Pridine Oxide Derivatives: Structure-Activity Relationship for Inhibition of Human Immunodeficiency Virus and Cytomegalovirus Replication in Cell Culture

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Dedicated to Prof. *Wolfgang Pfleiderer* on the occasion of his 75th birthday

Novel pyridine oxide derivatives have been discovered that are endowed with inhibitory potential against human immunodeficiency virus (HIV) and cytomegalovirus (CMV) in cell culture. The compounds show different antiviral specificities (solely active or concomitantly active against HIV-1 and/or HIV-2 and/or CMV) depending on the nature and the specific location of the substituents on the different parts of the molecule. The HIV-1-specific pyridine oxide derivatives have the highest selectivity index, and act as specific HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs). For other pyridine oxide derivatives, it cannot be excluded that a cellular target may be involved to explain the concomitant activity against HIV-1, HIV-2, and CMV. The latter compounds showed a relatively low selectivity index and were, in general, more cytostatic than the HIV-1-specific inhibitors.

1. Introduction. – To date, there are six nucleoside reverse transcriptase (RT) inhibitors (NRTIs), one nucleotide reverse transcriptase inhibitor (NtRTI), three non-nucleoside RT inhibitors (NNRTIs), and six protease inhibitors (PIs) approved to be included in HAART (highly active antiretroviral therapy) for the treatment of human immunodeficiency virus (HIV) infections [1]. These 16 drugs are targeted against two enzymes in the replication cycle of the virus: the virus-encoded reverse transcriptase and protease [2][3]. Given the long-term toxicity [4–6] and (cross)-resistance [7–10] of many of the drugs, there is a need for more drug leads that prevent or inhibit human immunodeficiency virus replication. Such novel drugs should not replace the currently existing drugs, but should rather be added to the drug cocktails to afford a better long-term suppression of the virus. There are examples of a variety of other interaction points in the infection cycle of HIV that can be targeted to efficiently suppress virus replication such as virus attachment to, or fusion with, the cell membrane, proviral DNA integration, or viral DNA transcription/translation [11][12]. It is too early to know whether and/or which of these new drug leads will eventually fill up a niche in the current drug armamentarium to combat HIV infections. Meanwhile, however, the search for new lead compounds for HIV therapy should be continued.

In this study, we report on an entirely novel class of pyridine oxide derivatives (*Fig. 1*) that are endowed with anti-HIV activity, and of which several members also show activity against cytomegalovirus (CMV) replication in cell culture. A profound structure-antiviral activity relationship has now for the first time been presented for

almost 200 compounds that belong to this class of antivirals. Our investigations resulted in the identification of new pyridine oxide derivatives that proved markedly active and selective for HIV-1, not being inhibitory to HIV-2 or any other virus, whereas other pyridine oxide derivatives inhibited HIV-1 and HIV-2 to a similar extent. Also, several members of the pyridine oxide derivatives showed selective inhibitory activity against CMV. This class of pyridine oxide derivatives thus includes compounds that are endowed with a different mechanism of antiviral action, different from the 'classical' mechanism of action of the non-nucleoside NNRTIs that are targeted only at the non-catalytic site of HIV-1 reverse transcriptase.

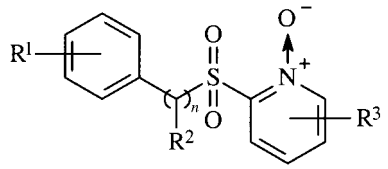


Fig. 1. *Basic structure of the pyridine oxide derivatives*

2. Experimental. – *Test Compounds.* The synthesis of the substituted pyridine oxide derivatives has been performed at *Uniroyal Chemical Ltd.* (Middlebury, CT).

Cells. Human lymphocyte CEM cells were obtained from the American Type Culture Collection and grown in *RPMI 1640* medium supplemented with 10% (*v*/*v*) inactivated fetal calf serum (*Gibco*), 2 mM L-glutamine (*Flow Laboratories*), and 0.075% (*v*/*v*) $NaHCO_3$ (*Flow Laboratories*). Cells were subcultured every 3 to 4 d. Human embryonic lung (HEL) fibroblasts were grown in minimum essential medium (MEM), supplemented with 10% inactivated fetal calf serum (FCS), 2 mM L-glutamine, and 0.3% $NaHCO_3$, and subcultured twice a week.

Cytostatic Activity of Test Compounds in Cell Culture. All assays were performed in 96-well microtiter plates (*Falcon 3072*; *Becton Dickinson*, Paramus, NJ). To each well were added *ca.* 6×10^4 CEM cells (100 µl) and a given amount of the test compound (100 µl). The cells were allowed to proliferate for 96 h at 37° in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a *Coulter* counter (model *ZB*; *Coulter Electronics Ltd.*, Harpenden, Hertfordshire, England). The 50% cytostatic concentration (CC_{50}) was defined as the concentration of compound that inhibited CEM cell proliferation by 50%.

Viruses. HIV-1(III_B) was kindly provided by *R. C. Gallo* and *M. Popovic* (at that time at the National Cancer Institute, Bethesda, MD). HIV-2(ROD) was from *L. Montagnier* (at that time at the Pasteur Institute, Paris, France). The cytomegalovirus strain AD-169 was used in the CMV inhibition assays.

Antiviral Activity of Test Compounds in Cell Cultures. CEM Cells were suspended at *ca.* 300,000 cells per ml of culture medium and infected with *ca.* 100 times the 50% cell-culture-infective doses of HIV-1(III_B) or HIV-2 (ROD). Then, 100 µl of the infected cell suspensions were added to 200-µl microtiter plate wells containing 100 µl of appropriate serial (five-fold) dilutions of the test compounds. The inhibitory effect of the test compounds on HIV-induced syncytium formation in CEM cells was examined microscopically on day 4 or 5 post infection. The EC_{50} was defined as the compound concentration that inhibits syncytium formation in the HIV-infected cell cultures by 50%.

The procedures of the anti-CMV assays were as follows: confluent HEL cells grown in 96-well microtiter plates were inoculated with CMV at an input of 100 PFU per well. After a 1 to 2-h incubation period, residual virus was removed, and the infected cells were further incubated with MEM (supplemented with 2% inactivated FCS, 1% L-glutamine and 0.3% $NaHCO_3$) containing varying concentrations of the compounds. Antiviral activity was expressed as EC_{50} (50% effective concentration), or concentration required to reduce virus-induced cytopathicity after 7 d by 50% compared to the untreated control.

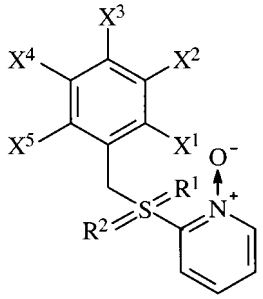
3. Results. – 3.1. *Antiviral Activity of Pyridine Oxide Derivatives with Modifications in the Phenyl Ring.* 3.1.1. *Activity against HIV.* A variety of unsubstituted pyridine oxide

derivatives have been synthesized in which a sulfone (SO_2), a sulfoxide (SO), or a thioether (S) links the pyridine oxide group through a CH_2 to a phenyl moiety (*Table 1*). Compounds **1**–**3** contain an unsubstituted phenyl and represent the simplest pyridine oxide derivatives synthesized. Whereas the SO_2- and SO-containing molecules, **1** and **2**, respectively, inhibited HIV-1 and HIV-2 replication in CEM cells to a similar extent at an EC_{50} of 2.1 to 4.4 μg/ml, compound **3** was virtually inactive at 100 μg/ml. The anti-HIV selectivity indices (SI; CC_{50}/EC_{50}) of **1** and **2** were at best *ca.* 8. Substitutions with one or several alkyl (Me, Et, i-Pr, *t*-Bu, or *t*-pentyl) groups at the phenyl part of the molecule (*i.e.,* **4**–**23**) did not markedly improve the antiviral activity of **1** and **2**. Among these, the 2,5-dimethyl-substituted **11** and the 2,4,6-trimethyl-substituted **17** were the most active compounds against HIV-1 (EC_{50} 1.5–1.8 μg/ml). Whereas **17** was equally active against HIV-1 and HIV-2, **11** was 3-fold less antivirally active (EC_{50} 5.8 μg/ml). The other Me-substituted compounds showed decreased antiviral activity and/or selectivity (*Table 1*). Alkyloxy (*i.e.*, MeO- and EtO-substituted pyridine oxide) derivatives tend to annihilate the antiviral activity of the compounds with the exception of the 2-methoxy-5-methyl-substituted **32**, which kept marked anti-HIV-1 activity (1.6 μg/ml) resulting in a further increased selectivity index (*SI:* 25). However, this compound was ninefold less active against HIV-2. The Ph moiety of the pyridine oxide derivatives has also been substituted by a variety of halogens (*i.e.*, F, Cl, Br, I; *Table 1*) (**36**–**51**). In general, toxicity increased for (especially) the Cl-substituted derivatives, and no increased antiviral efficiency was noted, except for **43** and **45** (EC_{50}: 0.63 to 1.0 μg/ml), albeit resulting in a very low selectivity (*SI:* 2–2.9; *Table 1*). Also, NO_2- and CN-substituted phenyl derivatives, **52**–**62**, did not result in improved antiviral activity or selectivity of the pyridine oxides. A carboxylic acid substituent (*i.e.*, **63**) completely abrogated anti-HIV activity. Ph, PhO, or $PhCH_2O$ derivatives were hardly active or selective against HIV. Finally, a OH group did not result in marked anti-HIV activity of the compounds. For most compounds where the corresponding SO_2, SO, and S derivatives could be compared, there was a trend of lower antiviral efficacy for the SO and/or S forms of the molecules.

3.1.2. *Activity against CMV.* Several pyridine oxide derivatives were also evaluated against CMV replication in cell culture. The antiviral activity ranged from 1.5 μg/ml for **23** to $>$ 50 μg/ml for **2** and **3**. However, anti-CMV selectivity was at most *ca.* 8- to 15-fold based on determinations of microscopically visible alterations of cell morphology (MCC). Alkyloxyphenyl- (*i.e.*, **24**–**35**), halogenophenyl- (*i.e.*, **36**–**51**), nitrophenyl- (*i.e.*, **52**–**59**) or cyanophenyl (*i.e.*, **60**–**62**)-substituted compounds showed invariably decreased antiviral activity/selectivity.

3.2. *Antiviral Activity of Pyridine Oxide Derivatives with Modifications in the Z-Part of the Molecule.* 3.2.1. *Activity against HIV.* A series of pyridine oxide derivatives was synthesized that contain a substitution (Z) at the CH_2 position linking the phenyl group to the other part of the molecule (*Table 2*). Among the alkyl-substituted derivatives (*i.e.*, **69**–**92**), several Me, Et, Pr, i-Bu, heptyl and undecyl substituents were introduced on the CH_2 group, as well as benzyl and phenyl, but in none of the cases, except for **82** (EC_{50} 17–35 μg/ml), was anti-HIV activity observed. None of the branched alkyl (*i.e.*, **92**) and allyl (*i.e.*, **93**) derivatives nor bromoalkyl (*i.e.*, **107**) derivatives were markedly inhibitory or selective against HIV. Substitution of a bulky phenyl or benzyl on the methylene completely annihilated anti-HIV activity. Most of the compounds described

Table 1. *Antiviral Activity of Pyridine Oxide Derivatives Modified in the Phenyl Moiety*



Compound No.	X^1	X^2	X^3	X^4	X^5	R^1	R^2	EC_{50} [a]) [μg/ml] HIV-1	HIV-2	CMV	IC_{50} [b]) [μg/ml] (CEM)	(HEL)	MCC[c]) [μg/ml] (HEL)
1	H	H	H	H	H	O	O	4.4	4.4	20	36	> 50	≥ 50
2	H	H	H	H	H	O	–	3.4	2.1	> 50	20	> 50	> 50
3	H	H	H	H	H	–	–	≥ 100	> 100	> 50	> 100	> 50	> 50
4	Me	H	H	H	H	O	O	11	7.5	12	23		50
5	Me	H	H	H	H	O	–	≥ 20	6	37	52		> 50
6	H	Me	H	H	H	O	O	2.4	9.0	16	23		50
7	H	Me	H	H	H	O	–	> 20	> 20	> 20	37		50
8	H	H	Me	H	H	O	O	13	10	29	24		> 50
9	H	H	Me	H	H	O	–	> 4	> 4	34.5	11		> 50
10	Me	H	Me	H	H	O	O	6.0	6.5	12	16		50
11	Me	H	H	Me	H	O	O	1.8	5.8	10	32	39	≥ 50
12	Me	H	H	Me	H	O	–	7.2	≥ 20	28	48	> 50	> 50
14	Me	Me	H	H	Me	O	O	5.0	4.7	8.6	11		50
15	Me	Me	H	H	Me	O	–	> 0.8	> 0.8	11	1.2		50
16	H	Me	Me	H	H	O	–	2.5	2.8	20	14		≥ 50
17	Me	H	Me	H	Me	O	–	1.5	1.5	25	12		> 50
18	H	H	Et	H	H	O	O	5.5	5.0	10	16		50
19	H	H	i-Pr	H	H	O	O	3.4	2.6	5	9.4		20
20	i-Pr	H	H	i-Pr	H	O	O	≥ 4	≥ 4	3.2	6.4		20
21	i-Pr	H	H	i-Pr	H	O	–	≥ 4	≥ 4	4.1	11		20
22	H	H	*t*-Bu	H	H	O	O	≥ 4	2.6	3.2	6.8		20
23	H	H	*t*-Pent	H	H	O	O	3.1	3.4	1.5	5.6		20
24	H	H	MeO	H	H	O	O	≥ 20	14	20	31		50
25	H	H	MeO	H	H	O	–	> 0.8	> 0.8	4.4	2.1		35
26	MeO	H	H	MeO	H	O	O	10	35	> 50	60		> 50
27	MeO	H	H	MeO	H	O	–	> 100	> 100	> 50	≥ 100		> 50
28	H	MeO	MeO	H	H	O	O	≥ 20	15	> 50	41		> 50
29	H	MeO	MeO	H	H	O	–	> 0.8	> 0.8	10	1.3		> 50
30	H	MeO	MeO	MeO	H	O	O	≥ 20	≥ 20	> 50	51		> 50
31	H	MeO	MeO	MeO	H	O	–	> 20	> 20	> 50	40		> 50
32	MeO	H	H	Me	H	O	O	1.6	14	37	43		> 50
33	MeO	H	H	Me	H	–	–	8	≥ 20	> 50	31		> 50
34	EtO	H	H	H	H	O	O	9.5	16	> 20	37		50
35	EtO	H	H	H	H	O	–	> 20	> 4	> 50	69		> 50
36	H	F	H	H	H	O	O	6.5	7.5	> 20	19		50
37	H	H	F	H	H	O	O	11	7.0	> 20	24		50
38	H	H	F	H	H	O	–	≥ 20	–	> 50	87		> 50
39	Cl	H	H	H	H	O	O	6.0	7.0	5	19		50

Table 1. *(cont.)*

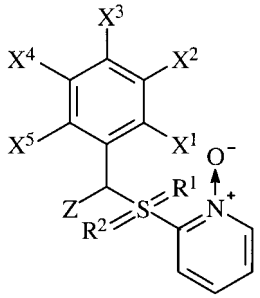
Compound No.	X^1	X^2	X^3	X^4	X^5	R^1	R^2	EC_{50} [a]) [μg/ml] HIV-1	HIV-2	CMV	IC_{50} [b]) [μg/ml] (CEM)	(HEL)	MCC[c]) [μg/ml] (HEL)
40	H	H	Cl	H	H	O	O	9.5	9.5	20	25		50
41	Cl	H	Cl	H	H	O	O	2.4	2.5	5	6.2		20
42	Cl	H	H	H	Cl	O	O	9.0	8.0	5	15		> 50
43	H	Cl	Cl	H	H	O	O	1.0	1.3	> 5	2.9		20
44	Cl	Cl	H	H	Cl	O	O	≥ 4	≥ 4	5	6.5		50
45	Cl	Cl	Cl	Cl	Cl	O	O	0.63	1.0	14	1.2		50
46	Cl	Cl	Me	Cl	Cl	O	O	≥ 0.8	≥ 0.8	5	2.0		20
47	Cl	H	NO_2	H	H	O	O	2.9	2.9	> 5	6.1		20
48	H	Br	H	H	H	O	O	2.5	≥ 4	5	7.4		20
49	Br	H	H	MeO	H	O	O	4.0	9.0	10	15		50
50	i-Pr	H	Br	i-Pr	H	O	O	2.3	2.5	3.7	4.4		> 50
51	I	H	H	H	H	O	O	5.3	3.4	> 20	25		50
52	NO_2	H	H	H	H	O	O	8.0	≥ 4	> 5	13		20
53	H	H	NO_2	H	H	O	O	> 4	> 4	7	7.6		50
54	H	NO_2	H	NO_2	H	O	O	2.8	3.5	5	4.4		20
55	H	NO_2	Me	H	H	O	O	2.3	2.4	> 50	5.8		20
56	H	Me	NO_2	H	H	O	O	3.1	2.5	5	4.9		20
57	Me	H	H	NO_2	H	O	O	73	100	> 50	57		> 50
58	MeO	H	H	NO_2	H	O	O	7.0	13	13	17		50
59	H	NO_2	Cl	H	H	O	O	1.5	1.9	> 5	4.3		20
60	CN	H	H	H	H	O	O	7.0	9.5	> 5	15		20
61	CN	H	H	H	H	O	–	15	11	35	≥ 100	> 20	> 50
62	H	H	CN	H	H	O	O	9.0	10	> 20	12		50
63	H	H	COOH	H	H	O	O	> 100	≥ 100	> 50	> 100		> 50
64	H	H	Ph	H	H	O	O	≥ 4	4.0	5	4.6		20
65	PhO	H	H	H	H	O	O	5.3	4.7	> 5	10		20
66	H	MeO	BzO	H	H	O	O	> 4	> 4	14	8.0		50
67	H	CF_3	H	H	H	O	–	13	17	> 50	54		> 50
68	OH	H	H	NO_2	H	–	–	10	> 20	> 50	37	> 20	> 50

[a]) EC_{50}, or 50% effective concentration required to inhibit HIV-induced giant-cell formation or CMV replication in CEM or HEL cell cultures, respectively.
[b]) CC_{50}, or 50% cytostatic concentration required to inhibit CEM or HEL cell proliferation by 50%.
[c]) *MCC*, or minimal cytostatic concentration required to cause a microscopically visible alteration of HEL cell morphology.

above contained also a variety of substitutents at the phenyl moiety. Introduction of a CM (*i.e.*, **99**–**102**), NH_2CO (*i.e.*, **103**–**105**), or MeOCO group (*i.e.*, **106**) on the methylene also did not result in active compounds. Instead, the MeOCO derivative **110** resulted in a pronounced antiviral activity against HIV-1 (EC_{50} 2.2 μg/ml) and HIV-2 (EC_{50} 6.7 μg/ml) (*Table 2*).

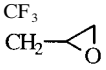
3.2.2. *Activity against CMV.* Alkyl substitution in the Z part of the pyridine oxide derivative molecule resulted in several highly active anti-CMV compounds with EC_{50} values lower than 1 μg/ml (*i.e.*, **83**, **90**, **91**, and **92**). The Z part could harbor small (Me) but also rather bulky (undecyl) alkyl groups. In virtually all these cases, a 2,5-dimethyl-substituted phenyl was present in the molecule. It should be emphasized that the antiviral selectivity for the most active compounds is not higher than 10, taking the

Table 2. *Antiviral Activity of Pyridine Oxide Derivatives Modified in the Z Part of the Molecule*



Compound No.	Z	X^1	X^2	X^3	X^4	X^5	R^1	R^2	EC_{50} [a]) [μg/ml] HIV-1	HIV-2	CMV	IC_{50} [b]) [μg/ml] (CEM)	(HEL)	*MCC* [c]) [μg/ml] (HEL)
69	Me	H	H	H	H	H	O	–	> 0.8	> 0.8	4.7	1.4		35
70	Me	H	H	Me	H	H	O	–	> 0.032	> 0.032	3	0.59	0.035	50
71	Me	Me	H	H	Me	H	O	O	> 100	> 100	> 50	> 100	50	> 50
72	Me	Me	H	H	Me	H	O	–	> 0.8	> 0.8	4.7	1.6		35
73	Me	Me	H	H	Me	H	–	–	6	> 20	> 20	74	> 20	50
74	Me	Me	Me	Me	Me	H	O	–	> 0.16	> 0.8	1.3	0.45	0.1	20
75	Me	H	H	F	H	H	O	–	> 0.16	> 0.16	2.3	0.33	0.036	20
76	Me	H	H	Cl	H	H	O	–	> 0.16	> 0.8	4.2	0.64	0.11	50
77	Me	Cl	Me	Cl	H	H	O	–	> 0.16	> 0.8	0.24	0.59	0.09	2
78	Me	Cl	H	Me	H	H	O	–	> 0.16	> 0.8	12	0.28	0.03	20
79	Me	Cl	H	H	Me	H	O	–	> 0.16	> 0.16	2	0.19	0.067	20
80	Me	H	H	$MeSO_2$	H	H	O	–	> 0.8	> 4	10	3.8	0.45	> 50
81	Me	Me	H	H	NH_2	H	O	O	20	41	> 50	≥ 100	> 50	> 50
82	Et	H	H	H	H	H	O	O	35	17	5.8	61	> 20	35
83	Et	Me	H	H	Me	H	O	O	> 20	> 20	0.4	80	> 20	3.5
84	Pr	H	H	H	H	H	O	O	> 20	13.5	3.2	43	> 20	35
85	Pr	H	H	H	H	H	–	–	> 20	> 20	11	62	> 20	> 50
86	Pr	Me	H	H	Me	H	–	–	> 4	> 4	12	9.2	1.3	50
87	Heptyl	H	Me	Me	Me	H	–	–	> 20	> 20	2	20	5	11.9
88	Heptyl	Me	H	H	Me	H	O	O	> 0.8	> 0.8	0.7	3.4	> 50	≥ 2
89	Heptyl	Me	H	H	Me	H	–	–	> 4	> 20	1.6	26	7.2	13
90	Undecyl	Me	H	H	Me	H	O	O	> 20	> 20	0.56	88	> 20	3.5
91	Undecyl	Me	H	H	Me	H	–	–	> 4	> 4	0.75	25	> 20	5
92	i-Bu	Me	H	H	Me	H	O	O	> 4	> 4	0.27	6.2	1.2	2
93	$CH_2=CHCH$	Me	H	H	Me	H	O	O	10	≥ 20		38	> 50	2
94	Ph	Me	H	H	H	H	O	O	> 20	> 20	11.5	47	> 50	> 50
95	Ph	Me	H	H	Me	H	O	O	> 20	> 20	20	43	> 20	50
96	Ph	Me	H	H	Me	H	–	–	≥ 20	> 20	> 5	34	> 20	20
97	$PhCH_2$	Me	H	H	Me	H	O	O	> 4	> 4	6.7	30	19	> 50
98	$PhCH_2$	Me	H	H	Me	H	–	–	> 0.8	> 4	10	2.6	1.6	50
99	CN	Me	H	H	H	H	O	–	> 0.16	> 0.8	6.3	0.67	0.12	50
100	CN	H	Me	H	H	H	O	–	> 0.16	> 0.8	2.8	0.74	< 0.2	> 50
101	CN	Me	H	H	Me	H	–	–	> 4	> 4	12	34	2	50
102	CN	H	H	F	H	H	O	–	> 0.16	> 0.8	5	0.72	0.28	> 50
103	NH_2CO	Me	H	H	H	H	O	–	> 100	> 100	> 50	> 100	> 20	> 50
104	NH_2CO	Me	H	H	H	H	–	–	> 100	> 100	> 50	> 100	> 50	> 50
105	NH_2CO	Me	H	H	Me	H	O	O	> 20	> 20	10	≥ 100	15.6	> 50
106	$HOOCCH_2$	Me	H	H	Me	H	O	O	> 20	> 20	24	87	> 50	> 50

Table 2. *(cont.)*

Compound No.	Z	X^1	X^2	X^3	X^4	X^5	R^1	R^2	EC_{50} [a]) [μg/ml] HIV-1	HIV-2	CMV	IC_{50} [b]) [μg/ml] (CEM)	(HEL)	*MCC* [c]) [μg/ml] (HEL)
106	$HOOCCH_2$	Me	H	H	Me	H	O	O	> 20	> 20	24	87	> 50	> 50
107	6-Br-hexyl	Me	H	H	Me	H	O	O	> 0.8	> 0.8	> 20	6.7	7.4	50
108	Br	Me	H	H	Me	H	O	O	20.7	85	> 50	81	16	> 50
109	MeOCO	Me	H	H	H	H	O	–	> 20	> 20	32	52	4.6	> 50
110	MeOCO	Me	H	H	Me	H	O	O	2.2	6.7	> 5	36	27	20
111	MeOCO	H	PhO	H	H	H	O	O	> 4	> 4	10	26	9.6	50
112	CF_3	Me	H	H	Me	H	O	O	12	> 20	> 20	41	> 20	50
113		Me	H	H	Me	H	O	O	16	> 20	10	24	14.5	50
114	Me, Cl	H	H	H	H	H	O	O	> 4	> 20	50	10	> 20	> 50
115	Me, Cl	Me	H	H	Me	H	O	O	> 4	> 4	20	23	> 20	≥ 50

[a]) EC_{50}, or 50% effective concentration required to inhibit HIV-induced giant-cell formation or CMV replication in CEM or HEL cell cultures, respectively.
[b]) CC_{50}, or 50% cytostatic concentration required to inhibit CEM or HEL cell proliferation by 50%.
[c]) *MCC*, or minimal cytostatic concentration required to cause a microscopically visible alteration of HEL cell morphology.

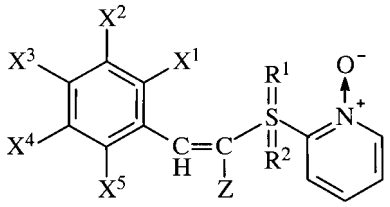
MCC into account. The pyridine oxide derivative **100** (the CH_2OH derivative) was slightly less inhibitory against CMV (2.8 μg/ml) but represented the most selective anti-CMV compound ($SI > 18$; *Table 2*).

3.3. *Antiviral Activity of Pyridine Oxide Derivatives Containing an Ethylene Linker between the Thioether and the Phenyl Ring of the Molecules.* 3.3.1. *Activity against HIV.* The pyridine oxide derivatives with an CH_2CH_2 bridge between the thioether and the phenyl moiety, and containing a CN group in the Z part of the molecule were not inhibitory against HIV at subtoxic concentrations, except for compound **122**, which showed a measurable anti-HIV activity (*Table 3*), but at compound concentrations that were only 2- to 3-fold lower than the cytostatic concentration. It was remarkable to note that an isopropyl bridge-containing compound with unsubstituted Ph proved exquisitely cytostatic (IC_{50} 0.22 μg/ml) but was also not antivirally active (data not shown).

3.3.2. *Activity against CMV.* The SO_2-containing pyridine oxide derivatives were not active against CMV. The other compounds (*i.e.*, **116**–**118**, **120**, **122**, and **123**) inhibited CMV-induced cytopathicity at concentrations well below the MCC. The i-Pr bridge-containing compound was also active against CMV (2.7 μg/ml) but – as found for CEM cell proliferation – proved also exquisitely cytostatic to HEL cell proliferation (data not shown).

3.4. *Antiviral Activity of Pyridine Oxide Derivatives Concomitantly Modified in the Pyridine Oxide Ring, the Phenyl Ring and/or the Z Part of the Molecule.* 3.4.1. *Activity against HIV.* Pyridine oxide derivatives, which contain an unsubstituted or substituted phenyl and/or Z part, and in which several substituents were also present in the pyridine oxide moiety of the molecule, were also considered. Introduction of one Me group in the pyridine oxide moiety resulted on several occasions in a markedly increased antiviral activity, particularly against HIV-1, while decreasing inhibitory activity against HIV-2. In particular, compounds **131** (EC_{50} 0.05 μg/ml), **129** (EC_{50} 0.42 μg/ml), and **128** (EC_{50} 0.75 μg/ml) are examples. In all cases, these compounds

Table 3. *Antiviral Activity of Pyridine Oxide Derivatives Containing an Ethylene Bridge between the Thioether and the Phenyl Moiety*



Compound No.	X^1	X^2	X^3	X^4	X^5	Z	R^1	R^2	EC_{50} [a]) [μg/ml] HIV-1	HIV-2	CMV	IC_{50} [b]) [μg/ml] (CEM)	(HEL)	*MCC* [c]) (μg/ml) (HEL)
116	H	H	H	H	H	CN	–	–	>4	>4	3.6	8.7	–	20
117	H	H	MeO	H	H	CN	–	–	>20	>20	10	35	–	≥50
118	H	MeO	MeO	MeO	H	CN	–	–	>20	>20	7.0	3.3	–	≥50
119	Cl	H	Cl	H	H	CN	O	O	>4	>4	>5	8.1	7.1	20
120	Cl	H	Cl	H	H	CN	–	–	>0.8	>0.8	1.2	5.9	3.5	50
121	Cl	H	H	H	Cl	CN	O	O	>4	>4	>20	11	3.4	50
122	Cl	H	H	H	Cl	CN	–	–	12	≥20	6.6	30	17	50
123	H	H	Octyloxy	H	H	CN	–	–	>20	>20	1.9	≥100	35	28

[a]) EC_{50}, or 50% effective concentration required to inhibit HIV-induced giant-cell formation or CMV replication in CEM or HEL cell cultures, respectively.
[b]) CC_{50}, or 50% cytostatic concentration required to inhibit CEM or HEL cell proliferation by 50%.
[c]) *MCC*, or minimal cytostatic concentration required to cause a microscopically visible alteration of HEL cell morphology.

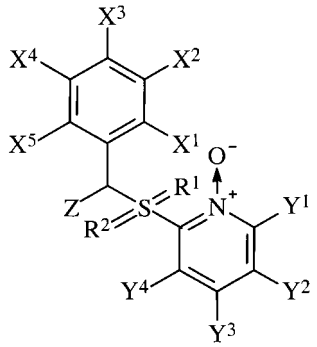
fully lost anti-HIV-2 activity, and should be considered highly specific non-nucleoside inhibitors of HIV-1 that resemble the well-known NNRTIs in terms of HIV specificity. Also, the cytotoxicity associated with several of the pyridine oxide derivatives is often decreased in these cases. This results in selectivity indices of compounds that may amount up to 300–750 (**128**, **129**, and **131**). The location of the Me group on the pyridine moiety seems less important to afford anti-HIV-1 potency and specificity, although it should be recognized that potency increases when the Me group is located in following order: $Y^2 < Y^1 < Y^3 < Y^4$.

Also, introduction of Cl substituents in the pyridine oxide part of the molecule tend to shift the anti-HIV-1/HIV-2 sensitivity spectrum of the compounds to a NNRTI spectrum (*i.e.*, **164** (EC_{50} 1.5 μg/ml) and **166** (EC_{50} 0.14 μg/ml)) although not always being the case (*i.e.*, **160**, **159**, **155**, and **157**). Also, in a number of cases, Cl substitutents (in particular in the Y^4 position) resulted in marked cytotoxicity of the test compounds (*i.e.*, **162**, **163**, **167**, and **164**) (*Table 4*).

3.4.2. *Activity against CMV.* The test compounds showed in many cases pronounced anti-CMV activity (*i.e.*, **131** (EC_{50} 4.1 μg/ml), **136** (EC_{50} 0.83 μg/ml), **164** (EC_{50} 4.7 μg/ml)), but, as found for the anti-CMV compounds described above, the antiviral selectivity did not exceed 10–15 (*Table 4*).

3.5. *Antiviral Activity of Reduced Pyridine Oxide Derivatives.* 3.5.1. *Activity against HIV.* Twenty-three compounds that lacked the O at the N-atom of the pyridine moiety

Table 4. *Antiviral Activity of Pyridine Oxide Derivatives Modified in the Pyridine Oxide and Phenyl and Z Part of the Molecule*



Compound No.	X1	X2	X3	X4	X5	Z	R1	R2	Y1	Y2	Y3	Y4	EC_{50} a) [μg/ml] HIV-1	HIV-2	CMV	IC_{50} b) [μg/ml] (CEM)	(HEL)	*MCC* c) [μg/ml] (HEL)
124	Me	H	H	Me	H	H	O	O	Me	H	H	H	4.0	> 100	> 50	≥ 100		> 50
125	Me	H	H	Me	H	Cl	O	O	Me	H	H	H	2.8	> 100	> 20	≥ 100		50
126	Me	H	H	Me	H	Me	O	O	H	Me	H	H	5.5	> 100	50	≥ 100		> 50
127	H	H	Cl	H	H	H	O	O	H	H	Me	H	> 14	11	> 5	20		20
128	Me	H	H	Me	H	Me	O	O	H	H	Me	H	0.75	≥ 20	38	25		> 50
129	Me	H	H	Me	H	Cl	O	O	H	H	Me	H	0.42	> 100	34.5	> 100		> 50
130	H	H	H	H	H	H	O	O	H	H	H	Me	15	> 20	> 20	28		50
131	Me	H	H	Me	H	Me	O	O	H	H	H	Me	0.05	> 20	4.1	38		35
132	Me	H	H	Me	H	H	O	–	H	H	H	Me	12	≥ 20	11	42		50
133	Me	H	H	H	H	H	O	O	H	H	H	Me	9.3	≥ 20	> 5	17		≥ 20
134	Me	H	Me	H	H	H	O	O	H	H	H	Me	6	≥ 20	12	11		50
135	Me	H	Me	H	H	Me	O	O	H	H	H	Me	1.4	≥ 100	10	≥ 100		50
136	Me	H	H	Me	H	Et	O	O	H	H	H	Me	1.4	≥ 20	0.83	20		13
137	Me	H	H	Me	H	Cl	O	O	H	H	H	Me	> 100	> 100	> 50	> 100		> 50
138	Cl	H	H	H	Cl	H	O	O	H	H	H	Me	> 20	> 20	43	70		> 50
139	Cl	H	H	H	Cl	H	O	–	H	H	H	Me	> 100	> 100	> 50	4 → 100		> 50
140	Cl	H	H	H	Cl	H	–	–	H	H	H	Me	> 20	> 20	28	31		> 50
141	H	H	H	H	H	Me	–	–	H	H	H	Me	> 20	> 20	> 50	60		> 50
142	Cl	H	H	H	H	H	O	O	H	H	H	Me	3.4	> 4	12	21		50
143	Me	NO_2	H	H	H	H	O	O	H	H	H	Me	≥ 4	> 4	> 5	9.6		20
144	Me	H	Me	H	H	Me	O	O	H	H	H	Me	2.4	> 20	12	45		> 50
145	Cl	H	H	H	H	Me	O	O	H	H	H	Me	3.3	> 20	12.5	38		> 50
146	Me	NO_2	H	H	H	Me	O	O	H	H	H	Me	11	60	> 50	96		> 50
147	Me	H	H	Me	H	H	O	O	H	H	H	MeO	0.70	3.3	> 5	8.0		20
148	Me	H	H	Me	H	H	O	–	H	H	H	MeO	> 20	> 20	11	62	1.6	> 50
149	Me	H	H	Me	H	H	–	–	H	H	H	MeO	1.4	> 20	8	70	2.0	> 50
150	Me	H	H	Me	H	H	O	O	H	H	H	OH	45	> 100	> 50	> 100	> 20	> 50
151	Me	H	H	Me	H	H	–	–	H	H	H	OH	> 20	> 20	> 5	65	7.7	20
152	Me	H	H	Me	H	Me	O	O	Me	H	Me	H	≥ 20	> 20	14	> 100		≥ 50
153	H	H	MeO	H	H	H	O	O	H	H	*t*-Bu	H	> 20	> 20	12.5	38		> 50
154	H	H	MeO	H	H	H	–	–	H	H	*t*-Bu	H	> 20	> 100	50	48		> 50
155	H	H	H	H	H	H	–	–	Cl	H	H	H	9.0	16	20	48		≥ 50
156	Me	H	H	Me	H	H	O	O	Cl	H	H	H	0.7	> 0.8	11	4.0		50
157	Me	H	H	Me	H	Me	O	O	Cl	H	H	H	0.9	2.4	9.1	4.5		50
158	H	H	H	H	H	H	–	–	H	Cl	H	H	> 20	> 20	> 20	66		50
159	Me	H	H	Me	H	H	O	O	H	Cl	H	H	1.9	2.6	12	4.9		50

Table 4. *(cont.)*

Compound No.	X^1	X^2	X^3	X^4	X^5	Z	R^1	R^2	Y^1	Y^2	Y^3	Y^4	EC_{50} [a]) [μg/ml] HIV-1	HIV-2	CMV	IC_{50} [b]) [μg/ml] (CEM)	(HEL)	*MCC* [c]) [μg/ml] (HEL)
160	Me	H	H	Me	H	H	O	–	H	Cl	H	H	2.1	3.0	11.5	14		> 50
161	Me	H	H	Me	H	H	–	–	H	Cl	H	H	> 100	> 100	> 50	> 100		> 50
162	H	H	H	H	H	Cl	O	O	H	Cl	H	H	> 0.8	> 0.8	> 20	2.3		50
163	H	H	H	H	H	H	O	O	H	H	H	Cl	> 0.8	> 0.8	> 5	1.5		20
164	H	H	H	H	H	H	O	–	H	H	H	Cl	1.5	> 4	4.7	3.7		20
165	H	H	H	H	H	H	–	–	H	H	H	Cl	2.4	> 20	> 50	> 100		> 50
166	Me	H	H	Me	H	H	–	–	H	H	H	Cl	0.14	> 20	25	42		> 500
167	Me	H	H	Me	H	Me	O	O	H	H	H	Cl	> 0.8	> 0.8	> 5	1.7		20
168	Me	H	H	Me	H	Cl	O	O	H	H	H	Cl	> 4	> 4	31.5	15		> 50
169	H	H	H	H	H	H	–	–	H	H	H	NO_2	2.3	≥ 20	40	31		> 50

[a]) EC_{50}, or 50% effective concentration required to inhibit HIV-induced giant-cell formation or CMV replication in CEM or HEL cell cultures, respectively.
[b]) CC_{50}, or 50% cytostatic concentration required to inhibit CEM or HEL cell proliferation by 50%.
[c]) *MCC*, or minimal cytostatic concentration required to cause a microscopically visible alteration of HEL cell morphology.

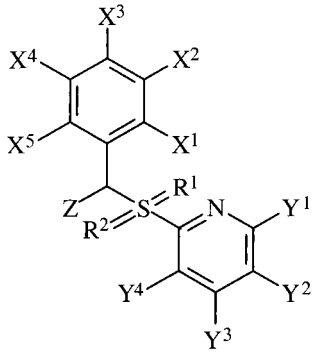
were evaluated for antiviral activity. Several reduced pyridine oxide derivatives were active against HIV. In many cases, selectivity for HIV-1 was observed (*i.e.*, **184**, **181**, and **190**), but, in a number of other cases, also anti-HIV-2 activity was found (*i.e.*, **173**, **175**, and **176**). The most antivirally selective compound was **184** (*SI:* 180; *Table 5*).

3.5.2. *Activity against CMV.* Many of the reduced pyridine oxide derivatives had no measurable anti-CMV activity. Only two compounds (**187** and **191**) showed pronounced anti-CMV activity (EC_{50} 1.2 and 0.31 μg/ml, resp.). However, the antiviral activity of these compounds was close to their toxicity threshold (*Table 5*).

Discussion. – Pyridine oxide derivatives represent an entirely new class of antiviral agents. They show specific activity against HIV and CMV. Several members were also evaluated against a panel of other DNA and RNA viruses (*i.e.*, herpes simplex virus type 1 and type 2, varicella-zoster virus, vaccinia virus, vesicular stomatitis virus, reovirus-1, polio virus, *Coxsackie* virus B4, *Semliki* forest virus, parainfluenza virus) and found inactive. Such antiviral activity spectrum is rather unusual.

Within the class of pyridine oxide derivatives, various subclasses can be considered. Several pyridine oxide derivatives show preference for HIV-1 and are not inhibitory against HIV-2, making the pyridine oxides members of a novel class of NNRTIs that are known to be highly specific for HIV-1 and targeted at HIV-1 RT (*i.e.*, nevirapine, delavirdine, and efavirenz). Indeed, *Stevens et al.* [13] recently reported that several of the HIV-1-specific pyridine oxide derivatives select for resistance mutations in HIV-1 RT that are characteristic for NNRTIs (*i.e.*, Tyr181Cys and Lys103Asn). They have also shown diminished antiviral activity against mutant HIV-1 strains that were selected in the presence of NNRTIs and contain NNRTI-characteristic mutations in the HIV-1 RT. The optimal structural requirements for NNRTI activity of the pyrimidine oxide derivatives are the presence of Me groups in preferably X^1 and X^3 positions of the phenyl ring, a SO_2 linker, a Me group in the Y^4 position of the pyridine oxide, and a Me

Table 5. *Antiviral Activity of Pyridine Derivatives*



Compound No.	X^1	X^2	X^3	X^4	X^5	Z	R^1	R^2	Y^1	Y^2	Y^3	Y^4	EC_{50} [a]) [μg/ml] HIV-1	HIV-2	CMV	IC_{50} [b]) [μg/ml] (CEM)	(HEL)	*MCC* [c]) [μg/ml] (HEL)
170	H	H	H	H	H	H	O	O	H	H	H	H	30	50	> 50	68	–	> 50
171	H	H	H	H	H	H	–	–	H	H	H	H	> 20	> 20	> 50	51	–	> 50
172	H	H	H	H	H	H	O	O	H	H	H	OH	60	> 100	> 50	> 100	> 20	> 50
173	H	H	H	H	H	H	–	–	H	H	H	OH	2.0	12	12	35	–	50
174	H	H	H	H	H	H	–	–	H	H	H	MeO	21	> 20	> 50	87	> 20	> 50
175	H	H	H	H	H	H	–	–	H	H	H	EtO	33	43	> 50	90	> 20	> 50
176	H	H	H	H	H	H	–	–	H	H	H	BuO	17	16	32	67	> 20	> 50
177	H	H	H	H	H	H	–	–	H	H	H	$PhCH_2O$	> 4	> 4	> 2	9.7	5.9	5
178	H	H	H	H	H	H	–	–	H	H	H	CN	12	≥ 20	20	33	> 20	50
179	H	H	H	H	H	H	–	–	H	NO_2	H	H	> 20	> 20	> 5	46	> 20	≥ 20
180	Me	H	H	Me	H	H	O	O	H	H	H	H	3.2	≥ 20	> 50	51	–	> 50
181	Me	H	H	Me	H	H	O	–	H	H	H	H	3.2	> 100	> 50	88	–	> 50
182	Me	H	H	Me	H	H	O	O	H	H	H	OH	24	100	> 50	> 100	> 20	> 50
183	Me	H	H	Me	H	H	O	–	H	H	H	OH	40	> 100	> 50	> 100	> 20	> 50
184	Me	H	H	Me	H	H	–	–	H	H	H	OH	0.22	> 4	> 5	39	16	20
185	Me	H	H	Me	H	H	–	–	H	H	H	MeO	1.7	40	> 50	78	> 20	> 50
186	Me	H	H	Me	H	Me	O	O	H	H	H	H	9.5	> 100	38	3.1	–	> 50
187	H	Me	Me	H	H	H	–	–	H	H	H	H	> 4	> 4	1.2	7.1	5.6	5
188	Me	H	H	Me	H	$HOCH_2$	O	O	H	H	H	H	> 100	> 100	> 50	≥ 100	> 20	> 50
189	Me	H	H	Me	H	$MeOCH_2$	O	O	H	H	H	H	60	> 100	> 50	≥ 100	–	> 50
190	Me	H	H	Me	H	Cl	O	O	H	H	H	H	0.9	> 4	20	38	–	50
191	H	H	Cl	H	H	H	–	–	H	H	H	H	≥ 1	≥ 0.8	0.31	1.2	0.35	1.3
192	H	H	Cl	H	H	H	–	–	H	H	Me_2N	CN	> 20	> 20	> 5	> 100	–	20

[a]) EC_{50}, or 50% effective concentration required to inhibit HIV-induced giant-cell formation or CMV replication in CEM or HEL cell cultures, respectively.
[b]) CC_{50}, or 50% cytostatic concentration required to inhibit CEM or HEL cell proliferation by 50%.
[c]) *MCC*, or minimal cytostatic concentration required to cause a microscopically visible alteration of HEL cell morphology.

substituent in the Z part of the molecule. It should be noted that the configuration on the methylene linker between the phenyl and the SO_2 has not yet been addressed. In case the pyridine oxide moiety is unsubstituted, poor, if any, NNRTI activity could be observed. Most remarkably, a number of selective anti-HIV-1 (NNRTI-like) com-

pounds show also concomitant activity against CMV (*i.e.*, **126**, **129**, **131**, **144**, **145**, and **166**) at drug concentrations that are well below the toxicity threshold. To the best of our knowledge, such dual antiviral activity has not been reported for any other well-known NNRTI. However, it should be kept in mind that the majority of NNRTIs may never have been investigated for their potential to exert concomitant anti-CMV activity. Therefore, our observations argue for revisiting these classes of NNRTIs to identify potential anti-CMV activity associated to these drugs.

Other pyridine oxide derivatives are also endowed with anti-HIV-2 activity, either at a 5- to 10-fold lower extent than their anti-HIV-1 activity or, in a number of cases, even to an equal extent. The mechanism of anti-HIV activity observed for this subclass of compounds is clearly different from the (HIV-1) RT inhibition and is currently under investigation in our laboratory.

As mentioned above, the observed antiviral activity spectrum (anti-CMV activity together with anti-HIV activity) found for the pyridine oxide derivatives is very unusual and led us to search for a correlation between anti-CMV activity and anti-HIV activity of the pyridine oxide derivatives. There was no correlation between anti-CMV activity and inhibition of HIV-1 replication in cell culture for those compounds that were HIV-1-specific (NNRTI-type compounds; *Fig. 2*,*a*; $r = -0.255$). Also, there was no correlation between the anti-CMV activity and the inhibitory activity against HIV-1 of those compounds that inhibited both HIV-1 and HIV-2 (*Fig. 2*,*b*; $r = -0.020$). In contrast, there seemed to be a significant but relatively weak correlation ($r = 0.556$) between anti-CMV and anti-HIV-2 activity of the pyridine oxide derivatives (*Fig. 2*,*c*). These observations may lead to the hypothesis that pyridine oxide derivatives – at least those that show activity against both HIV-1 and HIV-2 – may be targeted at a cellular event that may be in common between HIV and CMV and be required for efficient CMV and HIV replication in cell culture. Our findings that the compounds active against HIV-2 and also against CMV display a relatively low selectivity index, is not in disagreement with a potential cellular target for these compounds that is common between CMV and HIV. Whether a cellular target is actually involved in the eventual antiviral activity for these compounds is currently subject of in-depth investigation in our laboratory.

It should be recognized that the oxide part of the molecule may not always be necessary to ascertain anti-HIV-1 efficiency. Several reduced compounds indeed retain anti-HIV/CMV activity. The anti-HIV-1 activity of only one reduced pyridine oxide compound (**165**) could be compared with its corresponding oxide derivative (**1**) and found to be 10-fold less active an antiviral agent. Therefore, it would be interesting to synthesize the corresponding oxide derivatives of **187**, **185**, **180**, **181**, and **173** for evaluation of the antiviral activity against HIV and CMV, in an attempt to increase the antiviral activity of the pyridine oxide derivatives.

Several pyridine oxide derivatives showed exquisite inhibitory activity against HEL cell proliferation. Their IC_{50} values were markedly below the micromolar range and sometimes two orders of magnitude lower than the minimal cytotoxic concentration noticed for confluent HEL cell cultures (*i.e.*, **70**, **78**, and **80**). Also, the pyridine oxide derivatives most cytostatic against HEL cell proliferation were more than 10-fold less cytostatic to proliferating CEM cells. It cannot be excluded that such pyridine oxide derivatives may have intrinsic anti-cancer activity, and this issue should be further explored.

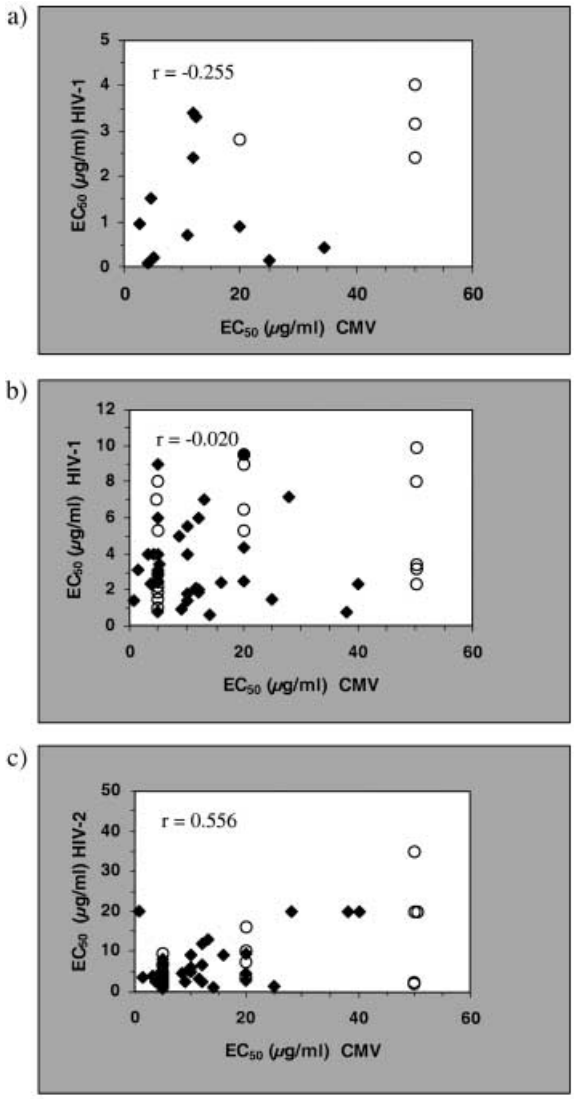


Fig. 2. *Correlation between anti-CMV activity in HEL cell cultures and anti-HIV activity in CEM cell cultures.* Closed symbols represent test compounds for which meaningful EC_{50} values for HIV and CMV could be determined. Open symbols represent test compounds for which no anti-CMV activity was noted at the indicated concentration on the *x*-axis. Higher compound concentrations could not be tested for anti-CMV activity due to toxicity of the test compounds. *a*) Correlation between the anti-CMV activity of pyridine oxide derivatives and the anti-HIV-1 activity of those compounds that display solely antiviral activity against HIV-1 but not HIV-2, and are endowed with an anti-HIV-1 activity at EC_{50} values equal to or lower than 10 μg/ml. *b*) Correlation between the anti-CMV activity of pyridine oxide derivatives and the anti-HIV-1 activity of those compounds that display antiviral activity against both HIV-1 and HIV-2 at EC_{50} values for HIV-1 equal to or lower than 10 μg/ml. *c*) Correlation between the anti-CMV activity of pyridine oxide derivatives and the anti-HIV-2 activity of compounds that displayed antiviral activity against HIV-2 at EC_{50} values equal to or lower than 50 μg/ml.

In conclusion, pyridine oxide derivatives represent a novel class of antiviral drugs with activity against HIV and CMV. Depending on the structure of the compounds, pyridine oxide derivatives may display antiviral selectivity for either HIV-1, or for HIV-1 and CMV, or for HIV-1 and HIV-2, or for HIV-1, HIV-2, and CMV. The molecular mechanism of antiviral action of the HIV-1-specific compounds is HIV-1 RT. The antiviral target for the pyridine oxide derivatives that, in addition to HIV-1, also inhibit HIV-2 and CMV, is currently unknown.

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REFERENCES

[1] J. Balzarini, *Int. Antiviral News* **2002**, *9*, 180.
[2] E. De Clercq, *J. Pharmacol. Exp. Ther.* **2001**, *297*, 1.
[3] J. H. Condra, J. P. Vacca, in 'The Human Immunodeficiency Virus: Biology, Immunology and Therapy', Ed. E. A. Emini, Princeton University Press, Princeton and Oxford, 2002, pp. 172–222.
[4] K. Brinkman, H. J. M. ter Hofstede, *AIDS Rev.* **1999**, *1*, 140.
[5] A. Carr, *AIDS Rev.* **1999**, *1*, 29.
[6] F. Blanco, A. Carr, *AIDS Rev.* **2001**, *3*, 98.
[7] E. Fontaine, K. Van Vaerenbergh, A.-M. Vandamme, J. C. Schmit, *AIDS Rev.* **1999**, *1*, 231.
[8] V. Miller, B. A. Larder, *Antiviral Ther.* **2001**, *6* (Suppl. 1), 25.
[9] J. Balzarini, *Biochem. Pharmacol.* **1999**, *58*, 1.
[10] R. W. Shafer, R. Kantor, M. J. Gonzales, *AIDS Rev.* **2000**, *2*, 211.
[11] E. De Clercq, *Nature Rev. Drug Discovery* **2002**, *1*, 13.
[12] E. De Clercq, *Curr. Med. Chem.* **2001**, *8*, 1543.
[13] M. Stevens, C. Pannecouque, E. De Clercq, J. Balzarini, Abstracts of the Fifteenth International Conference on Antiviral Research, Prague, Czech Republic, 17–21 March, 2002; *Antiviral Res.* **2002**, *54*, A42.

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