Drugs of the Future 1999, 24(11): 1189-1202

CCC: 0377-8282/99

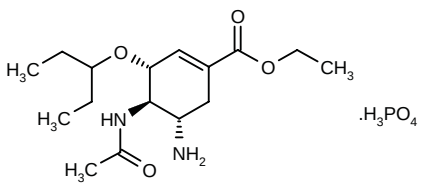
Oseltamivir Phosphate

Prop INNM

Anti-Influenza
Neuraminidase (Sialidase) Inhibitor

GS-4104/002
GS-4104 (as free base)
Ro-64-0796/002
Ro-64-0796 (as free base)
Tamiflu®

(3*R*,4*R*,5*S*)-4-Acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid ethyl ester phosphate (1:1)



$C_{16}H_{28}N_2O_4.H_3O_4P$ Mol wt: 410.4009

CAS: 204255-11-8
CAS: 196618-13-0 (as free base)
CAS: 209965-30-0 [as citrate (1:1)]
CAS: 204255-09-4 (as monohydrochloride)

EN: 241104

Synthesis

Oseltamivir phosphate can be obtained by two different ways:

1) The reaction of (–)-quinic acid (I) with 2,2-dimethoxypropane (II) and *p*-toluenesulfonic acid in refluxing acetone gives the protected lactone (III), which by treatment with sodium ethoxide in ethanol yields the ethyl ester (IV). The acylation of (IV) with mesyl chloride and TEA in dichloromethane affords the mesylate (V), which is dehydrated with SO_2Cl_2 in dichloromethane, giving the cyclohexenecarboxylate (VI). The transketalization of (VI) with 3-pentanone and $HClO_4$ affords the 3,4-pentylidene ketal (VII), which is cleaved with borane methyl sulfide complex to the 3-pentyl ether (VIII). The epoxidation of (VIII) by treatment with $KHCO_3$ in hot ethanol affords the epoxide (IX), which is opened with sodium azide and ammonium chloride in ethanol/water, resulting in the the azido alcohol (X). The cyclization of (X) with triphenylphosphine in refluxing THF/acetonitrile (1-3) or trimethylphosphine in anhydrous acetonitrile (4) yields aziridine (XI), which is opened by means of sodium azide in hot DMF to the azidoamine (XII). The acetylation of (XII) with acetic anhydride provides the azido-acetamide (XIII), which is reduced with H_2 over Lindlar catalyst (1-3) or over Ra-Ni (4) in ethanol and treated with 85% phosphoric acid. Scheme 1.

2) The esterification of Shikimic acid (XIV) with MeOH/TsOH gives the methyl ester (XV), which is treated with 2,2-dimethoxypropane (II) and TsOH to yield the acetonide (XVI). The mesylation of (XVI) with mesyl chloride and TEA in dichloromethane gives the mesylated acetonide (XVII), which is hydrolyzed with HCl, yielding the dihydroxy ester (XVIII). The epoxidation of (XVIII) with DBU in THF affords the epoxide (XIX), which is protected with methyl chloromethyl ether to give compound (XX). The reaction of (XX) with sodium azide in refluxing methanol/water provides the hydroxy azide (XXI), which is acylated with mesyl chloride to the mesylate (XXII). The cyclization of (XXII) by means of triphenylphosphine in THF affords the aziridine (XXIII), which is treated with sodium azide in hot DMF to give the amino azide (XXIV). The deprotection of (XXIV) with HCl followed by tritylation of the free amino group with trityl chloride and TEA yields compound (XXV), which is cyclized by means of mesyl chloride and TEA to afford the tritylaziridine (XXVI). The cleavage of the aziridine ring of (XXVI) with 3-pentanol, followed by acetylation of the resulting amino group, affords the acetamido aziridine (XXVII), which is hydrolyzed at the ester group with KOH in THF/water to give the carboxylic acid (XXVIII). The esterification of (XXVIII) with ethanol, DCC and DMAP in dichloromethane affords the azido ester (XIII), which is finally reduced with triphenylphosphine in hot THF/water. (5, 6). Scheme 2.

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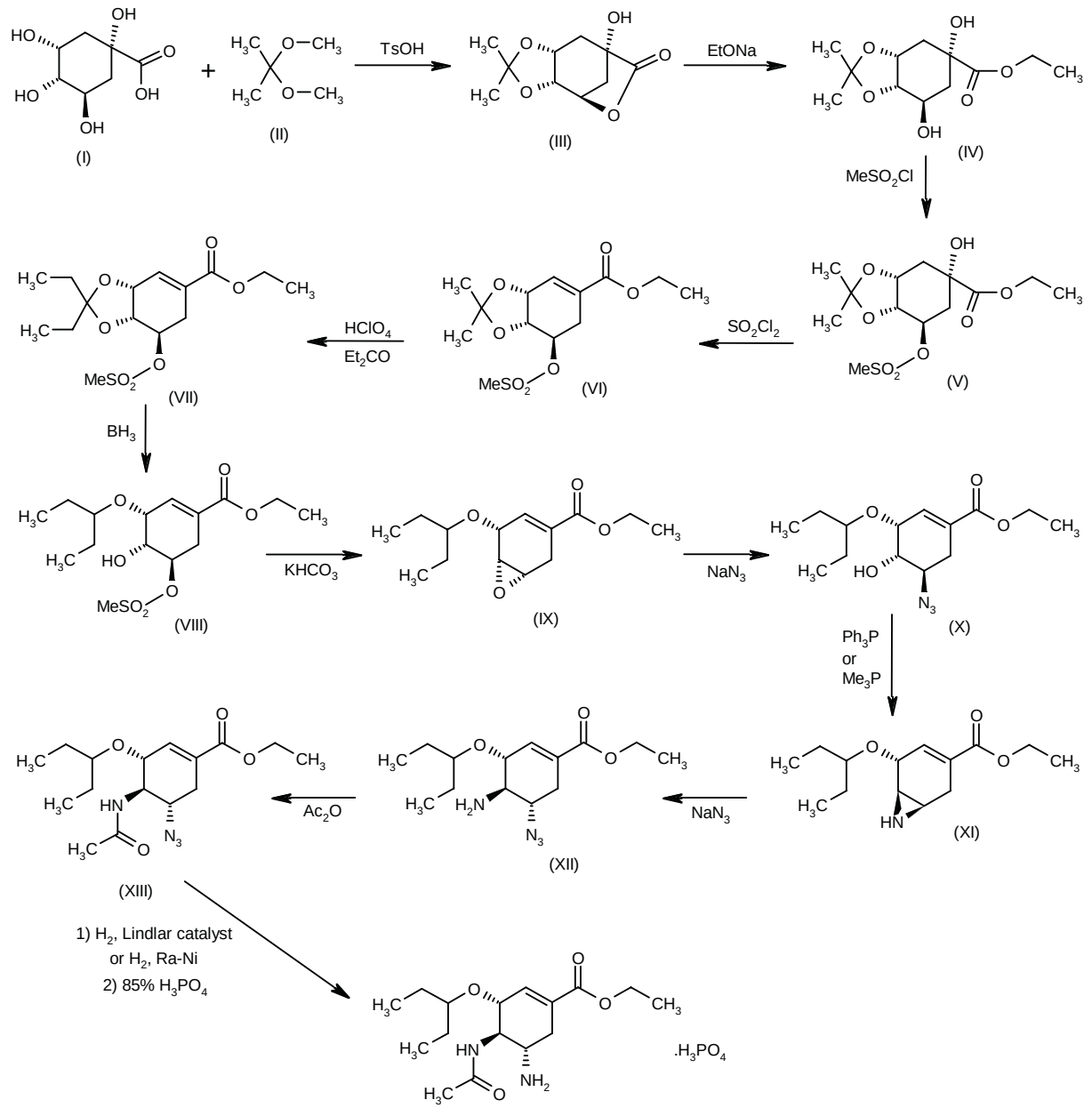
Scheme 1: Synthesis of Oseltamivir Phosphate



Description

Long white needles, m.p. 203-4 °C, $[\alpha]_D$ –39.9° (c 1, water) (1, 2, 4).

Introduction

Influenza A and B viruses belong to the *Orthomyxoviridae* family of viruses. Infection by influenza viruses is a major cause of morbidity and mortality, particularly among young children, the elderly, immunocompromised individuals and persons with chronic cardiovascular or respiratory diseases or diabetes (7). Serious complications from influenza include pneumonia and worsening of underlying medical conditions, resulting in an average of approximately 110,000 hospitalizations and 20,000 deaths annually in the United States (8). A pandemic of influenza which occurred in 1918 (Spanish influenza) was responsible for 20-40 million deaths worldwide. Other pandemics associated with high rates of mortality occurred in 1957 (Asian influenza) and in 1968

Scheme 2: Synthesis of Oseltamivir Phosphate

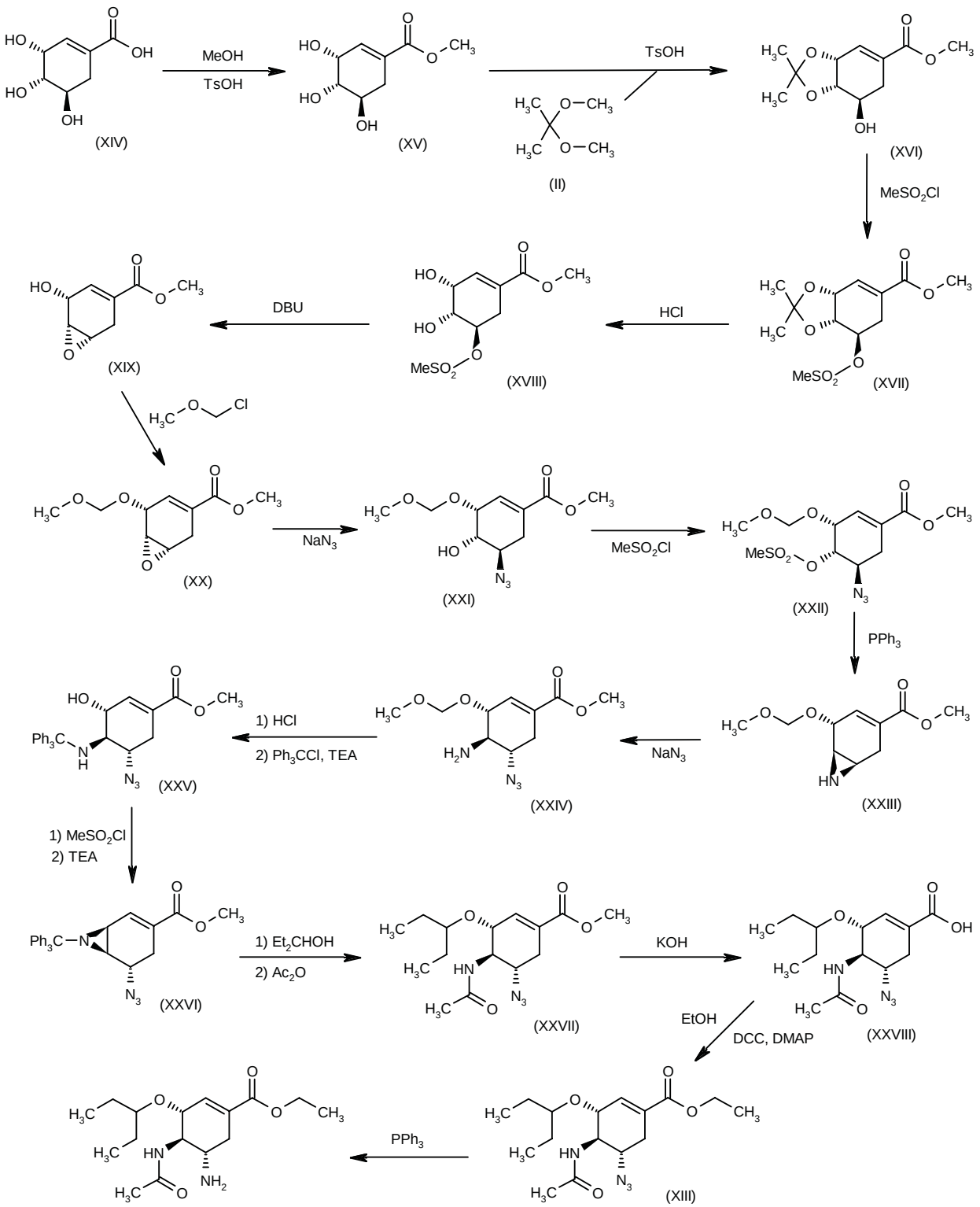
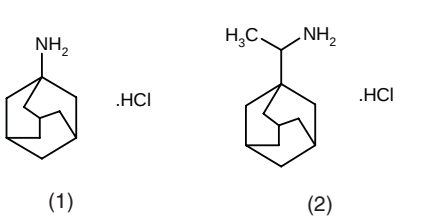


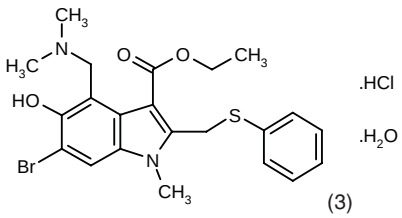
Table I: Antiinfluenza treatments marketed and under development (Prous Science Ensemble database).

Compound	Target	Status
1. Amantadine HCl	M2 ion channel protein ligand	L-1964
2. Rimantidine HCl	M2 ion channel protein ligand	L-1987
3. Arbidol (All Russian Chem. Pharm. Res. Inst.)	Inhibitor of the fusion of the influenza envelope with the host cell membrane; interferon inducer	L-1992
4. Zanamivir (Relenza®; Biota/Glaxo Wellcome)	Neuraminidase inhibitor	L-1999
5. Oseltamivir phosphate (Tamifu®; Gilead/Roche)	Neuraminidase inhibitor	L-1999
6. RWJ-270201/BCX-1812 (BioCryst/R.W. Johnson)	Neuraminidase inhibitor	Phase II
7. BCX-140 (BioCryst)	Neuraminidase inhibitor	Preclinical



(1)

(2)



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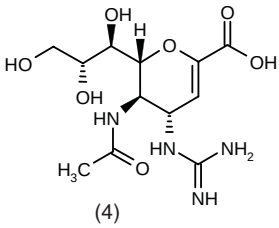

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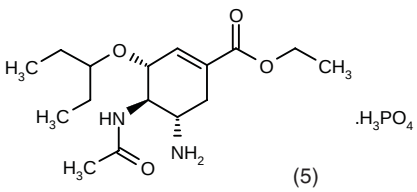

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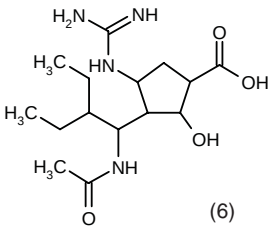

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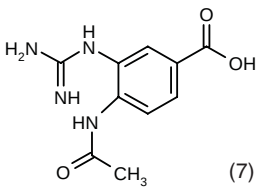


(7)

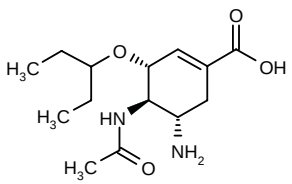
(Hong Kong influenza) and were responsible for 70,000 and 34,000 deaths, respectively, in the U.S. (9).

The development of vaccines for active immunization against influenza has been the most effective method of reducing the impact of influenza. Killed influenza vaccines have been available for nearly 70 years. However, efforts to control influenza through vaccination have been hampered due to a series of evolutionary mechanisms that have resulted in the formation of immunologically novel strains of influenza (10, 11).

Until this year, amantadine and rimantidine were the only antiviral agents approved by the FDA for the prophylaxis and treatment of influenza type A, but neither is effective against influenza type B viruses (8). Both compounds target the M2 ion channel protein of the type A influenza virus, which is missing in the type B virus (12). However, the effectiveness of these compounds is limited by adverse effects and the rapid emergence of resistant strains during clinical use (13). Another antiinfluenza agent, arbidol, was launched in the Russian Federation in 1992. Arbidol is an interferon inducer and has been reported to inhibit the fusion of the influenza envelope with the host cell membrane (9, 14).

Since the early 1970s, research efforts have focused on identifying novel antiviral agents directed against influenza A and B viruses. The influenza virus neuraminidase (sialidase), which is one of the two glycoproteins expressed on the virion surface, proved to be a valid target for antiviral intervention. The mushroom-shaped neuraminidase molecule cleaves terminal sialic acid residues from glycoconjugates and glycolipids and is essential for virus replication and infectivity (15-17). The neuraminidase active site is highly conserved across influenza A and B viruses (18-20), and several first-generation neuraminidase inhibitors were rationally designed based on the neuraminidase crystallographic structure (19, 21). At present, two neuraminidase inhibitors have been launched and others are in clinical and preclinical development (Table I).

The first of the neuraminidase inhibitors to reach the market, zanamivir (Relenza®), was launched in Australia in May 1999. The introduction of zanamivir represented a significant advance in the treatment of influenza. However, this second-generation neuraminidase inhibitor has the disadvantage of poor oral bioavailability and must be administered topically to the respiratory tract by intranasal spray or by inhalation (22). In the search for improved neuraminidase inhibitors that could be administered orally, scientists at Gilead synthesized a series of novel carbocyclic compounds incorporating various lipophilic alkyl side chains, from which GS-4104 (oseltamivir) was eventually selected for development (23, 24).



[I]

Pharmacological Actions

Oseltamivir is the ethyl ester prodrug of the potent influenza neuraminidase inhibitor GS-4071 [I] (IC_{50} = 1 nM) (24-26). The antiinfluenza activity of the active compound GS-4071 was comparable to that of zanamivir *in vitro* against various clinical strains and laboratory isolates of influenza A and B using a plaque inhibition assay in Madin Darby canine kidney (MDCK) cell monolayers (median ED_{50} values of <0.1, >10 and 0.1 μg/ml, respectively, for both drugs against 18 influenza A/H3N2 strains, 8 A/H1N1 strains and 10 B clinical isolates). In addition to having activity comparable to that of zanamivir against most common strains tested, GS-4071 was also active against certain viruses with reduced susceptibility to zanamivir due to a neuraminidase mutation (27). Potent synergistic antiviral activity was observed *in vitro* in combination with ribavirin (28).

Kinetic studies showed that GS-4071, a slow-binding neuraminidase inhibitor with biphasic kinetics, derives much of its binding energy through hydrophobic interactions with the region of the enzyme active site normally occupied by sialic acid (29). Resistance studies indicated that a resistant mutant strain of influenza A/Victoria/3/75 (H3N2) virus, which emerged following serial passage using 2-fold increased drug concentrations for each passage, has poor replicability and low infectivity *in vivo* in mice, and as such will have limited clinical significance (30, 31).

Although GS-4071 demonstrated potent antiinfluenza activity in the above studies, its development was hampered by poor absorption from the gastrointestinal tract and low oral bioavailability (~4% in mice). Thus, a series of prodrug analogs were designed with efforts focused on the carboxylate functionality. The preferred prodrug identified was GS-4104 (oseltamivir), which had a logP of +0.5 as compared to –1.4 for GS-4071, and oral bioavailability ranging from 30-100% in mice, rats and dogs. Twelve hours after oral administration of oseltamivir in dogs (5 mg/kg p.o.), plasma concentrations of GS-4071 were 90% higher than those required for neuraminidase inhibition (23, 32).

In mice infected with influenza A/NWS/33 (H1N1), A/Victoria/3/75 (H3N2), A/Shangdong/09/93 (H3N2) and B/HongKong/5/72 viruses, oral treatment with oseltamivir significantly inhibited the effects of infection, as seen by reduced incidence of death, increase in mean time to death, inhibition of decline of arterial oxygen saturation, reduced lung consolidation and inhibition of infectious virus recovered from lungs. Comparative studies in mice infected with influenza A (H1N1) indicated that the oral activity of oseltamivir was far superior to that of GS-4071 and zanamivir. The minimum effective dose of oseltamivir, administered b.i.d. for 5 days beginning 4 h prior to virus exposure, was 0.1 mg/kg/day p.o. Depending on dose of virus administered, treatment could be delayed for 48-60 h postexposure without loss of significant antiviral activity. When oseltamivir-treated, influenza-infected mice were caged with infected saline-treated animals, no influenza inhibitory activity was transferred from the former to the latter (33).

The ferret is frequently used as an animal model of influenza infection. In this animal, disease is self-limited with upper respiratory tract symptoms similar to those observed in humans. In the ferret model of influenza virus infection, oral administration of oseltamivir (25 mg/kg b.i.d.) decreased peak viral titers in nasal washings and abolished constitutional responses to infection, *i.e.*, fever, sneezing, nasal discharge, mouth breathing and lethargy (34).

The influence of treatment schedule and dose of viral challenge on the efficacy of oseltamivir were evaluated *in vivo* in mice. The compound was administered 1-4 times daily at doses of 5 or 32 mg/kg/day p.o. At the lower dose, oseltamivir was highly inhibitory against H1N1 virus using all four treatment schedules, although if treatment was discontinued too early in the course of the infectious process (*e.g.*, on day 2-3), efficacy was limited. Administration of a single low dose of oseltamivir at various time points postinfection was not effective. Administration of the higher dose of oseltamivir using any of the four treatment schedules produced significant antiviral effects. Administered twice daily for 5 days at a dose of 20 mg/kg/day p.o., oseltamivir completely prevented death in mice exposed to an otherwise lethal dose of influenza virus. The results indicated that, in order for oseltamivir to be effective, the drug must be present in the host at the time that viral titers reach a maximum level, and therefore a minimum of once-daily dosing is required (35).

Another study performed in mice was designed to determine whether treatment with oseltamivir would produce a resistant virus. Mice infected with influenza A/Shangdong/09/93 (H3N2) were treated with the study drug at doses of 100, 10 or 1 mg/kg/day p.o. x 5 days, beginning 4 h prior to virus exposure. The compound was protective at the two higher doses, although virus recovered from the lungs of 2 animals showed only 3-fold lower sensitivity to the active drug GS-4071 as compared to virus from placebo-treated mice. This was in contrast to the results seen with amantadine and rimantadine, in which virus from lungs of treated animals showed a 1000-fold decrease in sensitivity (36).

The *in vitro* pharmacological profile of selected neuraminidase inhibitors and other classes of antiinfluenza drugs is shown in Table II.

Table II: In vitro *pharmacological profile of selected neuraminidase (sialidase) inhibitors and other classes of antiinfluenza drugs (Prous Science MFLine database).*

Compound	Influenza type	Neuraminidase inhibition IC_{50}, nM (ref.)	Antiviral activity IC_{50}, μM (ref.)	Cytotoxicity CC_{50}, μM (ref.)
Neuraminidase inhibitors				
BCX-1827	A	NR	0.99 (58)	>1000 (58)
	B	NR	6.02 (58)	
BCX-1898	A	NR	3.79 (58)	>1000 (58)
	B	NR	3.11 (58)	
BCX-1923	A	NR	2.73 (58)	>1000 (58)
	B	NR	5.62 (58)	
GR-213982	A	12 (59)	0.006 (59)	NR
	B	2000 (59)	2.14 (59)	
GS-4071 (active metabolite of oseltamivir)	A	0.95 (23, 26, 34, 60-62)	0.030 (34)	>1000 (33)
	B	5.81 (23, 31, 34, 60, 62)	0.121 (34)	
RWJ-270201	A	0.1-1.4 (63)	1.71 (58)	>1000 (58)
	B	0.6-11.0 (63)	5.72 (58)	
Zanamivir	A	7.0 (34, 62, 64-67)	1.28 (34, 64, 65, 68)	>1000 (33)
	B	5.7 (34, 62, 65-67)	0.17 (34, 64, 65, 68)	
M2 channel ligands				
Amantadine HCl	A	–	4.65 (68)	40-1000 (68)
	B	–	>25 (68)	
Rimantadine HCl	A	–	2.47 (68)	10-4000 (68)
	B	–	>25 (68)	
Reverse transcriptase inhibitor				
Ribavirin	A	–	32.1 (68)	5-4500 (68)
	B	–	2.4 (68)	

NR: not reported. Most antiviral activity values refer to the mean calculated from a range of different values using a variety of virus strains and different methods from either one study or different studies assessing the same activities.

Toxicity

No significant drug-related toxicity was observed in rats administered oral doses of up to 800 mg/kg/day oseltamivir for 14 days (34).

Pharmacokinetics and Metabolism

Given that influenza virus replication takes place primarily in the surface epithelial cells of the respiratory tract, the capacity of oseltamivir to deliver the active drug GS-4071 to the bronchoalveolar lining fluid (BALF) is a significant indicator of its potential efficacy. Thus, the concentration-time profiles of GS-4071 were determined in the plasma and BALF of rats following oral administration of oseltamivir. Concentrations of the active drug in plasma and BALF peaked at 1 and 2 h, respectively, postdosing. Drug levels in BALF declined at a slower rate than in plasma, indicating slow clearance of the drug from lung acini. No unchanged prodrug was detected in BALF. Significant penetration of the parent drug into the lower respiratory tract was observed following oral administration of the prodrug, indicating the potential for sustained antiviral concentrations of GS-4071 in the fluid lining of the lungs (37) (Table III).

Table III: General pharmacokinetic profile of oseltamivir and its active metabolite GS-4071 in rats after oral administration of a 30-mg dose (37) [Prous Science PKline database].

Parameter	Oseltamivir		
	Plasma	BALF	GS-4071
C_{max} (mg/l)	7.8		
t_{max} (h)	1.0		
$t_{1/2}$ (h)	0.7	1.32	1.32
$AUC_{0-\infty}$ (mg·h/l)	19.0	28.7	52.6

Various preclinical pharmacokinetic studies have served to demonstrate the excellent bioavailability of oseltamivir and effective conversion to the active form in various animal species. The apparent terminal half-life was longer for the active form following administration of

the prodrug, indicating a slow rate of absorption and/or hydrolysis. No intact oseltamivir could be identified in rat plasma after oral dosing, although concentrations of the prodrug in marmoset and dog were 45% and 115%, respectively, of those of the active form. In all species tested (mice, rats, dogs and monkeys), the bulk of the absorbed prodrug was hydrolyzed to the active form (38).

The tissue distribution of total radioactivity was studied in rats administered [^{14}C]-labeled oseltamivir (10 mg/kg p.o.) 1, 6 and 24 h postdosing. The highest concentrations of total radioactivity were detected in the gastrointestinal tract and excretory organs. At 6-24 h, radioactivity levels in most tissues declined with a $t_{1/2}$ of approximately 5 h. At 6 h postdosing, concentrations in lung were approximately twice as high as in plasma; lung concentrations were 30 times those in plasma at 24 h postdosing. Twenty-four hours after administration of oseltamivir, 24.3% and 61.1% of dose was recovered in urine and feces, respectively; most of the radioactivity was attributable to GS-4071 (39).

The tolerability and pharmacokinetics of oseltamivir were investigated in healthy male volunteers following administration of single oral doses of 20-1000 mg under fasting conditions and at a dose of 200 mg in the presence of food. Good tolerance was reported, with no relationship between the dose and the number and incidence of side effects; no clinically relevant changes in vital signs, laboratory tests or ECG recordings were observed. Plasma concentration-time profiles for the prodrug and active form were similar at each dose level under fasting conditions. In the case of the prodrug, plasma concentrations peaked early after dosing and declined rapidly thereafter; maximum plasma concentrations of oseltamivir were approximately 15-30% those of GS-4071. The terminal half-life of GS-4071 was longer than that of the prodrug and the AUC for the prodrug was approximately 4% that of the active form at each dose level. Plasma concentrations of GS-4071 at 12 h postdosing were about 35% of those at C_{max}. Over the dose range tested, both C_{max} and AUC increased in a dose-proportional manner. Pharmacokinetic evaluation indicated that the prodrug oseltamivir is suitable for oral administration, giving high, dose-proportional and sustained plasma levels of the active compound (40).

Another pharmacokinetic study in healthy young and elderly subjects evaluated multiple doses of oseltamivir. The compound was administered twice daily for 7 days at doses of 50, 100, 200 and 500 mg in young subjects and at doses of 100, 150 and 200 mg in elderly subjects. Mean C_{max} values on day 7 at the 200-mg dose level were 1287 ± 316 and 1469 ± 160 ng/ml, respectively, in young and elderly subjects; the respective tmax values were 2.7 ± 1.2 and 2.8 ± 0.4 h. The half-life of GS-4071 in young and healthy subjects, respectively, was 8.7 ± 1.6 and 7.4 ± 0.9 h. Based on a calculation of AUC values, a dose of 150 mg b.i.d. in elderly subjects is expected to provide drug exposure comparable to that obtained at a dose of 200 mg b.i.d. in young subjects (41) (Table IV).

Table IV: Pharmacokinetic properties of GS-4071 after oral administration of oseltamivir 200 mg b.i.d. for 7 days in young and elderly healthy volunteers (41) [Prous Science PKline database].

Parameter	Young subjects	Elderly subjectes
C_{max} (ng/l)	1.3 ± 3.2	1.5 ± 0.2
t_{max} (h)	2.7 ± 1.2	2.8 ± 0.4
$t_{1/2}$ (h)	8.7 ± 1.6	7.4 ± 0.0

The bioequivalence of four different formulations of oseltamivir was studied in two randomized, single-dose, crossover trials. In the first study, 18 healthy volunteers received 150 mg of the prodrug in the capsule formulation used in phase III clinical trials and the capsule formulation proposed for marketing; in the other study, 18 healthy volunteers were administered the same dose of oseltamivir in a capsule formulation used in early clinical trials, the capsule formulation proposed for marketing and a liquid formulation. After dosing with all formulations, the prodrug was readily absorbed from the gastrointestinal tract and extensively converted to the active metabolite. All four formulations were shown to be bioequivalent and were well tolerated (42).

Since preclinical and early clinical data indicate that both oseltamivir and the active form GS-4071 are eliminated by renal tubular secretion, the effects on pharmacokinetics of coadministration of the prodrug with cimetidine or probenecid were evaluated in a randomized, crossover trial in 18 healthy volunteers. Subjects received a single 150-mg dose of oseltamivir either alone or during 5 days of treatment with cimetidine 400 mg q.i.d. or probenecid 500 mg q.i.d. No pharmacokinetic interaction was noted between cimetidine and either oseltamivir or GS-4071. Coadministration of probenecid had a negligible effect on the pharmacokinetics of oseltamivir, but it increased the AUC of the active compound by about 2-fold due to a reduction in renal clearance. All treatments were well tolerated. It was concluded that coadministration of these drugs should not result in clinically relevant effects on pharmacokinetics or tolerance (43).

Clinical Studies

An early placebo-controlled, double-blind efficacy study in 80 volunteers assessed nasal cytokine responses following experimental infection with influenza A/Texas/36/91 virus and subsequent 5-day oral treatment with one of four doses of oseltamivir (20, 100 or 200 mg b.i.d. or 200 mg once daily) or placebo. Time to cessation of viral shedding, symptom scores and nasal mucus weights were all reduced significantly in the oseltamivir treatment groups as compared to placebo. Viral titer AUC values for the combined active treatment groups were significantly lower than in the placebo group (median $\log_{10}$ $TCID_{50}$xh/ml = 79 and 273, respectively). Analysis of nasal wash samples, collected at baseline and on days 2,

Box 1: Effects of oseltamivir on cytokine responses in influenza A virus infection (44) [Prous Science CSline database].

Design	Randomized, double-blind, placebo-controlled clinical study
Population	Healthy volunteers (n = 80)
Treatments*	Oseltamivir (O), p.o. o.d. x 5 d (n = 64) Placebo (P) (n = 16)
Results	Time to cessation of viral shedding (h): P (107) > O (58) Symptom scores (AUC): P (400) > O (226) Nasal mucus weights (g): P (20) > O (9) Peak changes (d 2, 4 and 8 postinfection) in NW levels of IL-6 (pg/ml): P (15) > O (9) [$p = 0.05$] Peak changes (d 2, 4 and 8 postinfection) in NW levels of TNFα (pg/ml): P (0.5) > O (0) [$p = 0.02$]
Conclusions	Early treatment with oseltamivir reduced viral replication, illness measures and local production of proinflammatory cytokines in experimental human influenza A virus infection

*Administered 28 h after i.n. inoculation with virus

Box 2: Efficacy and tolerability of oseltamivir in the early treatment of influenza A (45) [Prous Science CSline database].

Design	Randomized, double-blind, placebo-controlled, dose-finding clinical study
Population	Healthy volunteers (n = 80)
Treatments*	Oseltamivir (O), 20 mg p.o. b.i.d. x 5 d (n = 16) O, 100 mg p.o. b.i.d. x 5 d (n = 16) O, 200 mg p.o. b.i.d. x 5 d (n = 16) O, 200 mg p.o. o.d. x 5 d (n = 16) Placebo (P) (n = 16)
Results	Viral titer AUC (median $\log_{10}$ TCID50xh/ml): P (273) > O (79) Viral titer AUC (median $\log_{10}$ TCID50xh/ml): Oo.d. (143) > Ob.i.d. (51-85) Time to cessation of viral shedding (h): P (107) > O (58) Time to cessation of illness (h): P (95) > O (53) Time to cessation of illness (h): O20 (94) $\geq$ O100 + O200 (47)
Conclusions	Early treatment with oseltamivir was associated with significant antiviral and clinical effects in experimental human influenza A virus infection

*Administered 28 h after i.n. inoculation with A/Texas/91 (H1N1) virus

4 and 8 postinfection, demonstrated that local production of the proinflammatory cytokines IL-6 and TNF-α increased only minimally in oseltamivir recipients, while significant increases were seen in the placebo group. Some subjects treated with the study drug experienced transient, mild to moderate nausea (44, 45) (Boxes 1 and 2).

Two randomized, double-blind, placebo-controlled studies were conducted at the University of Virginia School of Medicine between June and July 1997 to determine the efficacy of oseltamivir both as a preventive agent and in the treatment of influenza. In the prophylactic study, 37 volunteers were assigned to a 5-day treatment with oseltamivir (100 mg once daily or b.i.d.) or placebo. Treatment was initiated 26 h before inoculation by intranasal drops containing an infectious dose of the influenza virus. In the treatment study, 80 subjects were randomized to 1 of 4 active treatment groups (20, 100 or 200 mg b.i.d. or 200 mg once daily) or placebo. Treatment was initiated 28 h after inoculation with the influenza virus and continued for 5 days. In the prophylaxis study, 67% of subjects on placebo and 38% of those on oseltamivir became infected, and viral shedding was detected in 50% of patients in the placebo group compared to none of those on oseltamivir. Infection-related respiratory illness developed in 33% of those on placebo, while none of the study drug recipients became ill. In the treatment study, volunteers in the oseltamivir group had less severe symptoms and overcame their illness more rapidly than those in the placebo group. The average duration of infection was 53 h and 95 h in the oseltamivir and placebo groups, respectively. Nasal mucus was reduced by approximately 50% in the active treatment group. No important dose-related differences were noted. Transient, mild to moderate nausea was associated with treatment in 17% and 7% of subjects on active drug and placebo, respectively, but could be largely avoided by administering drugs with a meal (46).

Box 3: Efficacy of oseltamivir in the treatment of acute influenza (47) [Prous Science CSline database].

Design	Multicenter, randomized, double-blind, placebo-controlled clinical study
Population	Healthy nonimmunized adults with influenza illness (<36 h after symptom onset) (n = 374)
Treatments	Oseltamivir (O), 75 mg p.o. b.i.d. x 5 d O, 150 mg p.o. b.i.d. x 5 d Placebo (P)
Adverse Events	Gastrointestinal
Results	Duration of illness (d): P (4.3) > O150* (2.9) = O75* (2.9) [*p <0.001 *vs.* P] [30% reduction with O] Severity of illness (AUC): P (962.6) > O150* (626) ≥ O75* (597) [*p <0.001 *vs.* P] Duration of fever and use of acetaminophen: P > O Incidence of secondary complications (%): P (18) > O75* (10) ≥ O150* (7) [*p = 0.017 *vs.* P]
Conclusions	Oseltamivir significantly reduced severity and duration of influenza illness and decreased the incidence of secondary complications

Box 4: Effects of oseltamivir on duration and severity of influenza (48) [Prous Science CSline database].

Design	Multicenter, randomized, double-blind, placebo-controlled clinical study
Population	Healthy nonimmunized adults with influenza illness (<36 h after symptom onset) (n = 475)
Treatments	Oseltamivir (O), 75 mg p.o. b.i.d. x 5 d O, 150 mg p.o. b.i.d. x 5 d Placebo (P)
Adverse Events	Gastrointestinal
Results	Duration of illness (h): P (116.5) ≥ O75 (87.4) > O150* (81.8) [*p <0.007 *vs.* P] Duration of illness (h): P (117.7) > O75* (74.5) ≥ O150* (70.7) [*p <0.02] [40% reduction with O] Severity of illness (%), change *vs.* P: O ≥ –25 [p <0.007] Incidence of subjects remaining febrile after 36 h (%), change *vs.* P: –60 Severity of cough (%), change *vs.* P: –35
Conclusions	Oseltamivir significantly reduced severity and duration of influenza illness and was well tolerated

Healthy, nonimmunized adults (18-65 years) with febrile respiratory illness were treated with oseltamivir (75 or 150 mg p.o. b.i.d.) within 36 h of symptom onset in a placebo-controlled, double-blind, multicenter study. Of 629 subjects enrolled at 60 U.S. centers, 374 (60%) were infected with influenza. The median duration of illness in oseltamivir-treated patients was 2.9 days (both dose groups) compared to 4.3 days for those given placebo, representing a reduction of 30%. Severity of illness (AUC) was 597, 626 and 962.6 score-h in the 75-mg oseltamivir, 150-mg oseltamivir and placebo groups, respectively. Duration and severity of cough decreased in a similar fashion, and oseltamivir recipients reported less fever and less use of acetaminophen. The incidence of secondary complications, primarily bronchitis and sinusitis, was 18% in the placebo group and 10% and 7% in the low- and high-dose oseltamivir groups, respectively. The study drug was well tolerated, although some subjects experienced mild adverse gastrointestinal effects (47) (Box 3).

Another placebo-controlled multicenter study, also conducted during the 1997-1998 influenza season, enrolled 719 patients at centers in Canada, Europe and China and administered twice-daily oral doses of 75 or 150 mg oseltamivir. In this study, patients treated with either dose of the drug had an overall reduction of 30% in duration of illness; in a subgroup of patients initiating treatment within 24 h of symptom onset, a 40% reduction in duration of illness was obtained with oseltamivir. Disease severity was 25% lower in the active treatment groups and the proportion of subjects remaining febrile after 36 h was 60% lower. Duration and severity of cough was 35% lower with oseltamivir. Virus titers were reduced by 40% with the neuraminidase inhibitor, and use of antibiotics to treat bronchitis, otitis media, pneumonia and sinusitis was reduced significantly in the oseltamivir arms. Again, the drug was generally well tolerated, with only mild gastrointestinal effects (48, 49) (Boxes 4 and 5).

Data from 3 treatment studies were pooled for analysis. Of 1404 patients included, 887 had confirmed infection. Both doses of the study drug (75 and 150 mg b.i.d.)

Box 5: Effects of oseltamivir on reducing viral load and antibiotic use in patients with influenza (49) [Prous Science CSline database].

Design	Randomized, double-blind, placebo-controlled clinical study
Population	Healthy nonimmunized adults with influenza illness (<36 h after symptom onset) (n = 475)
Treatments	Oseltamivir (O), 75 mg p.o. b.i.d. x 5 d O, 150 mg p.o. b.i.d. x 5 d Placebo (P)
Results	Duration of illness (%), change *vs.* P: –30 Duration of illness (%), O initiated <24 h after symptom onset, change *vs.* P: –40 Severity of illness (AUC): P > O [p <0.05] [significant reduction with O] Use of antibiotics to treat symptomatic secondary complications, change *vs.* P: P > O75 [p <0.04]; P ≥ O150 [p = 0.57] Viral titer: P (131) > O150* (94) ≥ O75* (78) [*p <0.03 *vs.* P] [40% reduction with O]
Conclusions	Oseltamivir significantly reduced severity and duration of influenza illness and the need for antibiotic treatment for defined secondary complications

Box 6: Efficacy of oseltamivir in the treatment of influenza in adults (50) [Prous Science CSline database].

Design	Multicenter, randomized, double-blind, placebo-controlled clinical study
Population	Healthy nonimmunized adults with influenza illness (<36 h symptom onset) (n = 887)
Treatments	Oseltamivir (O), 75 mg p.o. b.i.d. x 5 d O, 150 mg p.o. b.i.d. x 5 d Placebo (P)
Results	Duration of illness (h): P (112.5) > O150* (78.5) = O75* (78.2) [*p <0.0001 *vs.* P] [30% reduction with O] Time to afebrile state over the dosing period (%), change: O150* (–38) = O75* (–38) [*p <0.0001 *vs.* P] Incidence of secondary complications associated with antibiotic use (%), change: O150* (–61) > O75* (–43) [*p <0.05] Reduction in antibiotic consumption: O75* (–80) [*p <0.04 *vs.* P]
Conclusions	Oseltamivir significantly reduced severity and duration of influenza illness, decreased the incidence of secondary complications and the use of antibiotics and was well tolerated

reduced duration of illness by 30%, severity of illness and time to afebrile state by 38% and incidence of secondary complications associated with antibiotic use by 43-61% compared to placebo; the low-dose oseltamivir group also showed an 80% decrease in antibiotic consumption. Treatment was well tolerated (50) (Box 6).

The efficacy of oseltamivir in preventing influenza infection has also been demonstrated in 2 large clinical studies. A total of 1559 healthy nonimmunized adults were treated during periods of local influenza activity with oseltamivir (75 mg once or twice daily) or placebo for 6 weeks. The incidence of illness (defined as temperature of 99 °F plus 1 respiratory and 1 systemic symptom) was 1.2% and 1.3% in the once- and twice-daily active treatment groups, as compared to 4.8% in the placebo arm. Overall, oseltamivir had a protective efficacy of 74% against influenza illness. In three centers with a combined attack rate of 7.1%, the study drug had a protective efficacy of 84%. Laboratory evidence of influenza illness was significantly lower in the oseltamivir groups than in the placebo group (6.3% *vs.* 11.9%). The overall dropout rate was higher for placebo than for oseltamivir (7.1% *vs.* 4.8%) and the study drug was well-tolerated over this long-term dosing period (51) (Box 7).

A systematic review of the impact of influenza on the workplace and the effects of treatment with oseltamivir has also been presented. Data on oseltamivir was obtained from a double-blind, randomized trial in 475 otherwise healthy adults with confirmed influenza infection, 353 of whom were employed at the time, receiving either the study drug (75 mg) or placebo twice daily for 5 days. The number of working days lost per influenza episode was found to be from 3-7 days and influenza infection was also found to adversely affect performance. Oseltamivir significantly reduced the duration and severity of illness compared with placebo and was generally well tolerated. Subjects receiving oseltamivir reported normalization of activity status 2 days earlier compared with placebo and treatment with oseltamivir also increased actual time at work by 63%. Overall, these data indicate a significant impact of influenza on work

Box 7: Oseltamivir for the prevention of influenza virus infection (51) [Prous Science CSline database].

Design	Multicenter, randomized, double-blind, placebo-controlled clinical study
Population	Healthy nonimmunized adults (n = 1559)
Treatments	Oseltamivir (O), 75 mg p.o. o.d. x 6 wks O, 150 mg p.o. o.d. x 6 wks Placebo (P)
Withdrawals	Similar among the three groups: 3.1-4%
Adverse Events	O75: nausea 12.1%, vomiting 2.5% O150: nausea 14.6%, vomiting 2.7% P: nausea 7.1%, vomiting 0.8%
Results	Risk of influenza (%): P (4.8) > O150* (1.3) ~O75* (1.2) [*p <0.001 *vs.* P] Overall protective efficacy (%): 74 Protective efficacy for culture proven influenza (%): 87 Rate of laboratory confirmed influenza infection (%): P (10.6) > O (5.3) [p <0.001
Conclusions	Oseltamivir was safe and effective for the prevention of influenza

Box 8: Effects of oseltamivir on daily performance and work absenteeism (52) [Prous Science CSline database].

Design	Multicenter, randomized, double-blind, placebo-controlled clinical study
Population	Healthy nonimmunized adults with influenza illness (n = 475)
Treatments	Oseltamivir (O), 75 mg p.o. b.i.d. x 5 d Placebo (P)
Results	Time return of ability to perform normal activities: O 2 d earlier *vs.* P [p = 0.0003] Significant difference between cumulative h worked with O *vs.* P [p = 0.006] Absence from work: O increased actual time at work by 63%
Conclusions	Treating influenza with oseltamivir could be a valuable addition to the management of influenza outbreaks in the workplace

absenteeism and activity, as well as a role for oseltamivir in the management of influenza outbreaks in the workplace (52) (Box 8).

In a preliminary analysis of ongoing treatment studies in the elderly and patients with chronic obstructive pulmonary disease and cardiovascular disease, oseltamivir (75 mg b.i.d. for 5 days) demonstrated a good tolerability profile, with no safety concerns. In a long-term prevention study involving 548 frail elderly patients, 80% of whom were vaccinated and nearly all of whom used concomitant medication, once-daily administration of oseltamivir (75 mg) for 6 weeks provided more than 90% protection from influenza illness as compared to placebo. The long-term prevention data support the use of oseltamivir as an additional safeguard to vaccination during influenza outbreaks in nursing homes (53).

Oseltamivir (Tamiflu®) has been approved and launched in Switzerland, its first market, for the treatment of all common strains of influenza. The drug was approved in late October by the U.S. FDA and is under regulatory review in Canada, where it has priority review status (54-56). Oseltamivir is supplied as capsules, 75 mg free base equivalent of the phosphate salt (57).

Manufacturer

Gilead Sciences, Inc. (US); licensed to F. Hoffmann-La Roche Ltd. (CH) for codevelopment and marketing worldwide.

References

1. Kent, K.M., Kim, C.U., McGee, L.R., Munger, J.D., Prisbe, E.J., Postich, M.J., Rohloff, J.C., Kelly, D.E., Williams, M.A., Zhang, L. (Gilead Sciences, Inc.). *Preparation of carbocyclic cpds.* US 5886213.

2. Kent, K.M., Kim, C.U., McGee, L.R., Munger, J.D., Prisbe, E.J., Postich, M.J., Rohloff, J.C., St. John, D.E., Williams, M.A., Zhang, L. (Gilead Sciences, Inc.). *Preparation of cyclohexene carboxylate derivatives.* WO 9807685.

3. Bischofberger, N.W., Dahl, T.C., Hitchcock, M.J.M., Kim, C.U., Lew, W., Liu, H., Mills, R.G., Williams, M.A. (Gilead Sciences, Inc.). *Cpds. containing six-membered rings, processes for their preparation, and their use as medicaments.* WO 9914185.

4. Rohloff, J.C., Kent, K.M., Postich, M.J. et al. *Practical total synthesis of the anti-influenza drug GS-4104.* J Org Chem 1998, 63: 4545-50.

5. Bischofberger, N.W., Kim, C.U., Lew, M., Liu, H., Williams, M.A. (Gilead Sciences, Inc.). *Carbocyclic cpds.* US 5763483.

6. Bischofberger, N.W., Kim, C.U., Lew, W., Liu, H., Williams, M.A. (Gilead Sciences, Inc.). *Cpds. and methods for synthesis and therapy.* EP 759917, JP 99501908, US 5952375, WO 9626933.

7. Lui, K.J., Kendal, A.P. *Impact of influenza epidemics on mortality in the United States from October 1972 to May 1985.* Am J Public Health 1987, 77: 712-6.

8. CDC. *Prevention and control of influenza recommendations of the Advisory Committee on Immunization Practices (ACIP).* MMWR 1999, 48 (no. RR-4).

9. Colacino, J.M., Staschke, K.A., Laver, W.G. *Approaches and strategies for the treatment of influenza virus infections.* Antivir Chem Chemother 1999, 10: 155-85.

10. Webster, R.G., Kawaoka, Y. *Influenza: An emerging and re-emerging disease.* Semin Virol 1994, 5: 103-11.

11. Cox, N.J., Fukuda, K. *Influenza.* Infect Dis Clin North Am 1998, 12: 27-38.

12. Pinto, L.H., Holsinger, L.J., Lamb, R.A. *Influenza virus M2 protein has ion channel activity.* Cell 1992, 69: 517-28.

13. Hayden, F.G., Couch, R.B. *Clinical and epidemiological importance of influenza A viruses resistant to amantadine and rimantadine.* Rev Med Virol 1992, 2: 89-96.

14. Glushkov, R.G. *Arbidol.* Drugs Fut 1992, 17: 1079-81.

15. Palese, P., Compans, R.W. *Inhibition of influenza virus replication in tissue culture by 2-deoxy-2,3-dehydro-N-trifluoroacetylneuraminic acid (FANA): Mechanism of action.* J Gen Virol 1976, 33: 159-63.

16. Klenk, H.D., Rott, R. *The molecular biology of influenza virus pathogenicity.* Adv Virus Res 1988, 34: 247-81.

17. Liu, C., Eichelberger, M.C., Compans, R.W., Air, G.M. *Influenza type A virus neuraminidase does not play a role in viral entry, replication, assembly, or budding.* J Virol 1995, 69: 1099-106.

18. Colman, P.M., Varghese, J.N., Laver, W.G. *Structure of the catalytic and antigenic sites in influenza virus neuraminidase.* Nature 1983, 303: 41-4.

19. Colman, P.M. *Influenza virus neuraminidase: Structure, antibodies, and inhibitors.* Protein Sci 1994, 3: 1687-96.

20. Varghese, J.N., McKimm-Breschkin, J.L., Caldwell, J.B., Kortt, A.A., Colman, P.M. *The structure of the complex between influenza virus neuraminidase and sialic acid, the viral receptor.* Proteins 1992, 14: 327-32.

21. Von Itzstein, M., Wu, W.Y., Kok, G.B. et al. *Rational design of potent sialidase-based inhibitors of influenza virus replication.* Nature 1993, 363: 418-23.

22. *Relenza receives approval in the E.U. for treatment of influenza.* DailyDrugNews.com (Daily Essentials) June 3, 1999.

23. Kim, C.U. *Rational drug design of orally active influenza neuraminidase inhibitors: Discovery and development of GS 4104.* Med Chem Res 1998, 8: 392-9.

24. Kim, C.U., Lew, W., Williams, M.A., Wu, H., Zhang, L., Chen, X., Escarpe, P.A., Mendel, D.B., Laver, W.G., Stevens, R.C. *Structure-activity relationship studies of novel carbocyclic influenza neuraminidase inhibitors.* J Med Chem 1998, 41: 2451-60.

25. Kim, C.U., Lew, W., Williams, M.A. et al. *Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: Design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity.* J Am Chem Soc 1997, 119: 681-90.

26. Williams, M.A., Lew, W., Mendel, D.B., Tai, C.Y., Escarpe, P.A., Laver, W.G., Stevens, R.C., Kim, C.U. *Structure-activity relationships of carbocyclic influenza neuraminidase inhibitors.* Bioorg Med Chem Lett 1997, 7: 1837-42.

27. Hayden, F.G., Rollins, B.S. *In vitro activity of the neuraminidase inhibitor GS4071 against influenza viruses.* Antivir Res 1997, 34(2): Abst 159.

28. Huffman, J.H., Morrison, A., Syndergaard, T., Sidwell, R.W. *Synergistic in vitro anti-influenza virus effects of the neuraminidase inhibitor GS4071 and ribavirin.* Antivir Res 1998, 37(3): Abst 172.

29. Mendel, D.B., Escarpe, P.A., Tai, C.Y., Williams, M.A., Lew, W., Wu, H., Kim, C.U., Laver, G., Graves, B.J. *Kinetic studies of the inhibition of influenza neuraminidases by GS4071.* Antivir Res 1998, 37(3): Abst 171.

30. Mendel, D.B., Tai, C.Y., Escarpe, P.A. *In vitro selection and characterization of a human influenza virus with decreased susceptibility to GS4071.* Antivir Res 1998, 37(3): Abst 113.

31. Tai, C.Y., Escarpe, P.A., Sidwell, R.W., Williams, M.A., Lew, W., Wu, H., Kim, C.U., Mendel, D.B. *Characterization of human influenza virus variants selected in vitro in the presence of the neuraminidase inhibitor GS4071.* Antimicrob Agents Chemother 1998, 42: 3234-41.

32. Li, W., Escarpe, P.A., Eisenberg, E.J. et al. *Identification of GS4104 as an orally bioavailable prodrug of the influenza virus neuraminidase inhibitor GS4071.* Antimicrob Agents Chemother 1998, 42: 647-53.

33. Sidwell, R.W., Huffman, J.H., Barnard, D.L., Bailey, K.W., Wong, M.H., Morrison, A., Syndergaard, T., Kim, C.U. *Inhibition of influenza virus infections in mice by GS4104, an orally effective influenza virus neuraminidase inhibitor.* Antivir Res 1998, 37: 107-20.

34. Mendel, D.B., Tai, C.Y., Escarpe, P.A. et al. *Oral administration of a prodrug of the influenza virus neuraminidase inhibitor GS4071 protects mice and ferrets against influenza infection.* Antimicrob Agents Chemother 1998, 42: 640-6.

35. Sidwell, R.W., Bailey, K.W., Bemis, P.A., Wong, M.H., Eisenberg, E.J., Huffman, J.H. *Influence of treatment schedule and viral challenge dose on the in vivo influenza virus-inhibitory effects of the orally administered neuraminidase inhibitor GS4104.* Antivir Chem Chemother 1999,10: 187-93.

36. Sidwell, R.W., Bailey, K.W., Wong, M.H., Huffman, J.H. *Lack of in vivo development of influenza A virus resistance to the orally effective neuraminidase inhibitor GS4104.* Antivir Res 1998, 37(3): Abst 112.

37. Eisenberg, E.J., Bidgood, A., Cundy, K.C. *Penetration of GS4071, a novel influenza neuraminidase inhibitor, into rat bronchoalveolar lining fluid following oral administration of the prodrug GS4104.* Antimicrob Agents Chemother 1997, 41:1949-52.

38. Cundy, K.C., Eisenberg, G., Bidgood, A., Li, W., Mendel, D., Francis, R.J., Angus, D., Sharp, S.R., Lee, W.A. *The novel influenza neuraminidase inhibitor prodrug GS4104 is highly bioavailable in animals.* 37th Intersci Conf Antimicrob Agents Chemother (Sept 28-Oct 1, Toronto) 1997, Abst H-135.

39. Cundy, K.C., Eisenberg, G., Bidgood, A., Lynch, G., Lee, W.A. *Enhanced delivery of the novel influenza neuraminidase inhibitor GS4071 to rat lung after oral administration of the prodrug GS4104.* 37th Intersci Conf Antimicrob Agents Chemother (Sept 28-Oct 1, Toronto) 1997, Abst A-122.

40. Wood, N.D., Aitken, M., Sharp, S., Evison, H. *Tolerability and pharmacokinetics of the influenza neuraminidase inhibitor Ro 64-0802 (GS4071) following oral administration of the prodrug Ro 64-0796 (GS4104) to healthy male volunteers.* 37th Intersci Conf Antimicrob Agents Chemother (Sept 28-Oct 1, Toronto) 1997, Abst A-123.

41. Algranati, N.E., Massarella, J.W., Wood, N.D., Rana, B., Aitken, M., Freestone, S., Brown, A., Ward, P. *Pharmacokinetics and tolerability of the influenza neuraminidase inhibitor Ro 64-0802 following multiple dose oral administration of the prodrug Ro 64-0796 to healthy young and elderly subjects.* Eur Repir J 1998, 12(Suppl. 28): Abst P1066.

42. He, G., Massarella, J., Schulz, R., Calvert, L., Woodruffe-Peacock, C., Dorr, A., Ward, P., Krimmer, D., Brown, A. *The relative bioavailability of early clinical and market formulations of the novel oral neuraminidase inhibitor Ro 64-0796/GS4104.* Fundam Clin Pharmacol 1999, 13(Suppl. 1): Abst PW177.

43. He, G., Massarella, J., Aitken, M., Dorr, A., Calvert, L., Butcher, R., Brown, A., Ward, P. *The pharmacokinetics and safety of the oral neuraminidase inhibitor Ro 64-0796/GS4104 when administered concurrently with cimetidine or probenecid in healthy subjects.* J Antimicrob Chemother 1999, 44(Suppl. A): Abst P17.

44. Hayden, F.G., Fritz, R.S., Lobo, M., Kinnersley, N., Mills, R.G., Straus, S.E. *Effects of the oral neuraminidase inhibitor GS4104 on cytokine responses during experimental human influenza A virus infection.* Eur Respir J 1998, 12(Suppl. 28): Abst 1791.

45. Hayden, F.G., Lobo, M., Treanor, J.J., Miller, M., Mills, R.G. *Efficacy and tolerability of oral GS4104 for early treatment of experimental influenza in humans.* 37th Intersci Conf Antimicrob Agents Chemother (Sept 28-Oct 1, Toronto) 1997, Abst LB-26.

46. Hayden, F.G., Treanor, J.J., Fritz, R.S., Lobo, M., Betts, R.F., Miller, M., Kinnersley, N., Mills, R.G., Ward, P., Straus, S.E. *Use of the oral neuraminidase inhibitor oseltamivir in experimental human influenza: Randomized controlled trials for prevention and treatment.* JAMA - J Am Med Assoc 1999, 282: 1240-6.

47. Treanor, J.J., Vrooman, P.S., Hayden, F.G., Kinnersley, N., Ward, P., Mills, R.G. *Efficacy of oral GS4104 in treating acute influenza.* 38th Intersci Conf Antimicrob Agents Chemother (Sept 24-27, San Diego) 1998, Abst LB-4.

48. Aoki, F., Osterhaus, A., Rimmelzwaan, G., Kinnersley, N., Ward, P. *Oral GS4104 successfully reduces duration and severity of naturally acquired influenza.* 38th Intersci Conf Antimicrob Agents Chemother (Sept 24-27, San Diego) 1998, Abst LB-5.

49. Oxford, J., Ward, P., Kinnersley, N. *Influenza treatment with GS4104 reduces viral load and antibiotic use related to respiratory complications.* Eur Respir J 1999, 14(Suppl. 30): Abst P1722.

50. Nicholson, K.G., Ward, P., Kinnersley, N. *Oral GS4104 in the treatment of influenza in adults is effective and reduces influenza-related complications and need for antibiotic treatment.* J Antimicrob Chemother 1999, 44(Suppl. A): Abst P5.

51. Hayden, F.G., Atmar, R.L., Schilling, M. et al. *Use of the selective oral neuraminidase inhibitor oseltamivir to prevent influenza.* New Engl J Med 1999, 341: 1336-43.

52. Jefferson, T., Wegmüller, Y., Barker, C., Ward, P. *The impact of influenza on the workplace and the effect of the antiviral GS4104 on daily performance and work absenteeism.* J Antimicrob Chemother 1999, 44(Suppl. A): Abst P23.

53. *Treatment with Tamiflu reduces secondary complications of influenza and need for antibiotics.* DailyDrugNews.com (Daily Essentials) Oct 14, 1999.

54. *Tamiflu, first oral influenza neuraminidase inhibitor, launched today in Switzerland.* DailyDrugNews.com (Daily Essentials) Oct 1, 1999.

55. *Roche's breakthrough flu treatment approved in Switzerland.* DailyDrugNews.com (Daily Essentials) Sept 27, 1999.

56. *Tamiflu approved in time for U.S. flu season.* DailyDrugNews.com (Daily Essentials) Oct 28, 1999.

57. *Tamiflu™ (oseltamivir phosphate) capsules.* Roche Laboratories Inc. Product Fact Sheet, Oct 1999.

58. Huffman, J.H., Morrison, A., Stowell, V., Barnard, D.L., Bush, K., Sidwell, R.W. *Potent in vitro infuenza virus-inhibitory activity of a series of cyclopentane neuraminidase inhibitors.* 39th Intersci Conf Antimicrob Agents Chemother (Sept 26-29, San Francisco) 1999, Abst F946.

59. Sollis, S.L.et al. *Novel inhibitors of influenza sialidase related to GG167 - Synthesis of 4-amino and guanidino-4H-pyran-2-carboxylic acid-6-propylamides, selective inhibitors of influenza A virus sialidase.* Bioorg Med Chem Lett 1996, 6: 1805.

60. Kim, C.U., Lew, W., Williams, M.A., Wu, H., Zhang, L., Chen, X., Escarpe, P.A., Mendel, D.B., Laver, W.G., Stevens, R.C. *Structure-activity relationship studies of novel carbocyclic influenza neuraminidase inhibitors.* J Med Chem 1998, 41: 2451-60.

61. Zhang, L. et al. *Synthesis and activity of C2-substituted analogs of influenza neuraminidase inhibitor GS4071.* Bioorg Med Chem Lett 1997, 7: 1847.

62. Smith, P.W., Robinson, J.E., Evans, D.N., Sollis, S.L., Howes, P.D., Trivedi, N., Bethell, R.C. *Sialidase inhibitors related to zanamivir: Synthesis and biological evaluation of 4H-pyran 6-ether and ketone.* Bioorg Med Chem Lett 1999, 9: 601-4.

63. Bantia, S., Ananth, S., Andries, K., Horn, L., Parker, C., Chand, P., Babu, Y. *In vitro activity of the neuraminidase inhibitor RWJ-270201 (BCX-1812) against influenza virus.* 39th Intersci Conf Antimicrob Agents Chemother (Sept 26-29, San Francisco) 1999, Abst F947.

64. Chandler, M., Bamford, M.J., Conroy, R. et al. *Synthesis of the potent influenza neuraminidase inhibitor 4-guanidino Neu5Ac2en. X-Ray molecular structure of 5-acetamido-4-amino-2,6-anhydro-3,4,5-trideoxy-D-erythro-L-gluconononic acid.* J Chem Soc - Perkins Trans I 1995, 9: 1173.

65. Bethell, R. *Sialidase as a target for inhibitors of influenza virus replication.* IBC Conf Antivir. Latest Preclin Clin Dev Infect Dis (June 26-27, Washington DC) 1997.

66. Smith, P.W. et al. *Novel inhibitors of influenza sialidases related to GG167. Structure-activity, crystallographic and molec ular dynamics studies with 4H-pyran-2-carboxylic acid 6-carboxamides.* Bioorg Med Chem Lett 1996, 6: 2931.

67. Starkey, I.D., Mahmoudian, M., Noble, D., Smith, P.W., Cherry, P.C., Howes, P.D., Sollis, S.L. *Synthesis and influenza virus sialidase inhibitory activity of the 5-desacetamido analogue of 2,3-didehydro-2,4-dideoxy-4-guanidinyl-N-acetylneuraminic acid (GG167).* Tetrahedron Lett 1995, 36: 299.

68. Woods, J.M., Bethell, R.C., Coates, J.A. et al. *4-Guanidino-2,4-dideoxy-2,3-dehydro-N-acetylneuraminic acid is a highly effective inhibitor both of the sialidase (neuraminidase) and of growth of a wide range of influenza A and B viruses in vitro.* Antimicrob Agents Chemother 1993, 37: 1473-9.