



HAL
open science

**In vitro activity of
2,4-diamino-6-[2-(phosphonmethoxy)ethoxy]-pyrimidine
against multidrug-resistant hepatitis B virus (HBV)
mutants.**

Marie-Noëlle Brunelle, Julie Lucifora, Johan Neyts, Stéphanie Villet, Antony Holy, Christian Trépo, Fabien Zoulim

► **To cite this version:**

Marie-Noëlle Brunelle, Julie Lucifora, Johan Neyts, Stéphanie Villet, Antony Holy, et al.. In vitro activity of 2,4-diamino-6-[2-(phosphonmethoxy)ethoxy]-pyrimidine against multidrug-resistant hepatitis B virus (HBV) mutants.. *Antimicrobial Agents and Chemotherapy*, 2007, 51 (6), pp.2240-3. 10.1128/AAC.01440-06 . inserm-00139473

HAL Id: inserm-00139473

<https://inserm.hal.science/inserm-00139473>

Submitted on 2 Apr 2007

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Revised manuscript # AAC01440-06 Version 3

1
2
3 ***In vitro* activity of 2,4-diamino-6-[2-**
4 **(phosphonomethoxy)ethoxy]-pyrimidine against**
5 **multidrug-resistant hepatitis B virus (HBV) mutants**

6
7 M.N. Brunelle^{1,2,3}, J. Lucifora^{1,2,3}, J. Neyts⁴, S. Villet^{1,2,3}, A. Holy⁵, C.Trepo^{1,2,3,6}, F.
8 Zoulim^{1,2,3,6}.

9
10 1) INSERM, U871, 151 Cours Albert Thomas, 69003 Lyon, France;

11 2) Université Lyon 1, 69008 Lyon, France;

12 3) IFR62 Lyon Est, Faculté de Médecine Laennec, 69008 Lyon, France ;

13 4) Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium;

14 5) Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech
15 Republic, Prague, Czech Republic;

16 6) Hospices Civils de Lyon, Hôtel Dieu Hospital, 69002 Lyon, France.

17
18
19
20 **Corresponding author:**

21 Fabien Zoulim

22 INSERM, U871, 151 Cours Albert Thomas, 69424 Lyon cedex 03, France

23 zoulim@lyon.inserm.fr

24
25
26
27 **Running title:** PME0-DAPym cross-resistance profile on HBV replication

28

ABSTRACT

The susceptibility of drug-resistant hepatitis B virus (HBV) mutants to lamivudine, adefovir, tenofovir, entecavir and 2,4-diamino-6-[2-(phosphonomethoxyethoxy)pyrimidine (PMEO-DAPym), a novel acyclic pyrimidine analogue, was assessed *in vitro*. Most drug-resistant mutants, including multidrug resistant strains, remained sensitive to tenofovir and PMEO-DAPym. Therefore, the latter molecule deserves further evaluation for the treatment of HBV infection.

1 Treatment of chronic hepatitis B virus (HBV) infection requires long term administration with
2 nucleos(t)ide analogs [lamivudine [(-)-β-L-2',3'-dideoxy-3' thiacytidine]), adefovir dipivoxil
3 (9-[(2-phosphonylmethoxy)ethyl]adenine), entecavir (2-amino-1,9-dihydro-9-[(1*S*, 3*R*, 4*S*)-4-
4 hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6*H*-purin-6-one, monohydrate) or
5 telbivudine (β-L-2'-deoxythymidine)] (28). However, this leads to the emergence of HBV
6 strains harbouring mutations within the reverse transcriptase (RT) sequence that confer
7 resistance to these drugs (14, 28, 29). The incidence of resistance increases progressively each
8 year, reaching 70 % after 4 years of lamivudine and 29% after 5 years of adefovir dipivoxil
9 therapy (9, 14). Currently, there are two options to treat patients who carry lamivudine-
10 resistant mutants. Lamivudine can be switched to adefovir-dipivoxil or entecavir with the risk,
11 however, of developing adefovir-resistance (7, 8) or entecavir-resistance (4, 19) in the long-
12 term. Adefovir dipivoxil can also be added to ongoing lamivudine monotherapy (7, 8) to
13 delay further resistance, as both drugs have a favorable cross-resistance profile when used in
14 combination (1, 22, 29). However, the emergence of HBV strains harbouring simultaneously
15 lamivudine- and adefovir-resistance mutations was recently reported within the viral
16 quasispecies of a patient who successively failed lamivudine and lamivudine plus adefovir
17 dipivoxil add-on therapy (24). The HBV resistant mutants that are selected after successive
18 failure to lamivudine and entecavir are resistant to both drugs (20, 23, 26). Thus, the
19 development of novel HBV inhibitors is needed to overcome HBV drug resistance, and to
20 design new combination strategies to delay or prevent drug resistance. Different nucleoside
21 analogs are currently in development. Recently, 2,4-diamino-6-[(2-
22 phosphonmethoxy)ethoxy]pyrimidine (PMEO-DAPym), an acyclic pyrimidine nucleoside
23 analog phosphonate, was shown to inhibit *in vitro* human immunodeficiency virus (HIV) and
24 HBV replication with a potency comparable to that of adefovir and tenofovir [(*R*)-9-[(2-
25 phosphonylmethoxy)propyl]adenine] (2, 11, 27). Tenofovir has been approved for HIV

1 therapy and is in phase III trial for HBV infection (25). Moreover, PMEO-DAPym proved to
2 have equipotent activity against wild-type (wt) and lamivudine-resistant rtM204V mutant
3 HBV in inducible transfected hepatoma cell lines (27). In the present study, we investigated,
4 in transiently transfected Huh7 cells, the cross-resistance profiles of a series of drug-resistant
5 HBV mutants, including multiple drug-resistant strains, to PMEO-DAPym and this in direct
6 comparison with other drugs in parallel assays.

7 First, we determined the effect of the compounds on Huh7 cell viability by
8 determining the concentration of drug that reduced the uptake of neutral red dye by 50 %
9 (CC_{50}), as described before (10). Transient transfection of Huh7 cells was then performed as
10 previously described with plasmids containing 1.1 genome unit of wt or mutant HBV strain
11 under the control of the chicken beta actin promoter (6). One group of constructs contained
12 the genome of HBV laboratory strains (genotype D, serotype ayw) including wt and resistant
13 HBV mutants obtained by site directed mutagenesis (lamivudine-resistant: rtL180M/M204V;
14 adefovir-resistant: rtN236T; lamivudine+adefovir-resistant: rtL180M/M204V/N236T) (3, 6,
15 17). The second group of constructs contained HBV genomes cloned from the viral
16 quasispecies of two HBV chronically infected patients who failed sequential therapy with
17 currently approved HBV inhibitors (23, 24). The following clinical isolates (cloned HBV
18 genomes) were studied: lamivudine-resistant mutants: rtL180M/M204V, rtL180M/A181V,
19 rtV173L/L180M/M204V; lamivudine+adefovir-resistant mutants: rtV173L/L180M/A181V,
20 rtV173L/L180M/A181V/M204V, rtV173L/L180M/A181V/M204V/N236T,
21 rtV173L/L180M/A181V/N236T; entecavir-resistant mutant: rtL180M/S202G/M204V.
22 Antiviral assays using transfected cells, purification of intracellular HBV DNA and its
23 analysis by southern blotting were performed as previously described (3, 6).

24 As shown in Table 1, in Huh7 cells, PMEO-DAPym had little or no effect on cell
25 viability [$CC_{50} > 1,000 \mu\text{M}$], as was also the case for lamivudine and tenofovir. The CC_{50}

1 value for entecavir and adefovir were $125 \pm 35 \mu\text{M}$ and $365 \pm 120 \mu\text{M}$, respectively.
2 Furthermore, PME0-DAPym had no effect on HBsAg production by WT HBV transfected
3 cells (data not shown). When the anti-HBV activity was assessed, entecavir proved to be the
4 most potent compound with the lowest 50% effective concentration (EC_{50}), followed by
5 lamivudine, PME0-DAPym, adefovir and tenofovir. The EC_{50} of PME0-DAPym was 3 to 4-
6 fold lower than that of adefovir and tenofovir, under our *in vitro* conditions (Table 1). The
7 EC_{50} of PME0-DAPym was higher under our *in vitro* conditions using Huh7 cells by
8 comparison with the results obtained in a stable cell line derived from HepG2 cells (27). This
9 type of EC_{50} variations between Huh7 and HepG2 cells has already been observed previously
10 with other nucleoside analogs (17); however the ranking of antiviral potency was not affected.
11 This may indicate that the intracellular metabolism including entry, transport,
12 phosphorylation, and pumping out of this nucleoside analogs may depend on the cell lines
13 used for the experiment.

14 PME0-DAPym inhibited the replication of both laboratory and clinical lamivudine-
15 resistant HBV variants, rtL180M/M204V and rtV173L/L180M/M204V strains, as efficiently
16 as wt HBV (Tables 2, 3). The rtL180M/A181V mutant displayed a 4.8-fold decreased
17 susceptibility to PME0-DAPym. However, among the drugs studied, tenofovir was the only
18 one which inhibited this mutant as well as wt HBV (Table 3). Lamivudine-resistant HBV
19 strains show decreased susceptibility to entecavir as compared with wild-type HBV strains
20 (Tables 2, 3). Interestingly, the laboratory HBV strain rtL180M/M204V engineered by site
21 directed mutagenesis (Table 2) is more susceptible to entecavir than its counterpart derived
22 from one patient (Table 3). Discrepancies between the susceptibility to entecavir of
23 laboratory- or patient- derived HBV rtL180M/M204V strains were already observed (20), and
24 may be explained by differences in the genetic background of the strains outside of the
25 polymerase region that has been cloned.

1 As previously reported, the rtN236T mutation identified in patients who failed
2 adefovir dipivoxil therapy decreased the sensitivity to adefovir by 3.2 to 7.3 (1, 3, 22) and to
3 tenofovir by 4.5-fold (3) (Table 2). The rtL180M/S202G/M204V mutant, identified in a
4 patient who failed successively lamivudine and entecavir therapy (23), displayed a 210-fold
5 resistance to entecavir and a >100-fold resistance to lamivudine (Table 3). Interestingly, both
6 adefovir- and entecavir-resistant HBV strains were sensitive to PMEODAPym (Tables 2, 3)

7 All four lamivudine+adefovir-resistant mutants, characterized in a patient who failed
8 sequential therapy, displayed a 2.1 to 5.1-fold decreased susceptibility to PMEODAPym
9 depending on the combination of mutations they harboured (Table 3). The EC_{50} of PMEODAPym
10 for mutants rtV173L/L180M/A181V, rtV173L/L180M/A181V/M204V and
11 rtV173L/L180M/A181V/M204V/N236T was lower than that of tenofovir and similar for
12 mutant rtV173L/L180M/A181V/N236T. However, the resistance factor observed for all four
13 lamivudine+adefovir-resistant mutants was slightly higher for PMEODAPym as compared to
14 tenofovir. PMEODAPym and tenofovir had a greater inhibitory activity on these multiple
15 drug-resistant mutants than lamivudine and entecavir; adefovir had slightly higher resistance
16 factors for these mutants but its *in vivo* pharmacological characteristics preclude its use at
17 higher dosage (15). The inhibitory activity of the evaluated compounds against the
18 rtV173L/L180M/A181V/N236T mutant (lamivudine and adefovir escape mutant) was ranged
19 in the following order of potency: tenofovir > PMEODAPym > entecavir > adefovir >
20 lamivudine.

21 Our results provide direct information regarding the cross-resistance profile of the
22 lamivudine-, lamivudine+adefovir- and entecavir-resistant HBV strains isolated from patients
23 who failed sequential therapy. Noteworthy, entecavir may not represent the best anti-HBV
24 agent to treat patients who failed a lamivudine therapy, as lamivudine may lead to the
25 emergence of HBV variants harbouring rtL180M/M204V or rtL180M/A181V mutations that

1 impair the antiviral effect of entecavir (Tables 2 and 3). Moreover, long-term entecavir
2 treatment of patients infected with lamivudine resistant HBV strains leads to the selection of
3 secondary mutations that, on a genetic background of lamivudine-resistant mutations, confer
4 increased resistance to entecavir (20, 23). Nevertheless, entecavir may be valuable for the
5 treatment of patients who failed adefovir dipivoxil therapy since mutants harbouring the
6 rtN236T mutation, in absence of the lamivudine-resistant mutation rtM204V, retained
7 susceptibility to entecavir (Tables 2 and 3) (3). Tenofovir displayed an antiviral activity
8 against wt HBV similar to adefovir (Table 1), and efficiently inhibited the replication of a
9 series of lamivudine-, adefovir-, lamivudine+adefovir- and entecavir-resistant HBV strains
10 (Tables 2 and 3). Clinically, tenofovir has been used successfully for the treatment of patients
11 who successively failed lamivudine and lamivudine+adefovir dipivoxil therapy (16, 23, 24).
12 Several clinical reports suggested a potent anti-HBV activity of tenofovir in patients failing
13 adefovir therapy and moreover a better anti-HBV activity of tenofovir over adefovir in
14 patients failing lamivudine therapy (13, 21), which may be due to better pharmacokinetic
15 properties. Whether tenofovir may select for drug-resistant mutants in patients remains a
16 matter of controversy (5, 18).

17 The development of novel strategies for HBV therapy that may be based on the
18 combination of various nucleoside analogs with different cross-resistant profile will require
19 the discovery of novel HBV inhibitors. We recently demonstrated the *in vitro* potency of the
20 2', 3'-dideoxy-3'-fluoroguanosine to inhibit wt, lamivudine-, adefovir- and lamivudine +
21 adefovir-resistant laboratory HBV strains (12). In the present study, we confirmed previous
22 studies that showed that PMEODAPym is a potent inhibitor of wt HBV *in vitro* (27).
23 Interestingly, we provide new information showing that PMEODAPym inhibits the
24 replication of lamivudine-, entecavir-, adefovir- and lamivudine+adefovir-resistant mutants
25 almost as efficiently as that of wt HBV (Tables 2 and 3). The *in vitro* cross-resistance profile

1 of PMEODAPym on the laboratory and clinical strains studied here proved to be more
2 favorable than that of lamivudine, adefovir and entecavir, and was more or less comparable to
3 that of tenofovir.

4 Interestingly, PMEODAPym efficiently inhibited all HBV variants harbouring the
5 rtL180M/M204V mutations which is the most frequently observed lamivudine-resistant
6 mutant in patients (14, 30) (Tables 2 and 3). Until now, only purine analogs, such as adefovir
7 or tenofovir, have shown activity against the replication of the lamivudine-resistant
8 rtL180M/M204V mutant which is resistant to lamivudine and all known pyrimidine L-
9 nucleosides (29). Thus, PMEODAPym, although not carrying a purine base, exhibits the
10 same cross-resistance profile as purine-based nucleoside phosphonate analogs. This supports
11 our earlier assumption that (based on molecular modelling) that the 2,4-diamino-substituted
12 pyrimidine ring of PMEODAPym can be viewed as a open-ring analog of the purine system
13 in the 2,6-diaminopurine acyclic nucleoside phosphonate derivatives (27). Although most
14 adefovir- and lamivudine+adefovir-resistant HBV strains retained some degree of
15 susceptibility to adefovir *in vitro* (Table 2 and 3), its clinical efficacy is limited by its
16 nephrotoxicity when the daily dose of adefovir dipivoxil is increased from 10 to 30 mg (15).
17 In our experimental conditions, tenofovir and PMEODAPym exhibited the most favourable
18 *in vitro* cross-resistance profiles as inhibitors of the replication of multiple drug-resistant
19 HBV genomes derived from clinical strains from patients who failed sequential therapy with
20 currently approved HBV inhibitors. Therefore, it will be interesting to determine the
21 pharmacodynamics of PMEODAPym *in vivo*.

22 In conclusion, the broad inhibitory activity of PMEODAPym against HBV drug-
23 resistant mutants and its favorable cytotoxicity profile, observed in tissue culture experiments,
24 warrants further pre-clinical evaluation of this compound in animal models of hepadnavirus
25 infection.

1 **Acknowledgements:** This work was part of the activity of the Network of Excellence,
2 “Virgil”, supported by the European Community (ViRgil LSHM-CT-2004-503359), and
3 Descartes Prize (HPAW-CT-2002-9001). This study is part of the research project
4 Z440250506 of IOCB Prague, within the frame of the activity of the Center for New
5 Antivirals and Antineoplastics 1M0508 of the Ministry of Education, Youth and Sports. It
6 was also supported in part by the Program of Targeted Projects of the Academy of Sciences of
7 the Czech Republic (#IQS 5400550501).

1 **Tables**

2

3 **Table 1: Activity of PME0-DAPym and selected compounds against wild-type HBV**
4 **replication and cell viability in Huh7 cells.**

5

6

7

8

9

10

11

12

13

Compound	EC ₅₀ (μM) ¹	CC ₅₀ (μM) ²
Lamivudine	1 ± 1.1	> 1000
Entecavir	0.3 ± 0.42	125 ± 35
Adefovir	13 ± 29	365 ± 120
Tenofovir	16 ± 7.9	> 1000
PME0-DAPym	4.9 ± 0.6	> 1000

14

¹ For each drug, the EC₅₀ value is the mean of the EC₅₀ of wild-type HBV that are shown in Tables 2 and 3.

15

16

² CC₅₀ are means values ± SD for 3 independent experiments performed in quadriplate.

17

Table 2: Effect of selected anti-HBV drugs on the replication of wild-type HBV and HBV laboratory strains of genotype D carrying lamivudine (LAM)-, adefovir (ADV)- or lamivudine+adefovir (LAM+ADV)-resistance mutations.

HBV strains	LAM ^b		ADV ^b		TDF ^b		ETV ^b		PMEO-DAPym	
	EC50 (μM)	FR ^a	EC50 (μM)	FR ^a	EC50 (μM)	FR ^a	EC50 (μM)	FR ^a	EC50 ^c (μM)	FR ^a
Wild-type	2.48 ± 0.67	1	15.8 ± 1.9	1	10.3 ± 1.3	1	0.8 ± 0.1	1	4.0 ± 0.51	1
ADV-R	2.65 ± 0.52	1.06	50.3 ± 11	3.2	46 ± 6	4.5	0.7	0.88	4.5 ± 0.35	1.1
LAM-R	>100	> 40	15.5 ± 1.8	0.98	35.2 ± 5.1	3.4	5 ± 0.25	6.25	4.7 ± 1.12	1.2
LAM+ADV-R	>100	> 40	100 ± 20	6.3	45.5 ± 6.1	4.4	5 ± 0.7	6.25	5.7 ± 0.77	1.4

^a: FR: Fold resistance = (mutant EC₅₀) / (wt EC₅₀).

^b: Data previously reported in (3).

^c: Values represent the mean of at least 3 independent experiments, each performed in triplicate. For each experiment, the drug-resistant HBV strains and their corresponding wt strain were treated simultaneously with the same range of drug concentrations (from 0 to 100 μM for PME0-DAPym, lamivudine, adefovir and tenofovir; from 0 to 10 μM for entecavir), and all the samples were extracted and analysed by southern blotting in parallel.

Lamivudine-resistant (LAM-R) mutant: rtL180M/M204V; Adefovir-resistant (ADV-R) mutant: rtN236T; Lamivudine+adefovir-resistant (LAM+ADV-R): rtL180M/M204V/N236T.

1 **Table 3: Effect of selected anti-HBV drugs on the replication of HBV mutants derived from the viral quaspecies of chronically infected patients.**

HBV strains	LAM		ADV		TDF		ETV		PMEO-DAPym	
	EC ₅₀ (μ M)	FR ^a	EC ₅₀ (μ M)	FR ^a	EC ₅₀ (μ M) ^c	FR ^a	EC ₅₀ (μ M) ^c	FR ^a	EC ₅₀ (μ M) ^c	FR ^a
wt 1	0.64±0.17 ^c	1	13.6±4.08 ^c	1	13.6±4	1	0.09±0.03	1	4.4 ± 0.5	1
wt 2	0.1±0.2 ^b	1	10 ± 3 ^b	1	25±7.1	1	0.06±0.01	1	5.6 ± 1.0	1
LAM-R										
rtL180M/M204V	>100 ^b	>1,000	15±6 ^b	1.5	27±10	1.1	10.5±2.2	175	5.3±0.88	0.9
rtL180M/A181V	80±9 ^b	800	27±16 ^b	2.7	36±13	1.4	1.5±0.6	28	27±10.3	4.8
rtV173L/L180M/M204V	>100 ^c	>156	9.8±2.5 ^c	0.7	16±5.8	1.2	3.7±1.4	43	4.1±0.4	0.9
LAM+ADV-R										
rtV173L/L180M/A181V	100±5 ^b	1,000	48±19 ^b	4.8	42±8.1	1.6	2.75±1.2	50	24±4.5	4.3
rtV173L/L180M/A181V/M204V	>100 ^b	>1,000	40±20 ^b	4.0	45±21.3	1.8	≥50±15.9	≥800	18±7.0	3.2
rtV173L/L180M/A181V/M204V/N236T	>100 ^b	>1,000	77±20 ^b	7.7	46±18.3	1.8	25.4±5.5	461	12±3.0	2.1
rtV173L/L180M/A181V/N236T	>100 ^b	>1,000	>100 ^b	> 10	28± 5.6	1.1	0.5±0.14	9.0	29 ± 5.3	5.1
ETV-R										
rtL180M/S202G/M204V	>100 ^c	>156	15±4.5 ^c	1.1	27±9.8	2	18±8.7	210	4.5 ± 0.9	1.0

2 ^a; FR: Fold resistance = (mutant EC₅₀)/(wt EC₅₀). For mutants rtV173L/L180M/M204V and rtL180M/S202G/M204V, the corresponding wt strain is wt1 (genotype H) and3 FR = (mutant EC₅₀)/(wt1 EC₅₀). For the other mutants, the corresponding wt strain is wt2 (genotype E) and FR = (mutant EC₅₀)/(wt2 EC₅₀).4 ^b; Data previously reported in (24).5 ^c; Values represent the mean of at least 3 independent experiments, each performed in triplicate. For each experiment, the drug-resistant HBV strains and their corresponding wt strain were treated simultaneously with the same range of drug concentrations (from 0 to 100 μ M for PMEO-DAPym, lamivudine, adefovir and tenofovir; from 0 to 10 μ M for entecavir), and all the samples were extracted and analysed by southern blotting in parallel.

8 Lamivudine: LAM; Adefovir: ADV; Tenofovir: TDF; Entecavir: ETV; Lamivudine-resistant: LAM-R; Lamivudine+adefovir-resistant: LAM+ADV-R; Entecavir-resistant:

9 ETV-R.

1 **References**

- 2 1. **Angus, P., R. Vaughan, S. Xiong, H. Yang, W. Delaney, C. Gibbs, C. Brosgart, D.**
3 **Colledge, R. Edwards, A. Ayres, A. Bartholomeusz, and S. Locarnini.** 2003.
4 Resistance to adefovir dipivoxil therapy associated with the selection of a novel
5 mutation in the HBV polymerase. *Gastroenterology* **125**:292-7.
- 6 2. **Balzarini, J., C. Pannecouque, E. De Clercq, S. Aquaro, C. F. Perno, H.**
7 **Egberink, and A. Holy.** 2002. Antiretrovirus activity of a novel class of acyclic
8 pyrimidine nucleoside phosphonates. *Antimicrob Agents Chemother* **46**:2185-93.
- 9 3. **Brunelle, M. N., A. C. Jacquard, C. Pichoud, D. Durantel, S. Carrouee-Durantel,**
10 **J. P. Villeneuve, C. Trepo, and F. Zoulim.** 2005. Susceptibility to antivirals of a
11 human HBV strain with mutations conferring resistance to both lamivudine and
12 adefovir. *Hepatology* **41**:1391-8.
- 13 4. **Chang, T. T., R. G. Gish, S. J. Hadziyannis, J. Cianciara, M. Rizzetto, E. R.**
14 **Schiff, G. Pastore, B. R. Bacon, T. Poynard, S. Joshi, K. S. Kleczewski, A. Thiry,**
15 **R. E. Rose, R. J. Colonno, and R. G. Hindes.** 2005. A dose-ranging study of the
16 efficacy and tolerability of entecavir in Lamivudine-refractory chronic hepatitis B
17 patients. *Gastroenterology* **129**:1198-209.
- 18 5. **Delaney, W. E. t., A. S. Ray, H. Yang, X. Qi, S. Xiong, Y. Zhu, and M. D. Miller.**
19 2006. Intracellular metabolism and in vitro activity of tenofovir against hepatitis B
20 virus. *Antimicrob Agents Chemother* **50**:2471-7.
- 21 6. **Durantel, D., S. Carrouee-Durantel, B. Werle-Lapostolle, M. N. Brunelle, C.**
22 **Pichoud, C. Trepo, and F. Zoulim.** 2004. A new strategy for studying in vitro the
23 drug susceptibility of clinical isolates of human hepatitis B virus. *Hepatology* **40**:855-
24 64.
- 25 7. **Fung, S. K., P. Andreone, S. H. Han, K. Rajender Reddy, A. Regev, E. B. Keeffe,**
26 **M. Hussain, C. Cursaro, P. Richtmyer, J. A. Marrero, and A. S. Lok.** 2005.
27 Adefovir-resistant hepatitis B can be associated with viral rebound and hepatic
28 decompensation. *J Hepatol* **43**:937-43.
- 29 8. **Fung, S. K., H. B. Chae, R. J. Fontana, H. Conjeevaram, J. Marrero, K.**
30 **Oberhelman, M. Hussain, and A. S. Lok.** 2006. Virologic response and resistance to
31 adefovir in patients with chronic hepatitis B. *J Hepatol* **44**:283-90.
- 32 9. **Hadziyannis, S. J., N. C. Tassopoulos, E. J. Heathcote, T. T. Chang, G. Kitis, M.**
33 **Rizzetto, P. Marcellin, S. G. Lim, Z. Goodman, J. Ma, S. Arterburn, S. Xiong, G.**
34 **Currie, and C. L. Brosgart.** 2005. Long-term therapy with adefovir dipivoxil for
35 HBeAg-negative chronic hepatitis B. *N Engl J Med* **352**:2673-81.
- 36 10. **Hantz, O., C. Borel, C. Traub-Pedatz, F. Zoulim, J. Desselin, M. Camplo, P. Vlieghe,**
37 **M. Bouygues, C. Trépo, and J. L. Kraus.** 1997. Selective inhibition of the duck
38 hepatitis B virus by a new class of tetraazamacrocycles. *Antimicrob. Agents*
39 *Chemother.* **41**:2579-2581.
- 40 11. **Holy, A., I. Votruba, M. Masojdkova, G. Andrei, R. Snoeck, L. Naesens, E. De**
41 **Clercq, and J. Balzarini.** 2002. 6-[2-(Phosphonomethoxy)alkoxy]pyrimidines with
42 antiviral activity. *J Med Chem* **45**:1918-29.
- 43 12. **Jacquard, A. C., M. N. Brunelle, C. Pichoud, D. Durantel, S. Carrouee-Durantel,**
44 **C. Trepo, and F. Zoulim.** 2006. In vitro characterization of the anti-hepatitis B virus
45 activity and cross-resistance profile of 2',3'-dideoxy-3'-fluoroguanosine. *Antimicrob*
46 *Agents Chemother* **50**:955-61.
- 47 13. **Lacombe, K., J. Gozlan, P. Y. Boelle, L. Serfaty, F. Zoulim, A. J. Valleron, and P.**
48 **M. Girard.** 2005. Long-term hepatitis B virus dynamics in HIV-hepatitis B virus-co-
49 infected patients treated with tenofovir disoproxil fumarate. *Aids* **19**:907-15.

- 1 14. **Lai, C. L., J. Dienstag, E. Schiff, N. W. Leung, M. Atkins, C. Hunt, N. Brown, M.**
2 **Woessner, R. Boehme, and L. Condreay.** 2003. Prevalence and clinical correlates of
3 YMDD variants during lamivudine therapy for patients with chronic hepatitis B. *Clin*
4 *Infect Dis* **36**:687-96.
- 5 15. **Marcellin, P., T. T. Chang, S. G. Lim, M. J. Tong, W. Sievert, M. L. Shiffman, L.**
6 **Jeffers, Z. Goodman, M. S. Wulfsohn, S. Xiong, J. Fry, and C. L. Brosgart.** 2003.
7 Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis
8 B. *N Engl J Med* **348**:808-16.
- 9 16. **Ratziu, V., V. Thibault, Y. Benhamou, and T. Poynard.** 2006. Successful rescue
10 therapy with tenofovir in a patient with hepatic decompensation and adefovir resistant
11 HBV mutant. *Comp Hepatol* **5**:1.
- 12 17. **Seigneres, B., C. Pichoud, P. Martin, P. Furman, C. Trepo, and F. Zoulim.** 2002.
13 Inhibitory activity of dioxolane purine analogs on wild-type and lamivudine-resistant
14 mutants of hepadnaviruses. *Hepatology* **36**:710-22.
- 15 18. **Sheldon, J., N. Camino, B. Rodes, A. Bartholomeusz, M. Kuiper, F. Tacke, M.**
16 **Nunez, S. Mauss, T. Lutz, G. Klausen, S. Locarnini, and V. Soriano.** 2005.
17 Selection of hepatitis B virus polymerase mutations in HIV-coinfected patients treated
18 with tenofovir. *Antivir Ther* **10**:727-34.
- 19 19. **Sherman, M., C. Yurdaydin, J. Sollano, M. Silva, Y. F. Liaw, J. Cianciara, A.**
20 **Boron-Kaczmarek, P. Martin, Z. Goodman, R. Colonna, A. Cross, G. Denisky,**
21 **B. Kreter, and R. Hindes.** 2006. Entecavir for treatment of lamivudine-refractory,
22 HBeAg-positive chronic hepatitis B. *Gastroenterology* **130**:2039-49.
- 23 20. **Tenney, D. J., S. M. Levine, R. E. Rose, A. W. Walsh, S. P. Weinheimer, L.**
24 **Discotto, M. Plym, K. Pokornowski, C. F. Yu, P. Angus, A. Ayres, A.**
25 **Bartholomeusz, W. Sievert, G. Thompson, N. Warner, S. Locarnini, and R. J.**
26 **Colonna.** 2004. Clinical emergence of entecavir-resistant hepatitis B virus requires
27 additional substitutions in virus already resistant to Lamivudine. *Antimicrob Agents*
28 *Chemother* **48**:3498-507.
- 29 21. **van Bommel, F., B. Zollner, C. Sarrazin, U. Spengler, D. Huppe, B. Moller, H. H.**
30 **Feucht, B. Wiedenmann, and T. Berg.** 2006. Tenofovir for patients with
31 lamivudine-resistant hepatitis B virus (HBV) infection and high HBV DNA level
32 during adefovir therapy. *Hepatology* **44**:318-25.
- 33 22. **Villeneuve, J. P., D. Durantel, S. Durantel, C. Westland, S. Xiong, C. L. Brosgart,**
34 **C. S. Gibbs, P. Parvaz, B. Werle, C. Trepo, and F. Zoulim.** 2003. Selection of a
35 hepatitis B virus strain resistant to adefovir in a liver transplantation patient. *J Hepatol*
36 **39**:1085-9.
- 37 23. **Villet, S., A. Ollivet, C. Pichoud, J. P. Villeneuve, C. Trepo, and F. Zoulim.** 2007.
38 Stepwise process for the development of entecavir resistance in a chronic Hepatitis B
39 virus infected patient. *J Hepatol* **46**(3):531-8.
- 40 24. **Villet, S., C. Pichoud, J. P. Villeneuve, C. Trepo, and F. Zoulim.** 2006. Selection of
41 a multiple drug-resistant hepatitis B virus strain in a liver-transplanted patient.
42 *Gastroenterology* **131**:1253-61.
- 43 25. **Wong, S. N., and A. S. Lok.** 2006. Tenofovir disoproxil fumarate: role in hepatitis B
44 treatment. *Hepatology* **44**:309-13.
- 45 26. **Yim, H. J., M. Hussain, Y. Liu, S. N. Wong, S. K. Fung, and A. S. Lok.** 2006.
46 Evolution of multi-drug resistant hepatitis B virus during sequential therapy.
47 *Hepatology* **44**:703-12.
- 48 27. **Ying, C., A. Holy, D. Hockova, Z. Havlas, E. De Clercq, and J. Neyts.** 2005. Novel
49 acyclic nucleoside phosphonate analogues with potent anti-hepatitis B virus activities.
50 *Antimicrob Agents Chemother* **49**:1177-80.

- 1 28. **Zoulim, F.** 2006. Antiviral therapy of chronic hepatitis B. *Antiviral Res* **71**:206-15.
- 2 29. **Zoulim, F.** 2004. Mechanism of viral persistence and resistance to nucleoside and
- 3 nucleotide analogs in chronic hepatitis B virus infection. *Antiviral Res* **64**:1-15.
- 4 30. **Zoulim, F., T. Poynard, F. Degos, A. Slama, A. El Hasnaoui, P. Blin, F. Mercier,**
- 5 **P. Deny, P. Landais, P. Parvaz, and C. Trepo.** 2006. A prospective study of the
- 6 evolution of lamivudine resistance mutations in patients with chronic hepatitis B
- 7 treated with lamivudine. *J Viral Hepat* **13**:278-88.
- 8
- 9