours. Parameters of renal function evaluated included water consumption, urine volume and the urinary excretions of sodium, potassium and chloride.

Results and Discussion: No notable changes in water consumption and urine volume were observed in any animals. Males exhibited statistically significant decreases in potassium excretion of 24%, 19% and 28% at low, middle and high doses. Females exhibited statistically significant increases in sodium excretion of 46%, 38% and 42% at low, middle and high doses and a decrease in potassium excretion of 39% at the high dose.

Gastrointestinal effects:

Study title: U-140690E: Isolated ileum profile.

Key study findings: TPV at 10^{-6} M did not contract or relax the tissue nor did it affect concentration-response curves for acetylcholine, histamine and barium chloride, indicating that TPV is devoid of significant agonist activity or antogonist effects on muscarinic or histamine receptors or membrane-mediated depolarization in the isolated guinea pig ileum..

Study no.: U00-3100 (Pharmacia & Upjohn Technical Report 1470-96-019)
Volume # and page #: Module 4, M002, vol.1.3, page 1.
Conducting laboratory and location: Pharmacia & Upjohn Ltd., Tsukuba Research Laboratories, 23 Wadai, Tsukuba, Ibaraki, Japan

Date of report: October 14, 1996

Objective: The objective of this GLP compliant study was to determine the effect of TPV on contractility and on agonist-induced contractions in ileum isolated from guinea pigs.

Materials and methods: The ileum was isolated from six male guinea pigs of the Hartley strain and cut into 1.5 cm sections. Isotonic contractions under 1 g tension were monitored in an organ bath filled with 10 ml of the modified Krebs solution, gassed with 95% O_2/5% CO_2 and maintained at 37° C. Concentration-response curves for acetylcholine, histamine and barium

chloride were obtained before and after 30 minutes of equilibration with TPV (Lot no. (A) 5075-AS-1768,[ ]purity) or vehicle (DMSO).

Results and Discussion: TPV at 10^{-6} M did not contract or relax the tissue nor did it affect concentration-response curves for acetylcholine, histamine and barium chloride.

Study title: U-140690E: Gastrointestinal profile following a single oral dose in Sprague-Dawley rats.

Key study findings: Gastric emptying and GI propulsion were significantly decreased at all doses in males and at middle and high doses in females. Gastric fluid volume was increased at the high dose in males and acid concentration of gastric fluid was decreased at the high dose in males and females. These changes were considered TPV-related.

Study no.: U00-3099 (Pharmacia & Upjohn Technical Report 1470-96-017)
Volume # and page #: Module 4, M002, vol. 1.3, page 1.
Conducting laboratory and location: Pharmacia & Upjohn Ltd., Tsukuba Research Laboratories, 23 Wadai, Tsukuba, Ibaraki, Japan

Date of report: October 14, 1996

Objective: The objective of this GLP compliant study was to determine the effect of a single oral dose of TPV on gastric emptying, gastrointestinal propulsion and gastric secretion.

Materials and methods: TPV (Lot No. (A)5075-AS-1768-J395,[ ]purity) in aqueous solution pH 10.5 was administered as single oral doses of 0, 62.5, 200, 500 (females) and 625 (males) to rats. Gastric emptying was studied in four groups with one group being euthanized without treatment. After dosing with vehicle or TPV, rats were fasted for four hours. All rats were euthanized and the weight of stomach contents was determined. Gastrointestinal propulsion was studied in four groups of rats. After an overnight fast without water, rats were dosed with vehicle or TPV. Six hours later, rats received a single oral dose of a liquid charcoal suspension as a marker of GI transit. Rats were euthanized 20 minutes after administration of the charcoal suspension. Gastric secretion was studied in four groups of rats. After an overnight fast without water, rats were dosed with vehicle or TPV. Three hours later, rats were subjected to pylorus ligation. Four hours later, rats were euthanized and gastric fluid volume, acid concentration and acid output were determined.

Results and Discussion: Gastric emptying and GI propulsion were significantly decreased at all doses in males and at middle and high doses in females. Gastric fluid volume was increased at the high dose in males and acid concentration of gastric fluid was decreased at the high dose in males and females. These changes were considered TPV-related.