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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	21-087, 21-246
Supporting document/s:	S062, S045
Applicant's letter date:	6/21/2012
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Product:	TAMIFLU®
Indication:	Treatment of influenza in infants with a post conceptual age of at least (b) (4) weeks to 1 year of age
Applicant:	Hoffman-La Roche, Inc.
Review Division:	Division of Antiviral Products (DAVP)
Reviewer:	Ita Yuen, PhD
Supervisor/Team Leader:	Hanan Ghantous, PhD, DABT
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1 Executive Summary

1.1 Introduction

Tamiflu™ is the tradename for oseltamivir phosphate (Ro 64-0796/002, GS4104), a prodrug of the influenza neuraminidase inhibitor, Ro 64-0802 (oseltamivir carboxylate). It was approved for marketing in the US for the treatment and prophylaxis of influenza infection in adults and children one year of age and older. However, for patients younger than 1 year old, there is currently no approved therapy for the treatment of viral influenza infection. The present supplemental NDA is submitted to support the approvability of Tamiflu™ for the unmet need in this patient population.

1.2 Brief Discussion of Nonclinical Findings

No new toxicity or target organ of toxicity was identified in the studies submitted in the present Supplemental New Drug Application (sNDA). In addition, no new safety signal was identified either by the clinical or nonclinical studies. The target organs of toxicities identified previously were gastrointestinal (GI) systems, kidneys, and bones as well as the lower tolerance of juvenile rats to Ro 64-0796.

The toxicity profile was similar between adult and juvenile rats. However, mortality was associated with a single oral dose of 500 mg/kg Ro 64-0796 in 7 day old rats while 2500 mg/kg Ro 64-0796 administered daily for fourteen days did not produce any toxicity in adult rats. The difference in the tolerance between the adult and juvenile rats may be partially explained by the difference in the exposures to Ro 64-0796 in the juvenile rat brain. Seven day-old rats had lower plasma esterase activity and renal clearance and a more porous blood-brain barrier, resulting in 12-fold higher brain exposure to Ro 64-0796 as compared to 42 day-old rats following a single oral administration of 1000 mg/kg. Pharmacokinetic data in infants less than 1 year old have shown that the age-related metabolic factors did not affect the systemic exposures to Ro 64-0796 and Ro 64-0802 in this population. How the porosity of the blood-brain barrier to Ro 64-0796 in 7 day old rats compared with blood-brain porosity in infants is not known. However, even assuming that human newborns' blood-brain barrier is as porous to Ro 64-0796 as that in 7 day-old rats, the safety margin calculated using the most conservative estimates from both clinical and nonclinical data is at least 120X. The high safety margin suggests low safety concern for infants less than 1 year of age.

Both Ro 64-0796 and Ro 64-0802 are not genotoxic or carcinogenic and considered a Pregnancy Category C.

In conclusion, the submitted nonclinical study results provide sufficient understanding of the toxicity and ADME profiles of both Ro 64-0796 and Ro 64-0802 to allow risk/benefit assessment of the usage and dosage of oseltamivir in children younger than 1 year old.

1.3 Recommendations

1.3.1 Approvability

Yes. There is no nonclinical safety concern for Tamiflu™.

1.3.2 Additional Non Clinical Recommendations

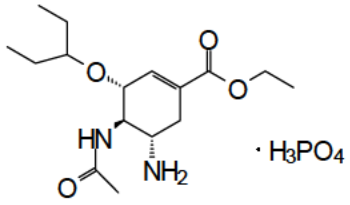
None.

1.3.3 Labeling

No new changes were made in the "Pregnancy", "Nursing Mother", or "Nonclinical Toxicology" sections.

2 Drug Information

2.1 Drug

CAS Registry Number:	204255-11-8
Generic Name:	Prodrug: Oseltamavir phosphate or oseltamavir Active drug: Oseltamavir carboxylate
Code Name:	Prodrug: Free base: Ro 64-0796/000; GS-4104 Phosphate salt: Ro 64-0796/002; GS-4104-02 Active drug: Phosphate salt: Ro 64-0802/002; GS-4017
Chemical Name:	Ro 64-0796: (3R,4R,5S)-4-(acetylamino)-5-amino-3-(1-thylpropoxy)-1-cyclohexene-1-carboxylic acid ethyl ester, phosphate (1:1)
Molecular Formula/Molecular Weight:	Ro 64-0796: $C_{16}H_{28}N_2O_4$ (free base)/M.W. = 312.41 $C_{16}H_{28}N_2O_4$ 1:1 H_3PO_4 (phosphate salt)/410.408
Structure or Biochemical Description:	Ro 64-0796: 
Pharmacologic Class	Influenza viral neuraminidase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND, (b) (4), NDA 21-087, DMF Type I #'s (b) (4), DMF Type III #'s (b) (4), DMF Type IV #'s (b) (4)

2.3 Drug Formulation

Tamiflu is available as capsules or powder for oral suspension. The capsule contains 30, 40, or 75 mg oseltamivir phosphate (free base equivalent), pregelatinized starch,

talc, povidone K30, croscarmellose sodium, and sodium stearyl fumarate. The powder contains oseltamivir phosphate than can be constituted with water to a concentration of 6 mg/ml oseltamivir base, sorbitol, monosodium citrate, xanthan gum, titanium dioxide, tutti-frutti flavoring, sodium benzoate, and saccharin sodium.

2.4 Comments on Novel Excipients

None.

2.5 Comments on Impurities/Degradants of Concern

None.

2.6 Proposed Clinical Population and Dosing Regimen

The present sNDA is submitted for Tamiflu™ for the treatment of influenza in infants with a post conceptual age of at least (b) (4) weeks to 1 year of age who have been symptomatic for 2 days or less. The proposed dose for this population is 3 mg/kg twice daily for 5 days by oral administration.

2.7 Regulatory Background

Tamiflu™ was approved for marketing on Oct. 27, 1999 under NDA 21,087 for the treatment of uncomplicated influenza infection in adults and adolescents who have been symptomatic for no more than 2 days. A Supplemental NDA (S-002) was submitted and Tamiflu™ approved on Nov. 17, 2000 for the prophylaxis of influenza viral infection in adults and adolescents 13 years and older. The recommended dose for this indication is 75 mg once a day and the therapy should commence within 2 days of exposure. The use of Tamiflu™ in patients older than 1 year of age was approved under NDA 21,246 on Dec. 14, 2000 with an oral suspension formulation containing 12 mg/ml Tamiflu™. The recommended dose is 30 mg twice a day for those with body weight ≤ 15 kg (33 lb.), 45 mg twice a day for those weighing between 15.1 and 23 kg (33 to 51 lb.), 60 mg twice a day for those weighing between 23.14 to 40 kg (51 to 88 lb.), and 75 mg twice a day for those whose weights are greater than 40 kg (88 lb.). The use of this agent for the prophylaxis of influenza viral infection in children 1-12 years of age was approved on Dec. 21, 2005 under NDA supplement # 017 and the dosages are a half of those recommended for treatment, i.e., once daily instead of twice daily dosing for 10 days.

3 Studies Submitted

3.1 Studies Reviewed

Effect of Ro 64-0796/002 (GS 4104.02) and Ro 64-0802/002 (GS 4017) in radio-ligand receptor binding assays (Study #'s 4055 and 4184; Report # RDR-1003006).

In-vitro pharmacology profile (not including neuraminidases) of oseltamivir phosphate and oseltamivir carboxylate (Report # 1026878).

The disposition of the pro-drug, oseltamivir, and its active metabolite, the influenza neuraminidase inhibitor, R00640802, in mice following single oral administration (b) (4) Study DDHN 1031) (Revised Report # 1004494).

RO0640796 (Tamiflu ®): intravenous single dose pharmacokinetics in rats following 30 mg/kg oseltamivir phosphate and distribution into the nonperfused and perfused brain (Study No. 08-0152 and 08-0153) (Report # 1027310).

RO0640802: Intravenous single dose pharmacokinetics in rats following 30 mg/kg oseltamivir carboxylate and distribution into the non-perfused and perfused brain (Study No. 08-0154 and 08-0155) (Report # 1027311).

Hydrolysis of RO0640796 (oseltamivir) to RO0640802 (oseltamivir carboxylate) by human brain S9 subcellular fractions from two individuals and a pool of four donors (Report # 1027737).

RO0640796 (Tamiflu, neuraminidase inhibitor): *In vitro* studies on the transport of RO0640796 (oseltamivir) and RO0640802 (oseltamivir carboxylate) by membrane vesicles expressing human MRP1, 2, 3 or BCRP and on the P-glycoprotein (MDR1) substrate and inhibition properties of RO0640796 (Report # 1029724).

RO0640796 (Tamiflu, neuraminidase inhibitor): Investigations on the transport of RO0640796 (Oseltamivir) and RO0640802 (Oseltamivir carboxylate) by polarized cell lines expressing human MDR1, BCRP or MRP2, or expressing mouse Mdr1a or Bcrp1 (Report # 1026298).

RO0640802 (Tamiflu®): Intravenous single dose pharmacokinetics in rats following 10 or 100 mg/kg oseltamivir carboxylate (Protocol No. 07-4814) (Report # 1026678).

RO0640796 (Tamiflu®, OP): Intravenous single dose pharmacokinetics in rats following 10 or 100 mg/kg oseltamivir phosphate (Protocol No. 07-4813) (Report # 1026679).

RO0640796-002: Single dose oral (gavage) toxicokinetic study in the male rat (Report # 1029359).

Determination of brain concentration of RO0640796-002 and RO0640802-002 following Administration by various routes in rats (Report # 1027859).

In vitro experiments of the metabolism of Ro 64-0796 by the marmoset (99/31/ROC/07) (Report # 1001666).

Hydrolysis of RO0640796 (oseltamivir) to RO0640802 (oseltamivir carboxylate) by brain S9 subcellular fraction from 7- and 42-day old rats (Report # 1027267).

RO0640802: Subcutaneous single dose pharmacokinetics in 7-day old juvenile rats following 10 mg/kg oseltamivir carboxylate and distribution into the perfused brain (Study No. 08-7367) (Report # 1032043).

Determination of blood and brain concentrations of Ro 64-0802/002 following subcutaneous dose administration (Report # 1029873).

RO0640802: Intraperitoneal single dose pharmacokinetics in 7-day old juvenile rats following 10 mg/kg oseltamivir carboxylate and distribution into the perfused brain (Study # 08-7366) (Report # 1031581).

RO0640796 (Oseltamivir, Tamiflu): influence of breastfeeding, milk, and GlySar on the oral absorption in juvenile Sprague Dawley rats (Report # 1035375).

Oseltamivir: Pharmacokinetics of oseltamivir and oseltamivir carboxylate after intravenous or oral administration of oseltamivir to neonatal marmoset monkeys (with adult control group) (Report # 1038614).

Ro 64-0796 (GS4104): Single dose pharmacokinetics of Ro 64-0796 and Ro 64-0802 in the marmoset (DM/96/018E) (reissued, replaces report dated 19/2/98) (Report # W-142687).

Tamiflu –: Physiologically based pharmacokinetic model development for marmoset and human and simulations in newborns (Report # 1018216).

RO0640796 (oseltamivir phosphate; Tamiflu™): Pharmacokinetics of the prodrug, oseltamivir, and active metabolite in the plasma and brains, and toxicity after a single oral administration of the prodrug to juvenile rats (b) (4) Report HRE 0089, Aptuit Report DDHN1041 (amended), Roche Study No. 7021K07) (Revised Report # 1008172).

3.2 Studies Not Reviewed

Many other submitted studies that are included in the present submission have been reviewed and the reviews were filed under NDAs 21,087 and 21, 246 and IND 53,093.

An integrated summary of the analytical methods used and data obtained during the clinical and pre-cliical development of Ro 64-0796/002 (Report # W-143081).

The neuraminidase inhibitor GS4104 (oseltamivir phosphate) is efficacious against A/Hong King/156/97 (H5N1) and A/Hong Kong/1074/99 *H9N2) influenza viruses (Report # W-1000496) – Should be reviewed by Virology reviewer.

Oseltamivir phosphate (Ro 64-0796/002) for the treatment and prophylaxis of influenza: written summary on absorption and disposition in animals (Report # 1003163).

An integrated summary of the analytical methods used and data obtained during the clinical and pre-clinical development of Ro 64-0796/002 (Report # W-143081).

Oseltamivir phosphate (Ro 64-0796/002) for the treatment and prophylaxis of influenza: Expert report on the pre-clinical documentation (Report # 1003164).

Written toxicology summary for Tamiflu™ (oseltamivir phosphate) in the treatment of children (Report # 1006338).

3.3 Previous Reviews Referenced

NDA 21,087.ori, NDA 21,087 SD-438, IND 53,093.551

4 Pharmacology

4.1 Primary Pharmacology

Please see Virologist's review.

4.2 Secondary Pharmacology

0.1, 1 and 10 μM Tamiflu™, GS 7061, GS 7172, GS 7293, Relenza™, and BCX-1812 were tested against receptors (mostly cloned from human tissues) known to be involved in the mechanism of nausea and vomiting in mammals (Report # RDR-1003006). The results indicated that, with the exception of Relenza™ which showed 91% inhibition to the H1 receptor-ligand binding at (10 μM), all of the compounds tested had very low affinity to these receptors.

Another *in vitro* study was conducted to assess the potential pharmacological activities for oseltamivir phosphate and oseltamivir carboxylate on a 155 molecular targets comprising of receptors and ion channels, as well as enzymes (excluding

neuraminidases) (Report # 1026878). The 155 molecular targets also covered those already evaluated in Report # 1003006 and included, for examples, dopamine-NMDA- and $GABA_A$-receptors (important in emotion, cognition, and behavior), hERG-, calcium-, and sodium-channels (important in cardiovascular functions), and mGlu2, and mGlu5 (metabotropic glutamate receptor family for excitatory neurotransmitter glutamate in CNS). The results showed that both oseltamivir phosphate and oseltamivir carboxylate (at 3 and 30 μM) lack any relevant pharmacological off-target activities defined as >50% inhibition/stimulation at any target tested.

4.3 Safety Pharmacology

See Appendix/Attachments.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Distribution in CNS:

There were two phases in a study, designed to assess distribution of Ro 64-0796 in the CNS (b) (4) Study DDHN 1031). During phase 1, blood and brains from several mice were pooled and spiked with [^{14}C]-labeled Ro 64-0796 (Revised Report # 1004494). Radioactivity in blood and brain samples were analyzed in order to determine the extraction efficiency. In phase 2 of the study, 21 male mice received an oral dose of 10 mg/kg Ro 64-0796 and the plasma and brain drug levels were determined using HPLC/MS/MS. In the original study report, there was a calculation error for the stock solution of Ro 64-0796, resulting in an 8% underestimation of drug concentration. The revised report contained the recalculation of Ro 64-0796 and Ro 64-0802 levels in mouse plasma and brain as well as the demonstration of stability of Ro 64-0796 in plasma containing dichlorvos, (an esterase inhibitor) and brain without esterase inhibitor. The results indicated that following an oral dose of 10 mg/kg Ro 64-0796, both the prodrug and active metabolite crossed the blood brain barrier, the former to a greater extent.

The pharmacokinetics of Ro 64-0796 and Ro 64-0802 in plasma, cerebral spinal fluid (CSF), and brain were determined following a single slow bolus intravenous administration of 30 mg/kg Ro 64-0796 to rats with and without brain perfusion (Report # 1027310). The results are presented in the following table:

Table 1. The pharmacokinetics of Ro 64-0796 and Ro 64-0802 in plasma, CSF, and brain following a single slow bolus intravenous dose of 30 mg/kg Ro 64-0796 to male rats.

	Ro 64-0796					
	Without Brain Perfusion			With Brain Perfusion		
	Plasma	CSF	Brain	Plasma	CSF	Brain
C_{max} (μg/ml)	12.00	0.90	0.71	12.50	0.92	0.49
T_{max} (hr)	0.083	0.083	0.083	0.083	0.083	0.083
$t_{1/2}$ (hr)	1.35	0.81	2.45	1.59	0.81	1.51
$AUC_{0-\infty}$ (μg-hr/ml)	5.33	0.45	1.31	5.33	0.47	1.16
CL (ml/min/kg)	93.9	-	-	93.9	-	-
Vc (l/kg)	1.57	-	-	1.59	-	-
MRT (hr)	0.54	-	-	0.52	-	-
Vss (l/kg)	3.04	-	-	2.90	-	-
T_{last} (hr)	8.00	8.00	8.00	8.00	8.00	8.00
AUC_{0-8h} (μg-hr/ml)	5.31	0.45	1.18	5.31	0.47	1.13
***-plasma ratio**	-	0.084	0.25	-	0.089	0.22
Ro 64-0802						
C_{max} (μg/ml)	6.88	0	0.16	6.30	0	0.076
T_{max} (hr)	0.083	0	0.25	0.083	0	0.25
$t_{1/2}$ (hr)	1.69	NC	0.51	1.71	NC	NC
$AUC_{0-\infty}$ (μg-hr/ml)	13.60	NC	0.15	12.80	NC	NC
T_{last} (hr)	8.00	NC	8.00	8.00	NC	8.00
AUC_{0-8h} (μg-hr/ml)	13.10	NC	0.15	12.40	NC	0.026
***-plasma ratio**	-	NC	0.011	-	NC	0.0021
AUC_{0796}:AUC_{0802} Ratio	1:4	-	8:1	1:2.4	-	43:1
* denotes CSF or brain						

Penetration through the blood brain barrier by Ro 64-0796 was evident. Little or no esterase activity was observed in CSF since Ro 64-0802 was not detectable. Brain perfusion did not affect the pharmacokinetics of either Ro 64-0796 or Ro 64-0802 in plasma or CSF. The systemic exposures to Ro 64-0796 were similar whether perfusion was performed or not. However, AUC value for Ro 64-0802 could not be calculated because it was not detectable in most of the samples collected where perfusion was performed. Plasma esterase in the residual blood in the brain can lead to ex vivo conversion of Ro 64-0796 to Ro 64-0802, leading to an overestimation of the brain Ro 64-0802 concentration. As observed previously, systemic exposures to Ro 64-0796 were about 1/3 of those to Ro 64-0802 in plasma.

The pharmacokinetics of Ro 64-0802 in plasma, CSF, and brain were determined following a single slow bolus intravenous administration of 30 mg/kg Ro 64-0802 to rats with and without brain perfusion (Report # 1027311). The results are presented in the following table:

Table 2. Pharmacokinetics of Ro 64-0802 in plasma, CSF, and brain following a single slow bolus intravenous dose of 30 mg/kg Ro 64-0802 to male rats.

	Without Brain Perfusion			With Brain Perfusion		
	Plasma	**CSF**	**Brain**	**Plasma**	**CSF**	**Brain**
C_{max} (μg/ml)	73.20	0.23	1.71	86.30	0.45	0.61
T_{max} (hr)	0.083	0.083	0.083	0.083	0.083	0.083
$t_{1/2}$ (hr)	1.64	0.34	0.38	1.69	0.38	0.48
$AUC_{0-\infty}$ (μg-hr/ml)	28.60	0.13	0.69	33.80	0.14	0.31
CL (ml/min/kg)	17.5	-	-	14.8	-	-
Vc (l/kg)	0.21	-	-	0.19	-	-
MRT (hr)	0.81	-	-	0.83	-	-
Vss (l/kg)	0.85	-	-	0.74	-	-
T_{last} (hr)	8.00	8.00	8.00	8.00	8.00	8.00
AUC_{0-8h} (μg-hr/ml)	28.30	0.13	0.69	33.40	0.14	0.31
***-plasma ratio**	-	0.0045	0.024	-	0.0041	0.0090
* denotes CSF or brain						

Penetration through the blood brain barrier by Ro 64-0802 was evident even though the concentrations in CSF were lower than those in brain, as observed with intravenous Ro 64-0796 administration. Brain perfusion did not affect the pharmacokinetics of Ro 64-0802 in plasma or CSF. However, the systemic exposures were lower in the perfused brain, suggesting that residual blood in blood vessels can lead to an overestimation of the brain drug concentrations.

Oseltamivir esterase activity was measured in S9 fraction prepared human brain (Report # 1027737). The esterase and paranitrophenyl acetate hydrolase activities in human liver S9 fraction served as control to show that the prepared S9 fraction was enzymatically active. The results indicated that human brain S9 fraction had very low oseltamivir esterase activity that is at least 300 times lower than that of the human liver S9 fraction. In comparison, paranitrophenyl acetate hydrolysis in human brain S9 fractions was only 3- to 10-fold lower than that in human liver S9 fractions.

Transport of Ro 64-0796 and Ro 64-0802 by the major human cellular export proteins expressed at the blood-brain barrier, in particular P-glycoprotein (P-gp or MDR1), breast cancer resistance protein (BCRP), and the human multi drug resistance related proteins (MRP1, 2, and 3) were studied *in vitro* using either membrane vesicles prepared from Sf9 cells or a polarized cellular system (Report # 1029724). The results indicated that both Ro 64-0796 and Ro 64-0802 are not substrates of MRP1, 2, 3, and BCRP since no active transport was observed in membrane vesicles expressing the respective transporters. However, Ro 64-0796 is a substrate of P-gp (MDR1) with IC_{50} values of 1.3 to 2.3 mM inhibiting the MDR1-mediated directional transport of digoxin (an MDR1 substrate).

The stability, passive permeability, and directional transport of Ro 64-0796 and Ro 64-0802 were measured by transport of radio-labelled Ro 64-0796 and Ro 64-0802 or model substrates (digoxin, PHIP, and saquinavir) by polarized cells stably expressing major cellular export proteins found at the blood-brain barrier (Report # 1026298). The proteins investigated were P-gp, human MDR1 and mouse MDR1a, human BCRP and

mouse BCRP1a, and MRP2. The results showed that Ro 64-0796 had an intermediate passive permeability, was a substrate of mouse MDR1a and human MDR1 P-gp, but was not a substrate of mouse BCRP1 or human BCRP and MRP2. Ro 64-0802 was poorly transported by all of the transporters studied either by passive or active transport.

The pharmacokinetics of Ro 64-0802 in plasma, brain, and CSF were studied following a single intravenous administration of 10 or 100 mg/kg Ro 64-0802 to male Sprague-Dawley rats (Report # 1026678). The results are presented in the following table:

Table 3. Pharmacokinetics of Ro 64-0802 in plasma, CSF, and brain after single intravenous slow injection of 10 and 100 mg/kg Ro 64-0802 to male rats.

	Plasma		CSF		Brain	
Dose (mg/kg)	**10**	**100**	**10**	**100**	**10**	**100**
$\mathbf{T_{max}}$ (hr)	0.083	0.083	0.083	0.083	0.083	0.083
$\mathbf{C_{max}}$ (µg/ml)	13.90	278.00	0.057	1.87	0.40	7.89
$\mathbf{AUC_{0-\infty}}$ (µg-hr/ml)	7.08	122.00	0.055	1.38	0.19	3.80
$\mathbf{t_{1/2}}$ (hr)	1.59	1.51	0.72	1.45	0.29	1.84
Clearance (l/min/kg)	0.024	0.014	-	-	-	-
Vss (l/kg)	1.10	0.46	-	-	-	-
$\mathbf{AUC_*/AUC_{plasma}}$ **ratio**	-	-	0.0078	0.011	0.027	0.031
* denotes CSF or brain						

The amounts of polar Ro 64-0802 in brain and CSF were low and dose related. The brain to plasma ratio was 1:30 (3%) which is in agreement with the published range of blood volume in the blood vessels of the brain. This suggests that the measured concentrations of Ro 64-0802 may be partly due to residual drug in cerebral blood vessels, especially since the brain was not perfused prior to harvesting for drug concentration determinations.

The pharmacokinetics of Ro 64-0796 and Ro 64-0802 in plasma, brain, and CSF were studied following a single intravenous slow injection of 10 or 100 mg/kg Ro 64-0796/002 to male Sprague-Dawley rats were investigated (Report # 1026679). The results are presented in the following table:

Table 4. Pharmacokinetic parameters of Ro 64-0796 and Ro 64-0802 in plasma, CFS, and brain after single intravenous slow injection of 10 and 100 mg/kg Ro 64-0796 to male rats.

Ro 64-0796						
	Plasma		CSF		Brain	
Dose (mg/kg)	**10**	**100**	**10**	**100**	**10**	**100**
T_{max} (hr)	0.083	0.083	0.083	0.083	0.083	0.083
C_{max} (µg/ml)	2.89	36.20	0.22	3.02	0.16	1.78
$AUC_{0-\infty}$ (µg-hr/ml)	1.43	18.90	0.11	1.82	0.13	3.64
$t_{1/2}$ (hr)	1.58	1.00	0.39	1.04	0.71	2.87
Clearance (l/min/kg)	0.12	0.088	-	-	-	-
Vss (l/kg)	4.25	2.98	-	-	-	-
AUC_*/AUC_{plasma} ratio	-	-	0.077	0.096	0.091	0.19
Ro 64-0802						
T_{max} (hr)	0.083	0.083	0	0.25	0.25	0.083
C_{max} (µg/ml)	2.15	25.90	0	0.11	0.071	1.01
$AUC_{0-\infty}$ (µg-hr/ml)	3.40	45.30	NC	NC	NC	1.35
$t_{1/2}$ (hr)	1.48	1.86	NC	NC	NC	1.39
AUC_*/AUC_{plasma} ratio	-	-	NC	NC	NC	0.030
AUC_{0796}:AUC_{0802} Ratio	1:2.4	1:2.4	-	-	-	2.7:1
* denotes CSF or brain NC = Not calculated						

As observed previously, rats hydrolyzed Ro 64-0796 less efficiently than primates. The prodrug to active drug AUC ratio being is 1:3 in rats while less than 5% of prodrug is detected in humans. Since Ro 64-0796 is more hydrophobic and a substrate for P-gp in the rat brain, It penetrates blood-brain barrier more readily than Ro 64-0802 with the prodrug to active drug AUC ratio being about 3:1, reverse of what's observed in plasma. The levels of Ro 64-0802 in brain and CSF were higher as compared to those from the previous study where Ro 64-0802 was administered intravenously. However, the brain to plasma AUC ratio was 3% (the estimated blood volume in the blood vessels of the brain), suggesting that the presence of Ro 64-0802 came from the residual blood in the brain rather than penetration through the blood-brain barrier.

The pharmacokinetics of Ro 64-0796 and Ro 64-0802 in plasma, brain, and CSF were studied following a single oral dose of 763 or 1000 mg/kg Ro 64-0796/002 in male Sprague-Dawley rats (Report # 1029359). The results are presented in the following table:

Table 5. Pharmacokinetics of Ro 64-0796 and Ro 64-0802 in plasma, brain, and CSF following a single oral dose of 763 or 1000 mg/kg Ro 64-0796 in male rats.

Ro 64-0796						
	Plasma		CSF		Brain	
Dose (mg/kg)	**763**	**1000**	**763**	**1000**	**763**	**1000**
T_{max} (hr)	6	2	6	2	8	8
C_{max} (μg/ml)	16.20	16.30	1.04	1.12	2.06	2.31
AUC_{0-8h} (μg-hr/ml)	109.00	107.00	7.16	7.47	12.20	13.60
AUC_*/AUC_{plasma} ratio	-	-	0.066	0.070	0.11	0.13
Ro 64-0802						
T_{max} (hr)	8	8	8	6	4	6
C_{max} (μg/ml)	41.70	49.70	0.35	0.36	0.54	0.64
$AUC_{0-\infty}$ (μg-hr/ml)	260.00	286.00	1.81	1.94	2.81	3.45
AUC_*/AUC_{plasma} ratio	-	-	0.0045	0.0068	0.010	0.012
AUC_{0796}:AUC_{0802} Ratio	1:2	1:3	1591:1	1099:1	4:1	4:1
* denotes CSF or brain						

The results of this study were consistent with a similar study of intravenous administration. Both prodrug and active metabolite were detected in brain and CSF, but at lower ratios to plasma as compared to those from the previous study. Ro 64-0802 in the brain likely came from the residual blood in the brain.

The exposures to Ro 64-0796 and Ro 64-0802 in various brain regions following a single intracerebroventricular (ICV; 0.08 and 0.2 μg/animals for both drugs), intravenous (IV; 5 and 30 mg/kg, respectively, for Ro 64-0796 and Ro 64-0802), or oral (200 mg/kg Ro 64-0796) administration in male, 9-week old Sprague-Dawley rats (Report # 1027859). Blood, CSF, and brain samples were collected for drug concentration determinations. Brains were weighed, then further dissected to olfactory bulb, hippocampus, and cerebellum regions and weighed. The results are presented in the table below. They demonstrated that ICV administration at drug concentrations up to 0.2 μg/animal resulted in very low exposures (≤ 2% those in plasma) to either Ro 64-0796 or Ro 64-0802 in CSF and the three brain regions examined.

Table 6. Exposures to Ro 64-0796 and Ro 64-0802 following a single dose of Ro 64-0796 or Ro 64-0802 via intracerebroventricular (ICF), intravenous (IV), or oral administration.

Ro 64-0796							
Route of administration	**ICV**	**ICV**	**ICV**	**ICV**	**IV**	**IV**	**Oral**
Test article administered	0796[a]	0802[b]	0796[a]	0802[b]	0796[a]	0802[b]	0796[a]
Dose level	0.08 µg/rat	0.08 µg/rat	0.2 µg/rat	0.2 µg/rat	5 mg/kg	30 mg/kg	200 mg/kg
C_{max} in plasma (µg/ml)	NC[c]	-	NC[c]	-	1.88	-	7.62
C_{max} in CSF (µg/m)	NC[c]	-	NC[c]	-	0.091	-	0.53
Brain-plasma ratio*							
Cerebellum	NC[c]	-	NC[c]	-	NC[c]	-	0.08
Hippocampus	NC[c]	-	NC[c]	-	NC[c]	-	0.016
Olfactory Bulb	NC[c]	-	NC[c]	-	NC[c]	-	0.04
Ro 64-0802							
C_{max} in plasma (µg/ml)	NC[c]	NC[c]	NC[c]	NC[c]	1.38	60.30	14.60
C_{max} in CSF (µg/m)	NC[c]	NC[c]	NC[c]	NC[c]	NC[c]	0.57	0.57
Brain-plasma ratio[d]							
Cerebellum	NC[c]	NC[c]	NC[c]	NC[c]	NC[c]	0.01	0.02
Hippocampus	NC[c]	NC[c]	NC[c]	NC[c]	NC[c]	0.009	0.01
Olfactory Bulb	NC[c]	NC[c]	NC[c]	NC[c]	NC[c]	0.02	0.02

a 0796 represents Ro 64-0796.
b 0802 represents Ro 64-0802.
c NC represents "not calculated" since most of the drug concentrations from the collected samples were below the limit of quantitation at 37.5 ng/g.
d The ratio was calculated based on AUC_{0-2h} values for drug administered via ICV and IV routes and AUC_{0-8h} values via oral route.

ADME in juvenile animals:

The metabolic profile of ^{14}C-labelled Ro 64-0796 was investigated using isolated perfused liver from marmoset and S9 supernatants isolated from frozen livers of female marmosets that were 1 day, 3 and 6 weeks, 3 months, and 4 years old (Report # 1001666). It was found that Ro 64-0796 was sequestered by the isolated marmoset liver rapidly ($t_{1/2}$ < 10 minutes, clearance 10 ml/min) while Ro 64-0802 was released by the liver slowly ($t_{1/2}$ = 6.5 hours). The experiments with S9 supernatant indicated that metabolic clearance of Ro 64-0796 was somewhat slower than its uptake. The results support the hypothesis that the long half-life of Ro 64-0802 following oral administration of Ro 64-0796 is governed by its slow release from hepatocytes. In addition, liver from marmosets less than 3 months old hydrolyzed Ro 64-0796 at a slower rate than adult animals. This slower rate would likely have a small effect on systemic exposure to Ro 64-0802 since the rate-determining step is the release of Ro 64-0802 from the hepatocyte, which is not age-dependent but formulation-specific.

Oseltamavir esterase activity was measured in brain and liver S9 fractions from 7- and 42-day old Sprague Dawley rats (Report # 1027267). Positive control for the esterase activity in S9 fraction was paranitrophyl acetate. The results showed that 7 day-old rats had very low brain and liver oseltamivir esterase activity as compared to 42 day-old (adult) rat, regardless of gender. In addition, the esterase activity in liver was greater

than in brain, regardless of age, and esterase activity in human liver S9 fractions was much greater than in liver from rat.

Ten mg/kg Ro 64-0802 (tartrate salt) was administered to 7 day-old rats as a single subcutaneous dose (Report # 1032043). Blood and brain samples were collected for drug concentration determinations. The results shown in the following table demonstrated that Ro 64-0802 was able to penetrate the blood-brain-barrier in 7 day-old rats following a single subcutaneous administration. However, the exposures in brain were only about 7% those in plasma.

Table 7. Pharmacokinetic parameters of Ro 64-0802 in plasma and brain following a single subcutaneous administration of 10 mg/kg Ro 64-0802 to 7 day-old male rats.

	Plasma	Brain	Brain:Plasma Ratio
C_{max} (μg/ml)	14.20	0.25	0.018
T_{max} (hr)	0.25	0.50	-
$t_{1/2}$ (hr)	2.91 (3-24 hr) 0.99 (0.5-3 hr)	3.63 (1-24 hr)	-
$AUC_{0-\infty}$ (μg-hr/ml)	23.40	1.69	0.072

Seven day-old rats received a single subcutaneous administration of 25 or 50 mg/kg Ro 64-0802 (Report # 1029873). Blood and brain samples were collected for drug concentration determinations. To remove cerebral blood content and reduce the possibility of overestimating drug concentrations in brain, the animals were perfused transcardially with saline following the blood collection for the pharmacokinetic analysis. The results showed again that Ro 64-0802 can penetrate blood brain barrier in the 7 day old rats, albeit at a low level, and the exposures were dose-related. The brain to plasma Ro 64-0802 ratio ranged from 8 to 10%.

Table 8. Pharmacokinetic parameters of Ro 64-0802 in plasma and brain following a single subcutaneous administration of 25 or 50 mg/kg Ro 64-0802 to 7 day-old rats (brain perfusion was performed).

	Plasma				Brain			
	Male		Female		Male		Female	
Dose (mg/kg)	**25**	**50**	**25**	**50**	**25**	**50**	**25**	**50**
T_{max} (hr)	0.25	0.25	0.25	0.5	0.25	4	0.25	0.25
C_{max} (μg/ml)	48.70	87.70	40.80	90.20	0.92	2.02	1.56	2.97
$AUC_{0-\infty}$ (μg-hr/ml)	68.80	150.00	67.50	155.00	6.02	14.50	6.10	14.90
AUC_{brain}/AUC_{plasma} ratio	-	-	-	-	0.088	0.097	0.090	0.096

Intraperitoneal injection was used to administer 10 mg/kg Ro 64-0802 to 7-day old male rats (Report # 1031581). Blood and brain samples were collected for drug concentration determinations. Transcardial perfusion was performed prior to the collection of brain to remove residual blood and avoid overestimation of Ro 64-0802 concentrations. Systemic exposures and the extent of penetration into brain following intraperitoneal administration were similar to those following subcutaneous administration in the 7 day old rats (~ 9%).

Table 9. Pharmacokinetic parameters of Ro 64-0802 in plasma and brain following a single subcutaneous administration of 10 mg/kg Ro 64-0802 to 7 day-old male rats.

	Plasma	Brain	Brain:Plasma Ratio
C_{max} (μg/ml)	16.60	0.31	0.019
T_{max} (hr)	0.25	0.50	-
$t_{1/2}$ (hr)	3.69 (3-24 hr) 2.00 (1-7 hr)	4.34 (1-24 hr)	-
$AUC_{0-\infty}$ (μg-hr/ml)	22.90	2.11	0.092

The potential effects of breast-feeding and the presence of milk or GlycylSarcosine (GlySar) on the oral absorption of Ro 64-0796 were studied in 7-day old (juvenile) Sprague-Dawley rats (Report # 1035375). Animals in groups 2-3 were fasted 8 hours predose until 5 hours post dose (14 hours total) while those in group 1 were breast-fed ad libitum. The results are shown in the following table:

Table 10. Systemic exposures of Ro 64-0796 and Ro 64-0802 in 7 day-old rats following oral administration of 30 mg/kg Ro 64-0796 (n=3/time point).

Group	Treatment	T_{max} (hr)	C_{max} (μg/ml)	AUC (μg-hr/ml)	$t_{1/2}$ (hr)
	Ro 64-0796				
1	Ro 64-0796 in aqueous soln. to breast-fed animals	1	6.14	31.60**	3.1
2	Ro 64-0796 in aqueous soln. to fasted animals (control)	0.5	10.50	38.90	2.3
3	Ro 64-0796 in emulsion in pasteurized bovine milk to fasted animals	1	7.99	38.80	2.7
4	Ro 64-0796 in aqueous soln. with 125 mM GlySar to fasted animals	0.5	10.40	34.20	2.1
	Ro 64-0802				
1	Ro 64-0796 in aqueous soln. to breast-fed animals	5	1.50	5.07***	-
2	Ro 64-0796 in aqueous soln. to fasted animals (control)	2	3.10	12.10	-
3	Ro 64-0796 in emulsion in pasteurized bovine milk to fasted animals	5	2.46	9.26*	-
4	Ro 64-0796 in aqueous soln. with 125 mM GlySar to fasted animals	2	2.71	10.90	-

* P < 0.05 ** P < 0.01 *** P < 0.001

As shown previously, juvenile rats cannot convert Ro 64-0796 to Ro 64-0802 efficiently. Breast-feeding significantly reduced the systemic exposures to both compounds (compare groups 1 and 2), although the presence of milk in the formulation did not affect the absorption of Ro 64-0796, but did reduce the conversion of prodrug to the active drug (compare groups 2 and 3). The presence of GlySar did not affect the systemic exposures to both compounds. The data indicated that the presence of milk reduced the ability of juvenile rats to convert Ro 64-0796 to Ro 64-0802. However, it's unclear whether the lower exposures in the breast-fed animals were due to the fed vs. fasted states or the presence of milk.

The pharmacokinetics of Ro 64-0796 and Ro 64-0802 were investigated in adult and neonate (2 to 4 days old) marmosets following a single oral (2 or 10 mg/kg) or intravenous (5 mg/kg) administration of Ro 64-0796 (Report # 1038614). Clinical signs

and body weights were recorded. Animals were sacrificed after the last blood collections for drug concentration determinations at 8 hours postdose. Liver samples were collected for quantification of Ro 64-0796 and Ro 64-0802 as well as carboxylesterase CES1 and CES2 mRNA levels. S9 fractions were also prepared from the liver for the assessment of Ro 64-0796 hydrolysis activity. P-nitrophenyl acetate and methyl anthranilate were used as positive controls for the non-specific and carboxylesterase-1 related enzyme activities. The pharmacokinetic data are presented in the following table. Values for the males and females were combined because of a lack of apparent gender differences. Systemic exposures to both Ro 64-0796 and Ro 64-0802 were greater (by 2-12 fold) in neonates than adult marmosets. Data from the intravenous administration showed slower clearance and the associated higher exposures to Ro 64-0796 in neonates, likely due to a combination of lower liver carboxylesterase expression and activity as well as reduced renal clearance. Higher systemic exposures to the active metabolite, Ro 64-0802, in neonates as compared to adult was likely caused by age-dependent reduction in renal excretion.

Table 11. Pharmacokinetic parameters of Ro 64-0796 and Ro 64-0802 in plasma following a single intravenous or oral administration of Ro 64-0796 to 2-4 day-old neonate or adult marmosets.

Ro 64-0796						
Dose (mg/kg)	**5**		**2**		**10**	
Route of admin.	**Intravenous**		**Oral**		**Oral**	
	Adult	**Neonate**	**Adult**	**Neonate**	**Adult**	**Neonate**
# of animals	4	4	4	2	4	4
C_{max} (µg/ml)	4.59	7.83	0.089	1.09	0.65	5.86
T_{max} (hr)	0.13	0.083	2.75	0.75	1.25	0.75
$t_{1/2}$ (hr)	0.88	1.19	1.45	0.90	1.25	0.99
$AUC_{0-\infty}$ (µg-hr/ml)	3.97	7.34	0.33	1.86	2.09	15.43
CL (ml/min/kg)	22.3	11.6	-	-	-	-
V_{ss} (l/kg)	0.78	0.70	-	-	-	-
AUC_{adult}:$AUC_{neonate}$ ratio	-	1:2	-	1:6	-	1:7
Ro 64-0802						
# of animals	4	4	4	2	4	4
C_{max} (µg/ml)	1.00	5.15	0.30	1.17	1.96	17.90
T_{max} (hr)	1	5.75	2.75	2	2.5	5
$t_{1/2}$ (hr)	2.24	2.66	2.18	7.01	1.54	3.10
$AUC_{0-\infty}$ (µg-hr/ml)	4.11	30.60	1.64	12.35	9.41	126.00
AUC_{0796}:AUC_{0802} ratio	1:1	1:4	1:5	1:7	1:5	1:8
AUC_{adult}:$AUC_{neonate}$ ratio	-	1:7	-	1:8	-	1:13

The pharmacokinetics of Ro 64-0796 and Ro 64-0802 were investigated in marmosets following a single intravenous or oral administration at 10 and 20 mg/kg Ro 64-0802, respectively, or a single oral dose of 20 mg/kg [^{14}C]-labeled Ro 64-0796 (Report # W-142687). The results are presented in the following table and demonstrate that the prodrug, Ro 64-796, provided much better oral bioavailability (52%) than that of the active drug, Ro 64-0802 (5%). Clearance of Ro 64-0802 following intravenous administration was moderate, the steady-state distribution volume relative large, and the elimination half-life very short. Substantial hydrolysis of Ro 64-0796 occurred likely during the absorption process since peak plasma levels for both Ro 64-0796 and Ro 64-

0802 took place rapidly and at same time (1 hour postdose). The longer apparent half life (2.8 hours) for Ro 64-0802 following oral administration of Ro 64-0796 as compared to the shorter one (0.5 hour) following intravenous administration of Ro 64-0802 was not due to ongoing hydrolysis of Ro 64-0796 after absorption since its concentrations were too low and its rate of elimination too rapid. It suggests that the oral absorption of Ro 64-0796 was probably the overall rate-limiting process for systemic exposures to Ro 64-0802 in this study.

Table 12. Pharmacokinetic parameters of Ro 64-0796 and Ro 64-0802 in marmosets following a single intravenous (10 mg/kg) or oral (20 mg/kg) administration of Ro 64-0802 or a single oral dose of 20 mg/kg [^{14}C]-Ro 64-0796.

Drug Administered	**Ro 64-0796**		**Ro 64-0802**	
Route of administration	**Oral**		**Oral**	**Intravenous**
Dose (mg/kg)	**20**		**20**	**10**
Drug monitored	**Ro 64-0796**	**Ro 64-0802**	**Ro 64-0802**	**Ro 64-0802**
C_{max} (μg/ml)	3.1	6.2	0.97	99
C_{24h} (μg/ml)	<0.001	0.016	0.005	0.017
T_{max} (hr)	1	1	0.5	0
$t_{1/2}$ (hr)	0.78	2.8	0.71	0.5
$AUC_{0-\infty}$ (μg-hr/ml)	3.3	21	2	20
Clearance (L/h/kg)	-	-	-	0.43
Vss (L/kg)		-	-	0.82
Bioavailability (%)	-	52	5.0	-

Pharmacokinetic processes, including metabolic conversion of the prodrug by carboxylesterases scaled from *in vitro* data, slow permeability-limited release of carboxylate from hepatocytes, renal clearance of Ro 64-0796 and Ro 64-0802, and age-related carboxylesterase activity were incorporated into physiologically based pharmacokinetic models for human and marmoset (Report # 1018216). Both clinical and nonclinical data from oral and intravenous administrations were used to validate the mathematical models. The results of model simulations suggest that infusion doses that deliver therapeutic levels of oseltemivir carboxylate (Ro 64-0802) in newborns are expected to result in ~ 3-fold higher exposures of oseltamivir (Ro 64-0796) than the same dose given orally. However, even with 3-fold higher exposures to Ro 64-0796, the safety margin is estimated to be greater than 800 X.

5.2 Toxicokinetics

None.

6 General Toxicology

6.1 Single-Dose Toxicity

Ro 640796 (oseltamivir phosphate; Tamiflu™): pharmacokinetics of the prodrug, oseltamivir, and active metabolite in the plasma and brains, and toxicity after a single oral administration of the prodrug to juvenile rats (b) (4) Report HRE 0089, Aptuit Report DDHN1041 (amended), Roche Study # 7021K07, Revised Report # 1008172;

GLP; With QA statement; Lot # BS99025046; Study initiation date 5/14/2001). The dosing groups and mortality rate are depicted in the following table:

Table 13. Group size, doses, mortality, and clinical signs following a single oral administration of Ro 64-0796 in juvenile rats that were 7, 14, 24, and 42 days old.

Age	7 Days Post-Partum				14 Days Post-Partum				Days Post-Partum			
									7	14	24	42
Dose (mg/kg/day)	0	500	700	1000	0	500	700	1000	1000	1000	1000	1000
Study Type	Toxicity						Toxicokinetics					
# of Animals	7 M 7 F	7 M 7 F	7 M 7 F	7 M 7 F	7 M 7 F	7 M 7 F	7 M 7 F	7 M 7 F	28 M 28 F	14 M 14 F	14 M 14 F	14 M 14 F
# of pups mortality	0 M 0 F	0 M 0 F	0 M 2 F	3 M 0 F	0 M 0 F	0 M 0 F	0 M 0 F	0 M 0 F	5 M 2 F	0 M 1F	1 M 0 F	0 M 0 F
# of pups w/ clinical signs* but no mortality	0 M 0 F	0 M 0F	2 M 2 F	4 M 7 F	0 M 0 F	0 M 0 F	0 M 0 F	3 M 1 F	3 M 3F	0 M 0 F	0 M 0 F	0 M 0 F
* Clinical signs included, hypoactivity, cold to touch, pale, and/or irregular breathing												

Mortality, morbidity, clinical signs, body weights, food consumption were monitored. Blood samples were collected from 2 pups/sex/time points at 0.25, 0.5, 1, 2, 4, 8, and 24 hours postdose. Necropsy was performed on all pups at time of deaths or scheduled termination (24 hours postdose). Microscopic examinations of the following tissues were performed on all animals that died or killed prematurely and those animals in the vehicle control and 1000 mg/kg/day groups: gastrointestinal tract (esophagus, stomach, duodenum, jejunum, cecum, colon, and rectum), liver, kidneys, lungs, cerebrum, cerebellum, thoracic spinal cord, and carcase. Mortality and adverse clinical signs were associated with doses ≥ 700 mg/kg Ro 64-0796 in 7 day-old pups, but only with 1000 mg/kg dose in pups older than 14 days of age. Pups that were 42 days and older could tolerate repeated administration of Ro 64-0796 at doses > 1000 mg/kg/day.

Table 14. Toxicokinetic parameters of Ro 64-0796 and Ro 64-0802 in plasma and brain following a single oral administration of 1000 mg/kg Ro 64-0796 to 7, 14, 24, and 42 day old rats.

Ro 64-0796								
	Plasma				**Brain**			
Age (days old)	**7**	**14**	**24**	**42**	**7**	**14**	**24**	**42**
T_{max} (hr)	3	2	0.75	2	6	16	5	3
C_{max} (μg/ml)	58.70	66.90	15.20	8.63	41.20	9.49	1.90	0.71
AUC_{0-24h} (μg-hr/ml)	676.00	352.00	156.00	79.60	489.00	177.00	22.80	9.99
$t_{1/2}$ (hr)	5.84	6.27	2.25	2.49	5.12	15.9	4.18	5.06
AUC_{brain}/AUC_{plasma} ratio	-	-	-	-	1.4:1	1:2	1:7	1:8
$AUC_{42\text{-}day\text{-}old}$: $AUC_{7\text{-}day\text{-}old}$	-	-	-	1:8.5	-	-	-	1:49
Ro 64-0802								
T_{max} (hr)	4	4	5	2	8	8	5	2
C_{max} (μg/ml)	26.20	142.00	31.00	45.20	1.85	1.18	0.45	0.62
AUC_{0-24h} (μg-hr/ml)	336.00	752.00	430.00	507.00	26.90	19.70	6.05	7.82
$t_{1/2}$ (hr)	6.74	5.86	2.71	2.78	10.60	82.60	4.02	4.75
AUC_{brain}/AUC_{plasma}	-	-	-	-	1:12	1:38	1:71	1:65
$AUC_{42\text{-}day\text{-}old}$: $AUC_{7\text{-}day\text{-}old}$	-	-	-	1:1.5	-	-	-	1:3
AUC_{0796}:AUC_{0802} ratio	2:1	1:2	1:3	1:6	18:1	9:1	4:1	1:1

As observed in other juvenile rat studies, 7 day-old pups were the most sensitive to the toxicity of Ro 64-0796. The brain to plasma ratio for the exposures to Ro 64-0796 was 0.72, indicating that the prodrug readily crossed the blood-brain barrier in the 7 day-old rats, but the ratios dropped to 0.13-0.15 for the 24 and 42 day-old rats. The results suggested that blood-brain barrier may have reached maturity in the 24 day-old rats. Even though Ro 64-0802 was detectable in the brain, the exposures in brain were less than 8% as compared to those in the plasma. Since the brains in these animals were not perfused to remove residual blood, the amounts of both drugs, especially Ro 64-0802 could be overestimated. Taken together, the results also suggested that the presence of Ro 64-0796 in brain may be one of the reasons why mortality was observed in the 7 and 14 day-old rats. Since ADME data suggested that rats convert Ro 64-0796 less efficiently than the primates and mostly in blood rather than in liver (major organ of conversion in primates), the results from this juvenile rat study may have little relevance to humans.

11 Integrated Summary and Safety Evaluation

No new toxicity or target organ of toxicity was identified in this supplemental NDA submission. The target organs of toxicities identified previously in the animals were the gastrointestinal (GI) system, kidney, and bone. In addition, juvenile rats were shown to be more sensitive to the toxicity of Tamiflu™. In the postmarketing experience, neuropsychiatric adverse events that have equivocal relationship to Tamiflu™ treatment were reported. Thus, the present submission contains many pharmacokinetic and distribution studies to identify possible mechanism of action of the toxicity and address the safety concern associated with the usage by infants younger than 1 year of age.

The submitted nonclinical study results provide sufficient understanding of the toxicity and ADME profiles of both Ro 64-0796 and Ro 64-0802 to allow risk/benefit assessment of the usage and dosage of oseltamivir in children younger than 1 year old.

Below are brief summaries of Tamiflu™ related toxicities identified thus far.

Juvenile Animals:

Mortality was associated with a single dose of 500 mg/kg Ro 64-0796 in 7 day old rats while 14 days of daily administration of 2500 mg/kg Ro 64-0796 did not produce any toxicity in adult rats. The toxicity profiles were similar in adult and juvenile rats. The major difference that may have caused the difference in tolerance to Ro 64-0796 between juvenile and adult rats was the high exposures to Ro 64-0796 in juvenile rat brain. Juvenile rats have lower esterase activity in plasma (AUC ratio for Ro 64-0796 and Ro 64-0802 was 2:1 in 7 day-old rats vs. 1:3 in adult rats) and lower renal clearance, resulting in much higher exposures to the prodrug (AUC ratio of 7 day-old to 42 day-old rats was 8.5:1). In addition, Ro 64-0796 crossed the blood brain barrier more readily in 7 day old rats which resulted in higher exposures to Ro 64-0796 in the brain (AUC ratio of 7 day-old to 42 day-old rats was 49:1). The immature and thus more porous blood brain barrier in juvenile rats also allowed penetration of more polar Ro 64-0802 into the brain. Maturation of these physiological functions was beginning to be observed in the 14 day old rats that converted prodrug more efficiently and allowed less penetration of drugs through blood-brain barrier, thus tolerated higher Ro 64-0796 doses.

The oral and intravenous pharmacokinetics of Ro 64-0796 and Ro 64-0802 were studied in adult and newborn marmosets, a species whose pharmacokinetic profile more closely resembles human's than does rat's. The results showed that following administration of oseltamivir to neonatal marmosets, significant concentrations of oseltamivir carboxylate are present in plasma. The resulting ratios of Ro64-0796:R0 64-0802 are lower in neonates than in adults, even though the *in vitro* data suggest reduced carboxylesterase activity in the newborns. The apparent discrepancy in neonates is likely reflective of less hepatic esterase activity and renal clearance of prodrug and slower renal clearance of active drug. The *in vitro* and *in vivo* information from marmosets was incorporated into a human PBPK model. Model simulations of pharmacokinetic parameters using data from marmosets predicted similar pharmacokinetic profiles between human adults and infants following oral and intravenous dosing.

The safety margins are 786-2358X for Ro 64-0796 and 17-50X for Ro 64-0802 via intravenous or oral administration. These safety margins are calculated using AUC values of 0.2848 and 4825.8 μg-hr/ml (based on clinical data) for prodrug and active drug, respectively, in infants < 1 year old and 676 and 336 μg-hr/ml, respectively, for 7 day-old rats. The reason for using the AUC values from the 7 day-old rats even though they were obtained after only a single oral dose, was the huge age-related reduction in exposures to prodrug. The systemic exposure to Ro 64-0796 in 14 day-old rats was about 50% that in 7 day-olds, suggesting that the AUC values obtained from multi-dose studies using older rats are inappropriate for estimating safety margin.

The following table contains safety margins provided by the sponsor:

Table 15. Sponsor calculated safety margins from preclinical data compared to the simulated steady state exposures following 3 mg/kg b.i.d. in infants < 1 year of age.

Species [Ref. no.]	Route; duration	Age in Days	NOAEL (mg/kg)	Plasma C_{max} (ng/mL)		Plasma AUC_{0-24h} (µg.h/mL)		Safety Margin C_{max}		Safety Margin AUC	
Juveniles				OP	OC	OP	OC	OP	OC	OP	OC
Rat [RDR W-0143066]	Oral; 2 weeks	20	500	16,600	10,100	59	71.2	200	15	187	11
Rat [RDR W-0143067]	Oral; 4 weeks	48	500	6880	15,400	37.9	103	83	23	120	16
Rat [RDR 1008172]	Oral; single dose	7	1000	55,870	26,200	676	336	673	39	2139	53
		14	1000	66,900	142,000	352	752	806	214	1114	118
		24	1000	15,200	31,000	156	430	183	47	494	68
		42	1000	8630	45,200	79.6	507	104	68	252	80
Rat [RDR 1027696]	Oral; single dose	7	394	42,400	9380	410	139	511	14	1297	22
		42	1314	11,500	38,400	82.7	467	139	58	262	73
Rat [RDR 1029873]	SC; single dose	7	50		88,900		152		134		24
Marmoset [RDR 1038614] (all single dose)	Oral (Males)	–	10	7130	19000	17800	89900	86	29	56329	14129
	Oral (Females)	–	10	4590	16900	12900	83600	55	25	40823	13138
	IV (Males)	–	5	6890	5280	7100	25600	83	8	22468	4023
	IV (Females)	–	5	8780	5020	7480	22500	106	8	23671	3536
Infants <1 yr				**83**	**664**	**0.316**	**6.363**				

[1] Simulated median exposure data in infants less than 1 year of age dosed 3 mg/kg b.i.d. extracted from the Oseltamivir Simulation Report . Table reports margins derived from median model-predicted (Individual Simulation Method) values for oseltamivir (OP) and OC steady-state AUC0-tau and Cmax from the most conservative under 1 sub-group (oseltamivir C_{max} = 83 ng/mL, AUC = 0.316 µg*h/mL from infants 9-12months; OC C_{max} = 664 ng/mL, AUC = 6.363 µg*h/mL from infants 0-1month).

OC, oseltamivir carboxylate; OP, oseltamivir phosphate/oseltamivir; SC, subcutaneous; IV, intravenous.

Even using the most conservative estimate, the safety margin for Ro 64-0796 was 120X, suggesting low safety concern for pediatric patients who are less than 1 year of age.

Neuropsychiatric Toxicities:

A variety of neurologic and behavioral symptoms including hallucinations, delirium, and abnormal behaviors that have resulted in fatal outcomes were reported in patients (primarily in the pediatric population) who took Tamiflu™. However, since influenza can also cause these symptoms, their causal relationship to Tamiflu™ administration is unclear. In addition, seizure has been reported as one of the postmarketing experiences. In order to investigate the potential for Ro 64-0796 and Ro 64-0802 to cause neurological or CNS toxicities, studies of the safety pharmacology as well as the distribution and exposures of both drugs in brain and CSF of both adult and juvenile animals were conducted.

Penetration into brain by Ro 64-0796 (oseltamivir) and Ro 64-0802 (oseltamivir carboxylate; OC) were observed in rodents. The extent of penetration depends on the age of the animals, physico-chemical properties (Ro 64-0802 being more polar than Ro 64-0796, thus lower levels in brain), PK properties (Ro 64-0802 with lower volume of distribution, hence lower levels in brain), and affinity to efflux transporter (only Ro 64-0796 with affinity to g-glycoprotein, thus more likely to cross blood brain barrier). Increased brain concentrations of Ro 64-0802 can occur following induction of inflammation by lipopolysaccharides, inhibition of active transport (MRP4 and OAT3) processes at the blood-brain barrier, or immature enzyme activities (low carboxylase expression). However, no neurological or CNS toxicity, nor any seizure potential was identified for Ro 64-0796 and Ro 64-0802 in any of the safety pharmacology and

general toxicity studies. Thus, the animal data suggest high safety margins for both Ro 64-0706 (2358X human exposure) and Ro 64-0802 (50 human exposure) even if the extent of blood-brain barrier penetration of both drugs in human infants is similar to that in juvenile rats following oral administration.

GI Toxicities:

Incidence of nausea, vomiting, and diarrhea is, in general, higher in both adults and children taking Ro 64-0796. Children were not more sensitive to GI toxicities caused by the prodrug, Ro 64-0796. In rabbits, GI irritation caused by Ro 64-0796 was the dose limiting toxicity. Death and premature sacrifice were seen in rabbits after only 4 oral doses of ≥750 mg/kg Ro 64-0796 because of the severe erosion/ulceration of the GI tissues and the associated symptoms (inappentence, reduced fecal output, body weight loss, etc.). The NOAEL for rabbits was 250 mg/kg following 10 days of dose administration. Similar irritation/ulceration/hemorrhage was seen in the stomach of marmosets that received 2000 mg/kg/day Ro 64-0796 once a day dose. But the severity was much milder and did not result in reduced food consumption or body weight loss. After 9 months of daily oral administration, the only GI toxicity associated with twice a day 500 mg/kg dose was salivation and emesis. Rodents were less sensitive to the GI irritation caused by Ro 64-0796. At 1000 mg/kg, the GI motility and gastric emptying were significantly reduced and stomach weights increased in rats. In addition, salivation was associated with 2000 mg/kg dose in the same species and this GI effect was managed by splitting the dose into two daily administrations. Using isolated guinea pig ileum, both Ro 64-0796 and Ro 64-0802 did not affect the resting tension and agonist response of smooth muscle.

Renal Toxicities:

Even though renal toxicities were observed in all species studied at high oral doses, it was not one of the common adverse reactions observed in the clinical studies or postmarketing experiences. These reversible renal toxicities were probably caused by saturation of renal glomerular filtration from the high plasma drug concentrations. The results from ADME studies suggested that renal glomerular filtration was the main route by which Ro 64-0796 and Ro 64-0802 are eliminated from the body. In addition, electrolyte excretion in urine was reduced in the presence of high drug concentrations. The renal toxicities in nonclinical studies likely had low clinical relevance since saturation of glomerular filtration is not expected at the recommended clinical dose in people with normal renal function. Dose adjustment is recommended in the label for patients with renal impairment.

Cardiovascular Toxicities:

Arrhythmia was reported as one of the postmarketing experiences. A variety of safety pharmacology studies investigated cardiovascular effects at doses similar to the clinical dose. Neither *in vitro* nor *in vivo* studies in rats and dogs definitively indicated that Ro 64-0796 or Ro 64-0802 may cause arrhythmia.

Bone Toxicities:

Three mortalities in low and mid dose groups in a 9 month oral toxicity study in marmosets were attributed to osteomalacia, slight but statistically significant elevation of alkaline phosphatase levels were noted at doses ≥ 1000 mg/kg/day Ro 64-0796 in rats, and abnormal ossification processes were seen in rat and rabbit fetuses that were exposed to Ro 64-0796 and Ro 64-0802 *in utero*. No additional nonclinical studies were conducted to address this safety concern. However, there is no clinical safety signal to date suggesting a potential for bone toxicity.

12 Appendix/Attachments

12.1 Studies Reviewed

NOTE: The studies listed below are not directly relevant to the proposed indication currently being assessed (i.e., the treatment of influenza in infants with a post conceptual age of at least (b) (4) weeks to 1 year of age who have been symptomatic for 2 days or less). However, these studies were submitted with this NDA and were reviewed below.

Ro 64-0796 (GS-4104.02) Irwin dose-range in mice following oral administration. (Report # W-142690).

Effect of Ro 64-0796/002 (Tamiflu) in a modified Irwin test in the mouse (DDHN1018) (Report # 1002630).

Ro 64-0796 (GS-4104.02) assessment of locomotor activity in mice following oral administration (Report # W-142691).

Effects of Ro 64-0796/002 (GS4104.02) in studies to assess anticonvulsant and pro-convulsant activity and hexobarbital-induced sleeping time in the mouse (DHB08602 and DHB08605) (Report # W-142971).

Effects of Ro 64-0796/002 (GS-4104.02) in studies to assess nociception, renal function, gastrointestinal transit and gastric emptying, respiratory rate and body temperature in the rat (DHB08603, 4, 6 and 8 (Report # W-142972).

Ro 64-0796 (GS-4104.02) cardiovascular and respiratory evaluation in the anaesthetized dog following intraduodenal administration (Report # W-142692).

Ro 64-0802/002 (GS-4071) cardiovascular and respiratory evaluation in the anaesthetized dog following intravenous administrations (DHB08601) (Report # W-142974).

Ro 64-0802/002 (GS 4071) on ECG interval parameters and heart rate in the conscious Beagle dog following intravenous administration (DDHN1027 and 28) (Report # 1003167).

Ro 64-0802/002 (GS-4071) *in vitro* evaluation in the isolated Purkinje fibre (DHB11801). (Report # W-143050).

Evaluation of the actions of Ro 64-0796/002 and its active metabolite Ro 64-0802/002 on action potentials in isolated rabbit cardiac Purkinje fibres (Report # 1003174).

Evaluation of the actions of Ro 64-0796/002 and it's active metabolite Ro 64-0802/002 on K currents through recombinant hERG channels expressed in CHO cells.

Ro 64-0796/002 (GS-4104.02) and Ro 64-0802/002 (GS-4071) *in vitro* evaluation in the isolated guinea-pig ileum (DHB08607) (Report # W-142973).
Effect of Ro 64-0802 (GS4071) on T cell proliferation *in vitro* (Report # W-143073).
RO0640796 (oseltamivir): Validation of a method for the determination of RO0640796 and RO0640802 in human plasma samples (Report # 1049241).
RO0640796 (oseltamivir): Stability of RO0640796 and RO0640802 in human EDTA plasma containing sodium fluoride (b) (4) (Report # 1039095).
An investigation into the site of absorption of oseltamivir (Ro 64-0796) in the dog after oral administration (Report # 1001668).
RO0640796 (Oseltamivir, Tamiflu®): influence of milk and GlySar co-administration on the oral absorption in adult, fed Sprague Dawley rats (Report # 1035034).
RO0640796 (Oseltamivir, Tamiflu®): Influence of milk and GlycylSarcosine on its oral absorption in adult fasted Sprague Dawley rats (Report # 1035372).
The absorption, metabolism and excretion of Ro 64-0796 in rats after oral and intravenous doses of 14-carbon labelled Ro 64-0796 (DHB08101) (Report # 143015).
The pharmacokinetics of Ro 64-0796 and Ro 64-0802 after oral and intravenous administration to the rat (DHBO8301) (Report # W-143046).
RO0640796 (Oseltamivir): Pharmacokinetic assessment after oral administration to wild type and Pept-1 knock out mice (Report # 1037322).
Tissue distribution of radioactivity in male Sprague Dawley rats following oral administration of 14-carbon labelled Ro 64-0796 (Report # W-142900).
Whole body phosphor imaging in the ferret following single oral administration of [^{14}C]Ro 64-0796, (DHB09201) (Report # W-142931).
The disposition and pharmacokinetics of Ro 64-0802 after intravenous administration, and after oral and intravenous administration of the pro-drug, Ro 64-0796, to female ferrets (Protocol No. 98/17/ROC/5) (Report # W-143018).
A cross-species comparison of the protein-binding and red cell partitioning of Ro 64-0796 and Ro 64-0802 (DHB08201 and DHB08202) (Report # W-143012).
Whole body phosphor imaging in the male and pregnant female rat following single oral administration of [^{14}C] Ro 64-0796 (DHB06001) (Report # W-142778).
Quantitative tissue distribution of radioactivity in male and pregnant female rats following single oral administration of [^{14}C]Ro 64-0796 (DHB09101) (Report # W-142967).
The disposition and pharmacokinetics of Ro 64-0796 and Ro 64-0802 after single oral administration of the pro-drug, Ro 64-0796, to pregnant rabbits (DHB07101) (Report # W-142986).
A reassessment of the metabolism of Ro 64-0796 by man (Report # 1001667).
Further *in vitro* studies on the metabolism of Ro 64-0796 in animals and man (DHB06803) (Report # 1001812).
The metabolism of Ro 64-0796 and Ro 64-0802 by human and canine hepatic “S9” and microsomal preparations (DDHN1030) (Report # 1003165).
Hydrolysis of RO0640796 (oseltamivir) to RO0640802 (oseltamivir Carboxylate) by recombinantly expressed human carboxylesterases 1 and 2 (HCE1 and HCE2) (Report # 1029175).

Ro 64-0796 (GS4104): The metabolism of Ro 64-0796 by rat, marmoset and human liver microsomes. Comparison with the metabolite profiles obtained from rat and marmoset urine following oral dosing with Ro 64-0796 (Report # W-142735).
Ro 64-0796: Excretion balance study in the marmoset after single intravenous and oral doses of 10 and 20 mg/kg, respectively (DM/96/017E) (Report # W-142663).
An *in vitro* study into the interaction of Ro 64-0802 and the human renal organic ion transporter (Report # 1003166).
Ro 64-0796/002 (oseltamivir phosphate): A 14-day rat study by the intravenous route (Report # 1001809; Study # 276/115).
Ro 64-0796i002: A 2-week intravenous toxicity and toxicokinetic study in the marmoset (Report # 1002143; Study # SAP718).
Local tolerance study with oseltamivir phosphate via perivenous and intraarterial Injections in rabbits (Report # 1025870).
Ro 64-0796 (also known as GS 4104): Oral (gavage) rabbit tolerance study (HRE/62/R) (Report # W-142792).
Ro 64-0796 (also known as GS 4104): A seven-day gastrointestinal tolerance study in dogs with the 75 mg capsule final formulation (Report # W-143072; Study # 276/104).

12.2 Safety Pharmacology Studies

12.2.1 Neurological/CNS functions

The effects of Ro 64-0796/002 on neurological and CNS functions were evaluated in mice and rats via oral and intravenous administration.

Mouse

Male ICR CD-1 mice were administered a single oral dose of 10, 100, or 1000 mg/kg Ro 64-0796 (Report # W-142690). The animals were monitored for general behavior and neurological state, motor functions, sensory responses, seizure potential, sleep, arousal, and body temperature using Irwin method of assessment at 30, 90, 150, and 300 minutes and 24 hours postdose. No change on gross physiology or behavior was noted.

Male CD-1 mice received a single intravenous dose of 3, 10, 30, or 100 mg/kg Ro 64-0796 (Report # W-1002630). Similar assessment as described above using Irwin method was carried out. No effect was associated with doses ≤ 30 mg/kg. However, all animals died within 5 minutes of administration at 100 mg/kg.

Ten male ICR CD-1 mice/dose received a single oral dose of 10, 100, or 1000 mg/kg Ro 64-0796 (Report # W-142691). Negative control group was dosed with water while the positive control group with 15 mg/kg diazepam. Spontaneous locomotive activity was monitored for each animal using an activity meter and infrared detection system at 10-minute interval from 30 to 90 minutes postdose. Ro 64-0796 did not exert any significant change on locomotor activity while diazepam caused 65% reduction in locomotor activity at 70-80 minutes postdose.

Eight male CD-1 mice/group received a single oral dose of 10, 100, or 1000 mg/kg Ro 64-0796 followed by a subcutaneous injection of pentylenetetrazole 2 hours later (Report # W-142971). The animals were observed for 45 minutes for signs of seizures

and loss of posture. Chlordiazepoxide and picrotoxin were used as positive controls for anti-convulsant and pro-convulsant, respectively. The results indicated that Ro 64-0796 had neither pro- nor anti-convulsant effects at doses up to 1000 mg/kg.

In the same study, another set of 8 male CD-1 mice/group received a single oral dose of 10, 100, or 1000 mg/kg Ro 64-0796 followed by an oral dose of hexobarbital 2 hours later (Report # W-142971). Chlorpromazine was given as the positive control at 30 minutes before the administration of hexobarbital. The animals were observed for 120 minutes for loss of righting reflex in addition to the determination of sleep duration. Plasma levels of the active drug, Ro 64-0802, were also determined. The C_{max} and AUC values were 87 μg/ml and 666 μg-hr/ml, respectively for the dose of 1000 mg/kg Ro 64-0796. These values that were at least two magnitudes of order higher as compared to 0.3 μg/ml (C_{max}) and 3.1 μg-hr/ml (AUC) following a single 100 mg dose of Ro 64-0796 in man. At these exposures, no significant effects on sleep time and righting reflex were found.

Rat:

A single oral dose of 10, 100, or 1000 mg/kg Ro 64-0796 was administered to 8 Sprague-Dawley rats/dose (Report # W-142972). The tails of the animals were exposed to an infrared heat source prior to dosing and at 130, 150, 180, 240, and 400 minutes postdose to determine the effect of compound on nociceptor. 20 mg/kg morphine given orally served as the positive control. Small, but statistical significant decrease in the response time to the painful stimuli was seen in all treatment groups. However, the effects were not time nor dose related and were similar to what had been observed in the pre-dose baseline response. Thus, the observed effect on the nociception by Ro 64-0796 was not considered to have any pharmacological significance. Morphine, as expected, increased the response time significantly.

12.2.2 Cardiovascular and respiratory functions

Rat:

A single oral dose of 10, 100, or 1000 mg/kg Ro 64-0796 was administered to 8 Sprague-Dawley rats/dose (Report # W-142972) and the effects on respiratory rate (in a plethysmorgraphy chamber) and body temperature (via rectum) were determined. 20 mg/kg chlorpromazine given orally served as the positive control. No treatment effect was observed for Ro 64-0796 while both respiratory rate and body temperature were reduced in the positive control group.

Dog:

Three anaesthetized female dogs received vehicle (water) followed two hours later by the administration of a single dose of 100 mg/kg Ro 64-0796 via intra-duodenal route (Report # W-142692). Systolic, diastolic, and mean arterial blood pressure, heart rate, left ventricular systolic pressure and dp/dt max, ECF, lead II, femoral blood flow and resistance, respiratory rate, minute volume, and tidal volume were measured for 2 hours after each dose. In addition, blood samples were collected for the determination of plasma levels of Ro 64-0796 and Ro 64-0802. It was found that C_{max} values for Ro 64-0796 and Ro 64-0802 were 23 and 9.7 μg/ml, respectively, as compared to 0.05 and 0.3

μg/ml following 100 mg oral dose of Ro 64-0796 in man. The pharmacokinetic data suggested that dogs do not hydrolyze the parent compound, Ro 64-0796, efficiently as compared to humans. At the exposure levels in dogs, no effects in the cardiovascular and respiratory parameters were detected.

Ro 64-0802/002 was administered continuously (30 minutes/dose) by intravenous route to 4 male anaesthetized Beagle dogs to achieve the accumulated single doses of 2, 15, and 100 mg/kg (Report # W-142974). ECG lead I, II, III, aVR, aVL, and aVF configurations (although these were not reported), lead II ECG variables (RR, PR, QT and QTc-intervals and QRS duration), arterial blood pressure (systolic, diastolic and mean), heart rate, mean femoral arterial blood flow, left ventricular pressures (systolic, end-diastolic, dP/dt max and dP/dt.P-1), cardiac output by thermodilution, respiratory rate, arterial blood gases (pCO_2 and pO_2), and pH as well as total peripheral resistance and femoral arterial conductance were measured for 4 hours post dose and analyzed. Small, but statistically significant increases in QTc-interval were associated with the 100 mg/kg dose as compared to the pre-dose values. No other cardiovascular and respiratory parameters were affected.

Six conscious Beagle dogs/sex received a dose of 248 mg/kg Ro 64-0802/002 by continuous intravenous infusion over a period of 150 minutes in order to achieve a steady state plasma level of 300 μg/ml (Report # 1003167). ECG was monitored since QTc prolongation was observed in the previous study. No treatment effect was observed.

In vitro:

The pro-arrhythmic potential of Ro 64-0796/002 and Ro 64-0802/002 were evaluated using isolated sheep (Report # W-143050) and rabbit (Report # 1003174) cardiac Purkinje fibers. Sheep Purkinje fibers were paced at a frequency 1 Hz and a supramaximal voltage for 60 minutes prior to exposure to 0.03, 0.3, 3, 30, and 100 μM Ro 64-0802/002. The frequencies of 1 and then 0.2 Hz were applied to the rabbit Purkinje fibers prior to exposure to 0.03, 0.3, 3, 30, and 100 μM Ro 64-0802/002 and 0.0075, 0.075, 0.75, 7.5 and 22.5 μM of Ro 64-0796/002. It was found that both Ro 64-0796 and Ro 64-0802 at concentrations up to 22.5 and 100 μM, respectively, did not have any effect on action potential parameters measured.

The potential of Ro 64-0796/002 and Ro 64-0802/002 to cause QT prolongation was evaluated using cloned hERG potassium channels expressed in Chinese Hamster Ovary cells . Concentrations of 0.03, 0.3, 3, 30 and 100 μM of Ro 64-0802/002 and 0.0075, 0.075, 0.75, and 7.5 μM of Ro 64-0796/002 were tested and found to exert no effects on inward or outward potassium current at the highest concentrations tested.

12.2.3 Renal functions

Eight male rats/dose received a single oral dose of 10, 100, or 1000 mg/kg Ro 64-0796 and the effects on urine and electrolyte excretion were evaluated (Report # W-142972). 20 mg/kg furosemide was used as the positive control. The results showed that, at 0-3 hour and 3-6 hour collection times, doses ≥ 100 mg/kg caused significant increases in electrolyte excretion (Na^+, Cl^-, K^+, and phosphate) which were accompanied by a

diuretic effect at 1000 mg/kg in the 6-24 hour collection. In addition, in the 6-24 hour collection time, 1000 mg/kg dose caused a significant reduction in K^+ excretion without any other effect. Increases in electrolyte excretion were reported in a 27-week rat oral toxicology study at 1000 mg/kg/day.

12.2.4 Gastrointestinal functions

Rat:

Eight male rats/dose received a single oral dose of 10, 100, or 1000 mg/kg Ro 64-0796 and the effects on gastrointestinal motility and gastric emptying were evaluated (Report # W-142972). Morphine at 20 mg/kg given orally served as the positive control. At 1000 mg/kg, gastrointestinal motility and gastric emptying were significantly reduced and stomach weights increased as compared to the vehicle control. Morphine exhibited similar effects, as expected. No such effects were observed in the 6-month oral toxicity study in rats, suggesting that these effects were transient without causing long-term changes.

Guinea pig:

Isolated guinea pig ileum was incubated with 0.47, 4.7, and 47 μM Ro 64-0796/002 and 1, 10, and 100μM Ro 64-0802/002 to evaluate the drugs' effects on resting tension and agonist response of smooth muscle (Report # W-142973). The agonists used in the study were acetylcholine, histamine 5-hydroxytryptamine, and barium chloride. No effect was detected at the highest concentrations tested.

12.2.5 Immune functions

Antigen specific T cell lines were prepared with influenza virus X31 (H3N2) before treatment with 0.1, 1, and 10 μM Ro 64-0802/002 (Report # W-143073). T-cell proliferation was measured by incorporation of ^{3}H-thymidine. Cyclosporine A (300 nMO was used as the positive control. Complete inhibition of T-cell proliferation was associated with 300 nM cyclosporine while the inhibition by 1 and 10 μM Ro 64-0802/002 was small but significant (15% and 20%, respectively). In the same study, peripheral blood lymphocyte antigen stimulated proliferation was also tested against 0.1, 1, and 10 μM Ro 64-0802/002. Peripheral blood lymphocytes were obtained from volunteer donor blood and prepared prior to incubation with Ro 64-0802. There was no significant inhibition of ^{3}H-thymidine incorporation at any of the concentration of Ro 64-0802 tested, while the positive control, cyclosporine A, exerted 90% inhibition.

12.3 PK/ADME

12.3.1 Analytical methods:

A quantitative analytical method was developed for the determination of Ro 64-0796 and Ro 64-0802 in human K_3-EDTA/NaF plasma samples using HPLC/MS/MS (Report # 10429241). The calibration ranges were 1 - 2500 ng/ml for Ro 54-0796 and 10 - 10000 ng/ml for Ro 64-0802. The method was shown to pass all the criteria for

selectivity and matrix variability, sensitivity, within- and between-run precision as well as total precision, within- and between-run accuracy, robustness, dilution, recovery, matrix effect, carry-over, autosample stability, plasma, and free/thaw stability. The method was used to determine the stability of 64-0796 and Ro 64-0802 in human plasma containing sodium fluoride (Report # 1039095). It was found that Ro 64-0796 was stable for at least 29 hours at room temperature, 27 hours at 5 ± 3°C, and 376 days at -20 ± 5°C and below -65°C. Ro 64-0802 was stable for at least 26 hours at room temperature, 24 hours at 5 ± 3°C, and 376 days at -20 ± 5°C and below -65°C. Stability after repeated freezing/thawing was determined to be 3 cycles for Ro 64-0796 and 2 cycles for Ro 64-0802 at -20 ± 5°C and below -65°C.

12.3.2 Absorption:

Four female beagle dogs received 75 mg Ro 64-0796 by oral, intra-duodenal, intra-ileal, and intra-colonic infusion using a cross-over design with a seven day washout period between each route of administration (Report # 1001668). The systemic exposure following intra-duodenal, intra-ileal, and intra-colonic infusion was 91%, 81%, and 62%, respectively, of that obtained from oral administration.

The effects of milk and GlycylSarcosine (PEPT-1 inhibitor) on the systemic exposures of Ro 64-0796 and Ro 64-0802 were investigated in rats under fed state (Report # 1035034). Three male fed Sprague-Dawley rats/group received an oral dose of 30 mg/kg Ro 64-0796 that were formulated in aqueous solution, as emulsion in pasteurized bovine milk, or in aqueous solution containing 125 mM GlySar. Blood samples were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 24 hours postdose. The results showed that both milk and GlySar did not significantly influence the systemic exposures (C_{max} and AUC values) to Ro 64-0796 and Ro 64-0802.

The effects of milk and GlycylSarcosine (PEPT-1 inhibitor) on the systemic exposures of Ro 64-0796 and Ro 64-0802 were investigated in rats under fasted state (Report # 1035372). Three male fasted, Sprague-Dawley rats/group received an oral dose of 30 mg/kg Ro 64-0796 that were formulated in aqueous solution, as emulsion in pasteurized bovine milk, or in aqueous solution containing 125 mM GlySar. Blood samples were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 24 hours postdose. The results showed that both did not significantly influence the systemic exposures (C_{max} and AUC values) to Ro 64-0796 and Ro 64-0802. However, slightly higher AUC values for both the prodrug and active metabolite were observed in the presence of 125 mM GlySar even though the plasma raw data and other derived pharmacokinetic parameters did not show statistically significant difference from those for the aqueous solution.

Two Sprague-Dawley rats/sex/group received [^{14}C]-labeled Ro 64-0796 as an oral dose at 0 (acetate buffer, pH 4), 10, 50, 250, or 1500 mg/kg or as an intravenous dose at 10 mg/kg (Report # 143015). Samples of urine and feces were collected and quantified for [^{14}C]-labeled Ro 64-0796 and Ro 64-0802. The major route of elimination following both oral (46-71%) and intravenous (~66%) administration was by urine. The [^{14}C]-labeled Ro 64-0796 was biologically stable since very little radioactivity was lost as CO_2 (~1%)

or retained in the carcasses (~0.5%) of the rats. The apparent absorption, based on urinary recoveries, was estimated to be 68%, 77%, 92%, and 105% for the doses of 10, 50, 250, and 1500 mg/kg, respectively. The major components in the urine and feces samples were Ro-64-0802 and Ro 64-0796, accounting for up to 69% of total radioactivity. In addition to Ro 64-0802, a number of monohydroxylated metabolites of both Ro 64-0796 and Ro 64-0802 were found mostly in feces, accounting for up to 20% of total radioactivity following the 10 mg/kg oral dose. The percentages of these metabolites were lower at higher oral doses. A metabolite, M3, which is ®-ω-carboxy-Ro 64-0796 was formed more readily in males and was a major metabolite (18%) following the 10 mg/kg dose by oral administration, but not intravenous administration. Saturation of cytochrome P450 metabolism was observed following the administration of the 1500 mg/kg dose since little oxidative metabolites were detected in urine and feces at this dose. Since all of the metabolites that were present in sufficient amounts were soluble in water, it's unknown what caused the renal mineralization following high doses of Ro 64-0796 in rats.

Five rats/sex/group received a single oral dose of 10 or 50 mg/kg [^{14}C]-Ro 64-0796 or a single intravenous dose of 10 mg/kg [^{14}C]-Ro 64-0796 or [^{14}C]-Ro 64-0802 (Report # W-143046). Blood samples for the drug concentration determinations were collected into tubes containing 5 μg/ml dichlovos to inhibit *ex vivo* hydrolysis of Ro 64-0796 by plasma esterase. The pharmacokinetics of Ro 64-0796 and Ro 64-0802 are presented in the following table. Rat is inefficient in converting Ro 64-0796 to Ro 64-0802. Systemic exposures of Ro 64-0802 was the same or lower than those for Ro 64-0796 following oral or intravenous administration of 10 mg/kg Ro 64-0796. As a comparison, the exposure ratio of Ro 64-0802 to Ro 64-0796 in humans is ~ 30:1 at the clinical dose. Clearance and steady-state volume of distribution for Ro 64-0796 were greater than those for Ro 64-0802 following intravenous administration, reflecting the facts that Ro 64-0796 were cleared by several pathways (hydrolysis to Ro 64-0802, cytochrome P450-catalyzed hydroxylation, glomerular filtration, and secretion by gut-wall) and it's lipophilic. The likely reason for lower oral bioavailability for Ro 64-0802 at 10 mg/kg as compared to 50 mg/kg was the saturation of cytochrome P450-mediated metabolism of Ro 64-0796 which allowed more to be cleared via conversion to Ro 64-0802.

Table 16. Pharmacokinetic parameters of Ro 64-0796 and Ro 64-0802 following intravenous administrations of 10 mg/kg Ro 64-0796 or Ro 64-0802 or oral administration of 10 or 50 mg/kg Ro 64-0796 in rats.

Route of admin.	**Intravenous**			**Oral**			
Drug admin.	**Ro 64-0796**		**Ro 64-0802**	**Ro 64-0796**			
Dose (mg/kg)	**10**		**10**	**10**		**50**	
Drug monitored	**Ro 64-0796**	**Ro 64-0802**	**Ro 64-0802**	**Ro 64-0796**	**Ro 64-0802**	**Ro 64-0796**	**Ro 64-0802**
T_{max} (hr)	0.5	0.5	0.3	0.5	1.5	0.5	0.5
C_{max} (μg/ml)	3.08	0.89	9.03	0.15	0.11	2.24	3.53
$AUC_{0-\infty}$ (μg-hr/ml)	2.42	1.61	6.49	0.75	0.78	4.47	13.1
$t_{1/2}$ (hr)	0.34 (.5-1.5h) 3.0(2.5-12.5h)	0.47 (.5-1.5h) 2.6(2.5-12.5h)	0.20 (.5-1h) 2.1(1.5-12.5h)	0.77 (1-3h) 5.9 (4-24h)	4.1 (2-24h)	1.1 (.5-6h) 5.8 (8-24h)	3.34 (1.5-24h)
CL (ml/min/kg)	4.13	-	1.54	-	-	-	-
Vss (l/kg)	3.84	-	1.86	-	-	-	-
Bioavailability (%)	-	24.8	-	31.0	12.0	36.9	40.7

The role that Peptide Transporter-1 (PEPT-1), a high-capacity low-affinity transporter that mediates the intestinal uptake of di- and tripeptides from the diet, plays on the oral absorption of Ro 64-0796 was studied in PEPT-1 knock out mice. A single 5 mg/kg dose of Ro 64-0796 was administered orally to male wild-type and Pept-1 knock out mice in the presence and absence of 125 mM glycylsarcosine, a substrate and inhibitor of PEPT-1 (Report # 1037322). Blood samples were collected for the concentration determinations of Ro 64-0796 and Ro 64-0802. The results are presented in the following table. The systemic exposures to Ro 64-0796 were a half of those in knock out mice than in the wild-type mice, indicating the uptake transporter PEPT-1 may be involved in the oral absorption of Ro 64-0796 in the GI system. 125 mM GlySar did not seemed to affect the systemic exposures to either Ro 64-0796 or Ro 64-0802 in either wild-type or knock out mice. It's expected that since the systemic exposures for Ro 64-0802 in the knock out mice would be a half those in the wild-type mice to reflect those for the parent compound, Ro 64-0796. However, the systemic exposures for Ro 64-0802 were the same in the presence and absence of PEPT-1 transporter in addition to substantial absorption of Ro 64-0796 in the absence of PEPT-1 transporter, suggesting other mechanism(s) are involved in absorption and metabolism of Ro 64-0796.

Table 17. Pharmacokinetic of Ro 64-0796 and Ro 64-0802 following a single oral dose of 5 mg/kg Ro 64-0796 in wild-type and PEPT-1 knock out mice.

	Ro 64-0796				**Ro 64-0802**			
Animal Type	**WT**	**WT**	**KO**	**KO**	**WT**	**WT**	**KO**	**KO**
125 mM GlySar	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
T_{max} (hr)	0.25	0.25	0.29	0.25	0.58	0.67	0.67	0.58
C_{max} (μg/ml)	9.57	7.63	4.21	6.43	7.74	6.57	5.25	6.72
$AUC_{0-\infty}$ (μg-hr/ml)	10.60	9.33	5.30	6.29	11.30	12.70	12.20	9.72
$t_{1/2}$ (hr)	1.85	3.10	2.17	1.65	1.85	2.70	1.93	2.34
MRT (hr)	1.22	1.76	1.51	1.08	1.52	2.23	2.22	1.52
KO = PEPT-1 knock out mice WT = Wild-type mice								

12.3.3 Distribution:

A single oral dose of 10 mg/kg [^{14}C] Ro 64-0796 (specific activity 10.4 μCi/mg) was administered to male Sprague-Dawley rats (Report # W-142900). Tissue, GI content, and carcass from 4 rats/time point were analyzed for radioactivity at 1, 6, and 24 hours postdose. In addition urine, feces, and cage-wash were collected 4, 8, 12, and 24 hours postdose for radioactivity determination. As seen in previous studies, drug-related material was well-distributed in all tissues except in brain. The radioactivity was high in lungs (twice that in plasma) during the early time points. As expected, radioactivity levels were high in all of the organs of metabolism and excretion. Excretion was rapid since only 3% of the original radioactivity remained in the animals 24 hours postdose. Twenty-nine % of total radioactivity was excreted through kidneys while 61% was found in feces.

A single oral dose of 5 mg/kg [^{14}C] Ro 64-0796 was administered to 3 ferrets (Report # W-142931). Tissue concentrations of radioactivity were quantified from 1 animal/time point at 0.5, 2, and 6 hours postdose by whole body phosphor imaging. Drug-related radioactivity was rapidly and widely distributed with the highest levels in the content of GI tract. Peak radioactivity was detected at 2 hours postdose in the majority of tissues with the highest levels in the wall of urinary bladder, followed by kidney cortex, liver, thyroid, pituitary, and lung. The amount of radioactivity in plasma was ~ 1/6 that in lung. Substantial amount of radioactivity was also detected in trachea, nasal mucosa, and the middle ear. The exposures ($AUC_{0\text{-}6h}$) in lungs were about 5.3X those in blood.

The excretion and pharmacokinetic profiles of Ro 64-0796 and Ro 64-0802 were studied in female ferrets (Report # W-143018). Ro 64-0796 were given as a single oral or intravenous dose at the level of 5 or 2 mg/kg, respectively, and Ro 64-0802 by intravenous route at a dose level of 2 mg/kg. Blood, urine, and fecal samples were collected at various time points up to 24 hours postdose for the determinations of Ro 64-0796 and Ro 64-0802 concentrations. Oral absorption for Ro 64-0796 was 73% and oral bioavailability was 80% in ferrets with minimal first-pass metabolism. The oral bioavailability of the active drug, Ro 64-0802, was 44%. About 63% of the administered dose was excreted in urine in which 10% were consisted of the prodrug, Ro 53-0796, and 82% Ro 64-0802. The clearance of Ro 64-0802 was slow (< 0.2 l/hr/kg) via renal route, in contrast of that of Ro 64-0796 (~ 1,1 l/hr/kg) which was eliminated both by hydrolysis to Ro 64-0780, and renal excretion. The steady-state volume of distribution for prodrug was 7 times that of the active drug (2.2 l/kg vs. 0.3 l/kg), reflecting its greater lipophilicity.

Protein binding and red cell partitioning of Ro 64-0796 and Ro 64-0802 were investigated using plasma proteins and erythrocytes isolated from man, rabbit, rat, and dog (Report # W-143012). Ro 64-0796 was 42%, 16%. 27%, and 40% protein-bound and plasma:blood ratios were 0.73, 1.2, 0.5, and 2.2 in man, rabbit, rat, and dog blood/plasma, respectively. No significant binding was detected for Ro 64-0802 in any species. The results indicated that cross-species comparisons of systemic exposure to Ro 64-0796 or Ro 64-0802 are appropriate. Ro 64-0796 also binds to a similar extent to albumin and α-1 acid glycoprotein at physiological levels.

Twenty mg/kg [^{14}C]Ro 64-0796 was administered orally to male and pregnant female (gestation day 16) Lister Hooded pigmented rats (Report # W-142778). The concentrations of radioactivity were determined by quantitative whole body phosphor imaging at 2, 7.5, 24, and 72 hours postdose. It was found that drug related radioactivity was well distributed except into the central nervous system. In addition, even though there was relative high radioactivity in the yolk sac, very little maternal-fetal transfer of radioactivity was observed. The highest drug exposures were found in kidney and liver, suggesting that Ro 64-0796 was excreted via both renal and hepatic routes.

Male and pregnant female (gestation day 16) Sprague-Dawley rats received a single oral dose of 20 mg/kg [^{14}C] Ro 64-0796 (Report # W-142967). The tissue concentrations of radioactivity were determined at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 48 hours postdose for male animals and at 1, 6, 12, and 24 hours postdose for pregnant females. Distribution pattern of radioactivity was similar between the male and pregnant female rats. As observed previously, radioactivity was rapidly and extensively distributed with maximum tissue concentrations achieved within 1 hour postdose. Transfer of radioactivity across placenta to fetus was seen, the concentrations of radioactivity in fetus was ½ of those found in the organ/tissue with the lowest radioactivity. Elimination of radioactive material was essentially complete at 48 hours postdose.

Female pregnant New Zealand White rabbits received a single oral dose of 50 mg/kg [^{14}C] Ro 64-0796 on gestation day 18 (Report # W-142986) and the disposition of radioactivity was examined in tissues. Greater than 84% of Ro 64-0964-related radioactivity was absorbed. Excretion was predominantly renal (84%). Radioactivity was widely distributed. Placenta transfer was limited since fetal exposures were estimated to be about 15-20% those of the dam. Exposures to Ro 64-0802 in lung were estimated to be 3 time those of plasma. Metabolite profile was similar to those in rodents. The metabolites included the hydroxylated derivatives of Ro 64-0796 and Ro 64-0802 at the pentyl side-chain. About 10% of metabolites in urine were glucuronides of Ro 64-0802. Additional unidentified and unquantifiable (by HPLC) metabolites were detected at 7 hours postdose.

12.3.4 Metabolism:

A pharmacokinetic study in man using had consistently recorded higher plasma levels (30-40%) of total drug-related material than that of Ro 64-0796 plus Ro 64-0802 (Report # 1001667). The observation implies the existence of additional circulating metabolites or systemic error in the analysis of the two compounds. A study was conducted subjecting plasma and urine samples from volunteers who took a dose of 75 mg ^{14}C-labelled Ro 64-0796 (orally) or Ro 64-0802 (intravenously) for analysis by HPLC/MS/MS and high sensitivity liquid scintillation counting. The results with more sensitive assay showed that the discrepancy between radioactivity and R 64-0796 plus Ro 64-0802 was smaller (13%) and metabolites other than Ro 64-0802 were not present to a significant degree.

The relative rate of hydrolysis of Ro 64-0796 and the involvement of esterases in this reaction were investigated in rat and mouse plasma, S9 supernatants from human liver, kidney, intestine wall, and lung, and rabbit liver (Report # 1001812). The results indicated that the liver, specifically hepatic esterases (from inhibition study with bis-nitrophenylphosphate), is mainly responsible for the hydrolysis of Ro 64-0796 *in vivo* in human. Metabolism of Ro 64-0796 in rabbits was both by hydrolytic and oxidative processes, although the latter process was more important since the rate of metabolism in the presence of NADPH regenerating system was 5 times that in its absence. The conversion rate was much slower by liver of rabbit (~2 pmole/min/mg protein) than those of human and marmoset (~1000 and 25 pmole/min/mg protein, respectively), but somewhat more quickly than those from the other species investigated. In rodent, the production of Ro 64-0802 from Ro 64-0796 occurs mainly in plasma (618 pmole/min/mg protein in rats and 462 pmole/min/mg protein in mice vs. < 1 pmole/min/mg protein in rodent liver). Hepatic metabolism in rodents is predominantly mediated by cytochrome P450. Simulations of hepatic insufficiency were possible by using oral bioavailability extrapolated from hepatic S9 fraction (consistent with available clinical data) and the results indicated that even severe hepatic disease should not lead to clinically important overexposure to Ro 64-0796 or underexposure to Ro 64-0802.

The metabolic profile of ^{14}C-labelled Ro 64-0796 and Ro 64-0802 were studied using canine and human S9 fractions or microsomal protein in the presence or absence of an NADPH-regenerating system (Report # 1003165). Human or canine S9 fractions or microsomes did not metabolize Ro 64-0802 at all. One hundred percent metabolism of Ro 64-0796 was observed 100 minutes after incubation while in canine fraction only 50% metabolism was seen. Liver S9 and microsomal fractions hydrolyzed Ro 64-0796 equally well both in the presence and absence of metabolic cofactors. An unknown compound with variable amounts was present in human incubations at 2 minutes and in most canine incubations. The experiment design did not allow elaboration of whether this compound is a real metabolite or an artifact arising from the termination methods used for the incubations.

It was reported in the literature that human carboxylase 1 (HEC1) but not HEC 2 is responsible for the hydrolysis of oseltamivir (Ro 64-0796) to oseltamivir carboxylate (Ro 64-0802). Using baculoviruses encoded recombinant HCE1 and HCE2 and the respective positive controls (methyl anthranilate for HCE1 and procaine for HCE2), it was demonstrated that HCE1, but not HCE2, was active in the conversion of oseltamivir to oseltamivir carboxylate (Report # 1029175).

Interspecies (rat, marmoset, and human) comparison of Ro 64-0796 metabolism by liver microsomes and following a single oral administration of 10 mg/kg [^{14}C]Ro 64-0796 were made (Report # W-142735). Cytochrome P450 was involved in the metabolism of Ro 64-0796 in rat but not human or marmoset livers. Two mono-hydroxylated metabolites, M4 and M5, were detected in the Ro 64-0796 incubations with rat liver microsomes. These two metabolites were also detected in rat urine following oral administration with Ro 64-0796 along with several other metabolites. But none were

detectable in marmoset urine. The most abundant metabolite was M3 which accounted for 3.8% of the dose or 18% of the radioactivity excreted in rat urine. In rat, unchanged prodrug, Ro 64-0796, accounted for 18% of radioactivity excreted while 43% was the active drug, Ro 64-0802. Very little prodrug was detected in the marmoset urine, suggesting that esterase activity was much higher in the marmoset than rat.

12.3.5 Excretion:

[^{14}C]Ro 64-0796 was administered as a single intravenous dose at 10 mg/kg or as a single oral dose at 20 mg/kg (Report # W-142663). Excreta were collected up to 3 days postdose. 78% of the dose was recovered in urine following intravenous administration, most of it recovered within 6 hours of dosing. Urinary recovery following oral dosing was lower, at 45%. Thus the estimated oral absorption was 57%. Low retention in carcasses was observed following either route of administration (9% via intravenous and 6% via oral). As expected, recovery of radioactivity in feces was higher (27%) following oral administration than intravenous one (7%).

12.3.6 Other studies:

Interaction of Ro 64-0802 and the human renal organic ion transporter 1 was studied using Chinese hamster ovary (CHO) cells expressing human renal organic anion transporter type 1(Report # 1003166). The inhibition of the uptake of p-aminohippuric acid in the presence of Ro 64-0802 (3 and 15 mM), probenecid (3 and 9 μM), or amoxicillin (6 and 10 mM) and the interaction of between Ro 64-0802 and amoxicillin were studied. The results showed that Ro 64-0802 was a weak substrate of the human renal organic anion transporter Type 1 and did not inhibit the transport of amoxicillin. Probenecid was a strong inhibitor of the uptake of p-aminohippuric acid. The results support the clinical data showing that probenecid increased exposure to Ro 64-0802 by inhibiting its active tubular secretion into urine but that there was a lack of interaction between amoxicillin and Ro 64-0802.

12.4 Repeat Dose Toxicology Studies

Study title: Ro 64-0796/002 (oseltamivir phosphate): A 14-day rat study by the intravenous route

Study no.:	(b) (4) 276/115-D6154
Report no.:	1001809
Study report location:	Electronic
Conducting laboratory and location:	(b) (4) (toxicity study); (b) (4) (plasma analysis)
Date of study initiation:	1/5/2000
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ro 64-0796/002, lot # BS99035346, & 100.2% pure

Key Study Findings

Except for a single death in the high dose group (female), no adverse effect was associated with the intravenous administration of Ro 64-0796. No cause of death could be identified for this rat and its relationship to the administration of Ro 64-0796 was unclear. Thus, the NOAEL for the study was likely to be 100 mg/kg/day.

Methods

Doses:	10, 50, and 100 mg/kg/day
Frequency of dosing:	Once a day
Route of administration:	Intravenous
Dose volume:	5 ml/kg at 0.5 ml/10 seconds
Formulation/Vehicle:	0.65% w/v NaCl, pH 4.0
Species/Strain:	Rats/Cr:CD® Sprague-Dawley
Number/Sex/Group:	5
Age:	6 weeks old
Weight:	Males: 177-222 g; Females: 136-185 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that affected the results

Observations and Results

Mortality

All animals were observed for mortality and morbidity twice a day. One high dose female was found dead on day 11 with unknown cause of death. Except for a tail lesion (sore) found on this animal, no other changes in the toxicological parameters could be found. Thus, it's unclear if the death was caused by Ro 64-0796.

Clinical Signs

All animals were observed for adverse clinical signs daily. A detailed physical examination was done for each animal weekly. No treatment effect was observed.

Body Weights

Body weights were recorded pretreatment on first day of dosing and weekly thereafter. No treatment effect was observed.

Feed Consumption

Food consumption was recorded weekly. No treatment effect was observed.

Ophthalmoscopy

Ophthalmic examinations were done on all animals pretreatment and in week 2. No treatment effect was observed.

Hematology

Blood samples were collected during week 2 for hematological determinations. No treatment effect was observed.

Clinical Chemistry

Blood samples were collected during week 2 for clinical chemistry determinations. No treatment effect was observed.

Urinalysis

Urine samples were collected overnight from all animals in week 2. No treatment effect was observed.

Gross Pathology

All surviving animals were sacrificed on day 15 and macroscopic examinations performed. No treatment effect was observed.

Organ Weights

Weights of adrenals, brain, heart, kidney, liver, ovaries, pituitary, prostate, spleen, testes/epididymides, and thyroids/parathyroids were recorded. No treatment effect was observed.

Histopathology

Adequate Battery: Yes. Adrenals, brain, cecum, colon, duodenum, eyes, femur, gross lesions, heart, ileum, jejunum, kidney, liver, lungs with mainstem bronchi, mammary glands, mandibular and mesenteric lymph nodes, esophagus, optic nerve, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerves, skin, spinal cord (cervical, lumbar, thoracic), spleen, sternum with bone marrow, stomach, testes/epididymides, thymus, thyroids/parathyroids, treachea,

urinary bladder, and uterus were examined microscopically.

Peer Review: No

Histological Findings: No treatment effect was observed.

Toxicokinetics

Blood samples for plasma drug concentration determination were collected at 5 and 15 minutes, 1, 2, and 8 hours postdose on days 1 and 14. Dichlorvos was added to the plasma samples to inhibit *ex vivo* esterase activity that converts Ro 64-0796 to Ro 64-0802. The pharmacokinetic data are presented in the following table:

Table 18. Pharmacokinetic parameters for Ro 64-0796 and Ro 64-0802 following intravenous administration of Ro 64-0796 to rats for 14 days.

Ro 64-0796						
	Male			**Female**		
Dose (mg/kg/day)	**10**	**50**	**100**	**10**	**50**	**100**
C_0 (µg/ml)	4.86	24.20	44.50	4.65	21.70	34.20
$AUC_{0-\infty}$ (µg-hr/ml)	1.62	9.22	16.7	1.97	9.26	16.7
$T_{1/2}$ (hr)	1.6	1.2	1.2	1.3	1.2	1.1
Clearance (l/hr/kg)	6.17	5.42	6.00	5.09	5.40	5.98
Vss (l/kg)	4.20	3.58	4.21	4.00	4.09	4.68
Ro 64-0802						
T_{max} (hr)	0.083	0.25	0.25	0.25	0.25	0.25
C_{max} (µg/ml)	2.34	12.60	23.30	2.78	13.50	16.40
$AUC_{0-\infty}$ (µg-hr/ml)	3.08	17.9	31.5	3.35	15.8	24.4
$T_{1/2}$ (hr)	2.2	1.8	1.7	1.8	1.7	1.5
AUC_{0802}/AUC_{0796} ratio	1.90	1.94	1.89	1.70	1.71	1.46

The results of the pharmacokinetic substudy were comparable to those in a previous intravenous study where a single dose of 10 mg/kg was administered. Systemic exposures to Ro 64-0802 were dose proportiona and were about twice those to Ro 64-0796l. The volume of distribution values were similar (4.1 vs. 3.8 l/kg previously) but that for clearance was higher (5.7 vs. 4.1 l/h/kg previously). Since the clearance for Ro 64-0796 was more rapid than liver blood flow (4 l/hr/kg), the results demonstrated that hydrolysis of Ro 64-0796 in rats occurs mostly in the plasma rather than in liver as in other species and supported the previous finding.

Dosing Solution Analysis

All dosing solutions were within acceptable range.

Study title: Ro 64-0796/002 (oseltamivir phosphate): A 2 week intravenous toxicity and toxicokinetic study in the marmoset

Study no.:	SAP718
Report no.:	1002143
Study report location:	Electronic
Conducting laboratory and location:	(b) (4) (toxicity study); (b) (4) (plasma analysis)
Date of study initiation:	2/17/2000
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ro 64-0796/002, lot # BS99035346, & 100.2% pure

Key Study Findings

No adverse effect was associated with the intravenous administration of Ro 64-0796 up to a dose of 50 mg/kg/day for 14 days in marmosets. Toxicokinetic data showed good exposures to Ro 64-0802, though hydrolysis of Ro 64-0796 was less efficient than via oral administration, supporting the findings that conversion of Ro 64-0796 occurs mostly in liver in primates. The NOAEL for the study was 50 mg/kg/day.

Methods

Doses:	0, 10, 25, and 50 mg/kg/day
Frequency of dosing:	Once a day
Route of administration:	Intravenous
Dose volume:	5 ml/kg at 2 ml/min
Formulation/Vehicle:	0.65% w/v NaCl, pH 4.0
Species/Strain:	Marmoset
Number/Sex/Group:	3
Age:	14-22 months old
Weight:	Males: 305-450 g; Females: 317-443 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that affected the results

Observations and Results

Mortality

All animals were observed for mortality and morbidity twice a day. No premature death was observed.

Clinical Signs

All animals were observed for adverse clinical signs twice daily. No treatment effect was observed.

Body Weights

Body weights were recorded prior to dosing (days -10 and 1) and twice weekly thereafter. No treatment effect was observed.

Hematology

Blood samples were collected on days -8 and 13 for hematological determinations. No treatment effect was observed.

Clinical Chemistry

Blood samples were collected on days -8 and 13 for clinical chemistry determinations. No treatment effect was observed.

Urinalysis

Urine samples were collected overnight from all animals prestudy and day 12. No treatment effect was observed.

Gross Pathology

All surviving animals were sacrificed on day 15 and macroscopic examinations performed. No treatment effect was observed.

Organ Weights

Weights of adrenals, brain, heart, kidney, liver, lungs, ovaries, spleen, testes/ epididymides, thymus, and thyroids/parathyroids were recorded. No treatment effect was observed.

Histopathology

Adequate Battery: Yes. Adrenals, aorta, brain, cecum, colon, duodenum, esophagus, gall bladder, gross lesions, heart, hind leg, ileum, jejunum, kidneys, liver, lungs, mammary glands, submandibular and mesenteric lymph nodes, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerves, seminal vesicles, skin, spinal cord (cervico- thoracic), spleen, stomach, thymus, thyroids/parathyroids, tongue, trachea, urinary bladder, uterus, and injection sites were examined microscopically.

Peer Review: No

Histological Findings: No treatment effect was observed.

Toxicokinetics

Blood samples for plasma drug concentration determination were collected at 5 and 15 minutes, 1, 3, and 8 hours postdose on day 14. The pharmacokinetic data are presented in the following table:

Table 19. Pharmacokinetic parameters for Ro 64-0796 and Ro 64-0802 following intravenous administration of Ro 64-0796 to marmosets for 14 days.

Ro 64-0796						
	Male			Female		
Dose (mg/kg/day)	**10**	**25**	**50**	**10**	**25**	**50**
C_0 (μg/ml)	5.84	13.10	21.70	7.85	12.10	26.60
C_{min} (ng/ml)	4.61	12.9	21.8	5.72	10.1	28.5
$AUC_{0-\infty}$ (μg-hr/ml)	4.61	8.52	16.4	4.80	9.89	18.9
$T_{1/2}$ (hr)	1.24	1.36	1.19	1.32	1.32	1.14
Clearance (l/hr/kg)	2.19	3.06	3.28	2.21	2.55	2.65
Vss (l/kg)	1.74	2.48	2.79	1.71	2.06	2.54
Ro 64-0802						
T_{max} (hr)	1.00	1.00	1.0	1.00	1.00	1.0
C_{max} (μg/ml)	2.37	4.46	11.10	2.30	4.85	19.50
C_{min} (μg/ml)	0.20	0.44	0.73	0.20	0.44	1.26
$AUC_{0-\infty}$ (μg-hr/ml)	8.12	17.1	36.2	7.89	17.5	46.3
App. CL (l/hr/kg)	1.23	1.49	1.44	1.28	1.43	1.10
$T_{1/2}$ (hr)	1.99	2.15	1.81	2.08	2.03	2.25
Bioavailability (%)	80	69	54	80	69	54
AUC_{0802}/AUC_{0796} ratio	1.76	2.01	2.21	1.64	1.77	2.45

There was no gender difference in the pharmacokinetics of either Ro 64-0796 or Ro 64-0802. Systemic exposures to both drugs were dose proportional. The ratios of Ro 64-0802 systemic exposures to those for Ro 64-0796 were about 2:1, similar to those found in the 14-day intravenous rat study and much lower than those by oral administration. The results support the findings in other ADME studies indicating that the hydrolysis of Ro 64-0796 following oral administration in primates occurs mostly in liver while this process in rodents takes place mostly in plasma. The average clearance was 2.7 l/h/kg and the steady-state volume of distribution was 2.2 l/kg, about twice lower than those in rats. Bioavailability for Ro 64-0802 at the 10 mg/kg dose was about 80%.

Dosing Solution Analysis

All dosing solutions were within acceptable range.

12.5 Special Toxicology Studies

Local tolerance study with oseltamavir phosphate via perivenous and intraarterial injections in rabbits (Report # 1025870; (b) (4); GLP; With QA statement; Study initiation date 12/7/2006; lot # GSI0053, 94.6% pure). Four male Hra:(NZW)SPF rabbits/group received one bolus injections per day of vehicle (saline, pH 4) or Ro 64-0796/002 at concentrations of 4 (2 mg/site), 8 (4 mg/site), or 16 (8 mg/site) mg/ml for 5 days via intraarterial and perivenous administrations. The dosing volume was 0.2 ml/site for perivenous (right marginal ear vein) and 0.5 ml/site for intraarterial (right central ear artery) injections. For the 1st 2 rabbits/group, saline was given by perivenous injection into left marginal ear vein and intraarterial injection into the left central ear artery. Mortality, clinical observations, local irritation at the injection site, body weight gain, food consumption, and macroscopic changes were monitored. Microscopic evaluations were performed for the injection sites. Perivenous

injection of 16 mg/kg/day Ro 64-0796 caused erythema, discoloration, and an increased incidence and severity (minimal to moderate) of hemorrhage at the injection site.

Ro 64-0796 (also known as GS 4104): Oral (gavage) rabbit tolerance study (HRE/62/R) (Report # W-142792; Study # HRE/62/R; (b) (4) [plasma analysis]; GLP; With QA statement; Study dates 5/9/1997; lot #'s 4104-02-D-1 & 4104-02-B-4, 100.2% pure). Three female non-pregnant New Zealand White rabbits/group, 4 months of age, weighed 3-4 kg, received oral doses of vehicle (acetate buffer, pH 4.0), 50, 250, or 1500 mg/kg/day Ro 64-0796 for 10 days. Because of severe deterioration in clinical conditions in 2 out of 3 animals receiving the 1500 mg/kg/day dose after 3-4 days of dosing, dosing for this group was terminated and 2 more dosing groups, 500 and 750 mg/kg/day, were added. Clinical signs, body weight changes, and food consumptions were monitored. Necropsy was performed at the termination. Blood samples were collected for plasma drug concentration determinations on day 10 at 1, 2, 4, 8, and 24 hours postdose.

All 3 animals in the 1500 mg/kg/day group and one animal in the 750 mg/kg/day had to be sacrificed prematurely, mostly after receiving 4 doses. They commonly had notable weight loss, inappentence, reduced or no fecal output following 1 or 2 doses and exhibited hypoactivity, prostration, tremors, few feces, hunched posture, and salivation prior to death. Few feces, inappentence, and body weight loss or reduce body weigh gain were associated with dosed ≥ 500 mg/kg/day Ro 64-0796. At doses ≥ 750 mg/kg/day, GI abnormalities including erosion and reddening of the stomach mucosa, dark stomach contents, ulceration around the pyloric sphinter, distension of the intestines with fluid, and hard and dehydrated feces in the colon were common necropsy findings. The results suggested GI intolerance in rabbits at doses ≥ 500 mg/kg/day Ro 64-0796.

The toxicokinetic data are presented in the following table. The results showed rapid conversion of Ro 64-0796 to Ro 64-0802. Systemic exposures to both drugs were dose proportional. The ratio of systemic exposures of Ro 64-0796 to Ro 64-0802 was about 1:3, similar to those seen in rats and lower than those in primates.

Table 20. Pharamcokinetic parameters of Ro 64-0796 and Ro 64-0802 following oral administration of 50, 250, 500, and 500 mg/kg/day Ro 64-0796/002 to female rabbits.

	Ro 64-0796				Ro 64-0802			
Dose (mg/kg/day)	**50**	**250**	**500**	**750**	**50**	**250**	**500**	**750**
T_{max} (hr)	1.0	1.0	1.0	2.0	1.0	1.0	2.0	2.0
C_{max} (µg/ml)	2.37	13.30	28.00	23.30	7.74	37.70	67.90	63.60
C_{min} (µg/ml)	0.013	0.12	0.45	0.89	0.035	0.38	1.44	4.04
AUC_{0-24h} (µg-hr/ml)	3.97	22.5	101	222	13.3	82.5	251	627

Ro 64-0796 (also known as GS 4104): A seven-day gastrointestinal tolerance study in dogs with the 75 mg capsule final formulation (Report # W-143072; Study # 276/104 (b) (4) [in-life phase], (b) (4),

(b) (4) [plasma analysis]; GLP; With QA statement; Study dates 5/9/1997; Ro 64-0796/V14-22, lot # GMZ0129/03, 98% pure). Two beagle dogs/sex/group, 5 months old, weighing 6.05-8.6 kg (males) and 6.5-8.0 kg (females), received either empty control capsules or capsules containing 75 mg Ro 64-0796 orally twice a day 12 hours apart for 7 days. Animals were monitored for mortality, morbidity, and ill health twice daily, for body weight changes and food consumption weekly, Blood samples for drug plasma concentration determinations were taken on days 1 and 7 at 0.5, 1, 2, 4, 6, 8, and 12 hours postdose. Fecal occult blood tests were performed on two consecutive days prior to the start of the study. A full observation of the entire GI tract was performed after necropsy at day 8. Histopathological examinations were done for cecum, colon, duodenum, ileum, jejunum, esophagus, rectum, and stomach. No irritation to the tissues in the GI tract was observed.

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/s/

ITA S YUEN
11/30/2012

HANAN N GHANTOUS
12/03/2012
I agree with the conclusion of the reviewer Dr. Ita Yuen.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 21-246 **Applicant: Roche** **Stamp Date: 6/21/2012**

Drug Name: Tamiflu™ **NDA/BLA Type: Supplemental**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?		X	
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		X	

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___X____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

ITA S YUEN
11/30/2012

HANAN N GHANTOUS
11/30/2012