Journal of Antimicrobial Chemotherapy (2006) **57**, 472–481
doi:10.1093/jac/dki481
Advance Access publication 29 December 2005

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Pyridine *N*-oxide derivatives are inhibitory to the human SARS and feline infectious peritonitis coronavirus in cell culture

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Received 7 July 2005; returned 5 October 2005; revised 14 October 2005; accepted 12 December 2005

***Objectives*: Evaluation of a wide variety of pyridine *N*-oxide derivatives on their inhibitory activity against feline coronavirus (FIPV strain) and human SARS-CoV (Frankfurt strain-1) in cell culture.**

***Methods*: FIPV and SARS-CoV were exposed to confluent Crandel feline kidney (CRFK) and simian kidney (Vero) cell cultures in the presence of serial concentrations of the test compounds. The anti-cytopathic activity of the pyridine *N*-oxide derivatives was monitored by spectrophotometric analysis.**

***Results and conclusions*: A wide variety of pyridine *N*-oxide derivatives have been found to be inhibitory against feline coronavirus (FIPV strain) and human SARS-CoV (Frankfurt strain-1) in CRFK and simian kidney (Vero) cell cultures, respectively. The oxide part on the pyridine moiety proved indispensable for anti-coronavirus activity. The potency and virus specificity of the pyridine *N*-oxide derivatives varied depending the nature and specific location of substituents (i.e. alkyl, halogeno, nitro, etc.) on the different parts of the molecule. The most selective compounds were active in the higher microgram per litre range, being non-toxic at 50–100 mg/L. There was a poor structure-antiviral activity relationship (SAR) for the pyridine *N*-oxide derivatives against Fe-CoV and SARS-CoV. One of the most active and selective compounds was shown to inhibit Fe-CoV replication at the transcriptional level.**

Keywords: severe acute respiratory syndrome, feline coronavirus, FIPV

Introduction

The discovery of a novel human coronavirus (H-CoV) as the cause of the newly recognized severe acute respiratory syndrome (SARS)[1–4] provides a new challenge to the medical community to keep control on this disease. Although human coronaviruses cause up to 30% of colds, they rarely cause a lower respiratory tract disease, and never have a devastating effect as seen with the SARS-CoV.[5] In contrast, animal coronaviruses are known to cause devastating epizootics of respiratory or enteric diseases in livestock and poultry.[5] In fact, coronaviruses have been isolated from avian, porcine, feline, murine, bovine and canine species.[5] All known coronaviruses are categorized in three serologically well-defined and unrelated groups. The SARS coronavirus is clearly new to the human population and its RNA genome differs substantially from sequences of all known coronaviruses.[6,7] The natural host of the SARS-CoV is most likely a Civet cat, whose virus had acquired the ability to infect humans. Interestingly, SARS-CoV can be readily isolated and grown in monkey kidney Vero cell cultures.[8]

Vaccines are available for some animal coronaviruses. However, although vaccination with live, attenuated virus is effective against porcine epidemic diarrhoea virus and avian infectious bronchitis virus, recombination of the genome of vaccine strains with wild-type coronavirus is a potential risk when applied in humans.[5] Moreover, some vaccines against feline coronaviruses have been proven to enhance disease when vaccinated animals were exposed to wild-type virus, and thus antibody enhancement of disease is a potential risk of SARS-CoV vaccines in humans.[5] Therefore, it is prudent to develop safe and effective drugs against SARS-CoV as quickly as possible in case a novel wide-spread outbreak would occur. The development of effective drugs against SARS-CoV may also provide new strategies for the prevention or treatment of other coronavirus diseases in animals or humans. Indeed, there are no approved drugs with proven efficacy against coronaviruses. Ribavirin has been given to SARS patients but its efficacy is unclear. Several other compounds have been recently mentioned as potential drug leads against SARS-CoV.[9–15]

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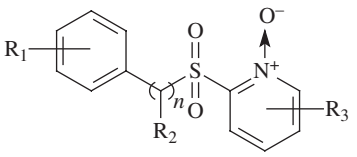


Figure 1. Basic structure of the pyridine *N*-oxide derivatives. The '*n*' annotation indicates that the carbon that is linked to the aryl, R_2 and pyridinesulphone moieties can be extended to a longer alkyl chain.

In this study, we evaluated a variety of 192 compounds that all belonged to the class of the pyridine *N*-oxide derivatives (Figure 1) against both SARS-CoV and the type II strain of feline infectious peritonitis virus (FIPV). FIPV virus causes a severe disease in cats characterized by vasculitis and disseminated pyogranulomatous lesions in various tissues and organs. The pyridine *N*-oxide compounds have previously been demonstrated to be inhibitory against the human immunodeficiency virus (HIV) in cell culture.[16–19] Several of the pyridine *N*-oxide derivatives have been demonstrated to act at a post-integrational event in the replication cycle of HIV, i.e. HIV gene expression.[18] Prolonged exposure of one of the prototype compounds (JPL-32) to DBA/2 and SCID mice demonstrated lack of acute toxicity. Moreover, a preliminary efficacy experiment showed protective activity against HIV-induced destruction of CD4-positive human T-lymphocytes in SCID mice.[19]

Our aim in the present study was to reveal whether pyridine *N*-oxide derivatives are endowed with inhibitory activity against SARS-CoV and FIPV, and whether the antiviral potencies of the pyridine *N*-oxide derivatives previously reported for HIV could be correlated with their antiviral activity against SARS-CoV and FIPV.

Materials and methods

Compounds

The structures of the compounds have been reported previously.[16]

Cell culture and viruses

The SARS-CoV (Frankfurt-1 strain) was kindly provided by Prof. Dr H. F. Rabenau (Johann Wolfgang Goethe University, Frankfurt, Germany). Vero E6 cells were propagated in minimal essential medium (MEM; Gibco Life Technologies, Rockville, MD, USA) supplemented with 10% fetal calf serum (FCS; Integro, Zaandam, The Netherlands), 2 mM L-glutamine (Gibco) and 1.4% sodium bicarbonate (Gibco Life Technologies, Rockville, MD, USA). Virus-infected cells were maintained at 37°C in a 5% CO_2 atmosphere in MEM supplemented with 2% FCS. The Fe-CoV (FIPV strain 1146) was originally isolated by McKeirnan *et al.*[20] and propagated from Crandel feline kidney (CRFK) cells maintained in RPMI-1640 medium (Gibco) and supplemented with 10% fetal bovine serum (Harlan Sera-Lab Ltd, Loughborough, UK), 2 mM L-glutamine (Gibco) and 0.075% sodium bicarbonate (Gibco). Virus-infected cells were maintained at 37°C in RPMI-1640 medium supplemented with 2% FCS.

Antiviral and cytostatic activity assays

Antiviral activity and cytotoxicity measurements were based on the viability of Vero cells that had been infected or mock-infected with 100 $CCID_{50}$ (50% cell culture infective dose) of the SARS-CoV or CRFK cells infected or mock-infected with 100 $CCID_{50}$ FIPV in the presence of various concentrations of the test compounds. The virus–drug mixture was not removed after the adsorption phase of the virus infection. The compounds were present throughout the whole time period of the experiment. Three days (SARS-CoV) or four days (Fe-CoV) after the infection, the number of viable cells was quantified by a tetrazolium-based colorimetric method as previously described for HIV by Pauwels *et al.*[21] The medium was aspirated and replaced by a solution of MTT [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazonium]/PMS (phenazinemethosulphate) according to the manufacturer's instructions (Promega Corporation, Madison, WI, USA). The results are given as the mean (±SD) of at least 2–3 independent experiments. The cytotoxic concentration was determined as the concentration of the compound that reduced cell viability by 50% (CC_{50} or 50% cytotoxic concentration), and the antivirally effective concentration was determined as the compound concentration that prevented the viral cytopathic effect by 50% of the control value (EC_{50} or 50% effective concentration). Thus, both the CC_{50} and the EC_{50} values were calculated from the absorbance values, and the EC_{50}s were obtained after deduction of the absorbance value of the control cultures from the absorbance value of the virus-infected cultures.

Time-of-addition experiments

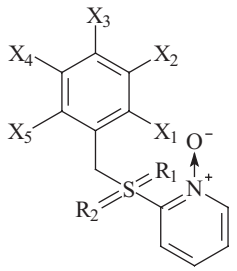
To reveal at what stage in the infection cycle the compounds are inhibitory, a time-of-addition (TOA) experiment was carried out in which CRFK cell cultures were infected with a high dose (≥1000 $CCID_{50}$) of FIPV. The pyridine *N*-oxide **45** was used at 50 mg/L. At different time points post-infection (i.e. 0, 1, 2.5, 5, 7, 14, 25, 34, 49 and 72 h), the drug was added to the infected cell cultures. After 72 h, full cytopathic activity was noticed for the control cultures. Optical reading of the MTT-exposed cell cultures was then performed to quantify the protective effect of the test compounds added at the different time points against virus-induced cytopathicity.

Results

Antiviral activity of pyridine N-oxide derivatives with modifications in the phenyl ring

A variety of substituted pyridine *N*-oxide derivatives have been evaluated in which a sulphone (SO_2), a sulphoxide (SO) or a thio ether (S) links the unsubstituted pyridine *N*-oxide group through a CH_2 to the phenyl moiety (Table 1). The anti-coronavirus activities ranged between 0.29 and >100 mg/L for FIPV and between 4.2 and >100 mg/L for SARS-CoV depending on the nature and the locations of the substituents in the phenyl moiety. As a rule, when antiviral activity was recorded, the anti-FIPV activity was always more pronounced than the anti-SARS-CoV activity. Compounds that were weakly inhibitory against FIPV did not show detectable antiviral activity against SARS-CoV. Generally, sulphide and sulphoxide derivatives showed poor, if any, antiviral activity against both coronaviruses except for **27** and **35** that were inhibitory to Fe-CoV (EC_{50}: <10 mg/L), but not to SARS-CoV (EC_{50}: >100 mg/L). The position of one or more alkyl or alkoxy groups on the phenyl moiety does not play a marked role in the eventual anti-coronavirus potency of the test compounds (compare compounds **4–35**). This was generally also observed for the halogen-, nitro- and cyano-substituted compounds. The most potent antiviral activity was noted for the trichloro (**44**), pentachloro (**45**), methyl/tetrachloro (**46**) and nitro (**52**) derivatives

Table 1. Antiviral activity of pyridine *N*-oxide derivatives modified in the phenyl moiety



								EC_{50}[a] (mg/L)			IC_{50}[b] (mg/L)	CC_{50}[c] (mg/L)	
Code	X_1	X_2	X_3	X_4	X_5	R_1	R_2	HIV-1	FIPV	SARS-CoV	CEM	CRFK	Vero
1	H	H	H	H	H	O	O	4.4 ± 3.1	16 ± 9	32 ± 1	36 ± 2	>100	>100
2	H	H	H	H	H	O	–	3.4 ± 3.2	–	–	≥20	–	–
3	H	H	H	H	H	–	–	≥100	>100	>100	>100	>100	>100
4	Me[d]	H	H	H	H	O	O	11 ± 4	6.6 ± 0.0	42 ± 12	23 ± 0.4	>100	76 ± 6
5	Me	H	H	H	H	O	–	≥20	13 ± 11	>100	52 ± 19	>100	>100
6	H	Me	H	H	H	O	O	2.4 ± 0.2	1.8 ± 0.6	65 ± 27	23 ± 1.6	82 ± 5	78 ± 16
7	H	Me	H	H	H	O	–	>20	>4	>50	37 ± 0.1	12 ± 0	50 ± 3
8	H	H	Me	H	H	O	O	13 ± 6	3.4 ± 0.8	36 ± 1	24 ± 1.5	>100	>100
9	H	H	Me	H	H	O	–	>4	>4	>50	11 ± 4	12 ± 0	52 ± 4
10	Me	H	Me	H	H	O	O	6.0 ± 2.8	3.9 ± 1.1	30 ± 7	16 ± 2	>100	>100
11	Me	H	H	Me	H	O	O	1.8 ± 0.9	5.8 ± 6.1	55 ± 23	32 ± 4	≥100	84 ± 11
12	Me	H	H	Me	H	O	–	7.2 ± 5.3	11 ± 7	>50	48 ± 5	60 ± 0	62 ± 16
14	Me	Me	H	H	Me	O	O	5.0 ± 1.7	2.4 ± 1.8	>70	11 ± 1	77	80 ± 8
15	Me	Me	H	H	Me	O	–	>0.8	>0.8	>5	1.4 ± 0.5	1.2 ± 1.0	13 ± 9
16	H	Me	Me	H	H	O	–	2.5 ± 0.4	>4	>50	14 ± 8	12 ± 1	59 ± 4
17	Me	H	Me	H	Me	O	–	1.5 ± 0.0	>20	46 ± 5	12 ± 4	66 ± 7	>100
18	H	H	Et	H	H	O	O	5.5 ± 2.1	3.7 ± 1.7	38 ± 16	16 ± 3	>100	>100
19	H	H	iProp	H	H	O	O	3.4 ± 0.9	4.3 ± 1.0	55 ± 33	9.4 ± 0.2	>100	>100
20	iProp	H	H	iProp	H	O	O	≥4	1.3 ± 0.4	>30	6.4 ± 0.1	60	34 ± 1
21	iProp	H	H	iProp	H	O	–	≥4	>4	>30	10 ± 4	12 ± 0	37 ± 2
22	H	H	t-But	H	H	O	O	≥4	2.2 ± 0.4	49 ± 9	6.8 ± 0.4	78	>100
23	H	H	t-Pent	H	H	O	O	3.1 ± 1.3	2.5 ± 0.9	>40	5.6 ± 0.2	39 ± 11	46 ± 3
24	H	H	OMe	H	H	O	O	≥20	12 ± 3	51 ± 19	31 ± 2.4	>100	>100
25	H	H	OMe	H	H	O	–	>0.8	>0.8	4.2 ± 3.2	2.1 ± 0.5	2.2 ± 1.8	11 ± 9
26	OMe	H	H	OMe	H	O	O	10 ± 3	2.5 ± 2.0	>100	60 ± 0.3	>100	>100
27	OMe	H	H	OMe	H	O	–	>100	9.8 ± 4.6	>100	≥100	>100	>100
28	H	OMe	OMe	H	H	O	O	≥20	3.8 ± 16	>100	41 ± 4	>100	>100
29	H	OMe	OMe	H	H	O	–	>0.8	>0.8	>2	1.4 ± 0.4	2.2 ± 1.5	7.0 ± 45
30	H	OMe	OMe	OMe	H	O	O	≥20	3.7 ± 2.4	≥100	51 ± 4	>100	>100
31	H	OMe	OMe	OMe	H	O	–	>20	35 ± 19	>100	50 ± 14	60 ± 0	>100
32	OMe	H	H	Me	H	O	O	1.6 ± 0.9	1.2 ± 0.1	68 ± 28	43 ± 0.2	>100	>100
33	OMe	H	H	Me	H	–	–	6.7 ± 4.6	>4	>40	24 ± 16	12 ± 0	46 ± 1
34	OEt	H	H	H	H	O	O	9.5 ± 3.5	8.0 ± 1.8	>100	37 ± 1.1	>100	>100
35	OEt	H	H	H	H	O	–	>20	6.3 ± 1.1	>100	62 ± 34	60 ± 0	>100
36	H	F	H	H	H	O	O	6.5 ± 0.7	3.7 ± 1.9	59 ± 21	19 ± 1.4	>100	75 ± 10
37	H	H	F	H	H	O	O	11 ± 1.4	5.3 ± 2.4	>60	24 ± 2	65 ± 0	69 ± 2
38	H	H	F	H	H	O	–	≥20	16 ± 5	>100	87 ± 19	>100	>100
39	Cl	H	H	H	H	O	O	6.0 ± 0.0	5.5 ± 1.6	>70	19 ± 1.3	62 ± 3	72 ± 19
40	H	H	Cl	H	H	O	O	9.5± 3.5	6.2 ± 1.4	35 ± 6	25 ± 2	>100	>100
41	Cl	H	Cl	H	H	O	O	2.4 ± 1.4	3.2 ± 1.1	11 ± 4	6.2 ± 0.5	59 ± 1	57 ± 1
42	Cl	H	H	H	Cl	O	O	9.0 ± 4.2	5.9 ± 0.4	46 ± 1	15 ± 0.4	>100	≥100
43	H	Cl	Cl	H	H	O	O	1.0 ± 0.0	3.3 ± 2.9	>50	2.9 ± 0.2	54	70 ± 1
44	Cl	Cl	H	H	Cl	O	O	≥4	0.87 ± 0.10	12 ± 7	6.5 ± 0.4	60	72 ± 14
45	Cl	Cl	Cl	Cl	Cl	O	O	0.63 ± 0.29	0.79 ± 0.18	17 ± 7	1.2 ± 0.1	>100	>100

Table 1. (*Continued*)

Code	X_1	X_2	X_3	X_4	X_5	R_1	R_2	EC_{50}[a] (mg/L) HIV-1	FIPV	SARS-CoV	IC_{50}[b] (mg/L) CEM	CC_{50}[c] (mg/L) CRFK	Vero
46	Cl	Cl	Me	Cl	Cl	O	O	≥0.8	0.29 ± 0.02	17 ± 5	2.0 ± 0.1	≥20	>100
47	Cl	H	NO_2	H	H	O	O	2.9 ± 1.9	12 ± 9	49 ± 40	6.1 ± 1.2	46 ± 25	>100
48	H	Br	H	H	H	O	O	2.5 ± 0.0	2.3 ± 1.8	56 ± 44	7.4 ± 0.1	60	82 ± 15
49	Br	H	H	OMe	H	O	O	4.0 ± 0.0	2.9 ± 2.2	38 ± 4	15 ± 1.1	>100	≥100
50	iProp	H	Br	iProp	H	O	O	2.3 ± 0.4	2.8 ± 1.3	16 ± 5	4.4 ± 0.7	>100	>100
51	I	H	H	H	H	O	O	5.2 ± 2.3	41 ± 32	>100	25 ± 4.7	>100	>100
52	NO_2	H	H	H	H	O	O	8.0 ± 0.0	0.91 ± 0.36	20 ± 6	13 ± 1.1	65 ± 2	>100
53	H	H	NO_2	H	H	O	O	>4	10 ± 7	43 ± 33	7.6 ± 0.3	58 ± 12	74 ± 6
54	H	NO_2	H	NO_2	H	O	O	2.8 ± 1.0	3.3 ± 1.6	>20	4.4 ± 1.1	55 ± 7	63 ± 23
55	H	NO_2	Me	H	H	O	O	2.3 ± 0.4	3.3 ± 1.3	>50	5.8 ± 0.6	60	62 ± 9
56	H	Me	NO_2	H	H	O	O	3.1 ± 1.3	4.2 ± 2.4	11 ± 1	4.9 ± 1.2	54 ± 3	85 ± 10
57	Me	H	H	NO_2	H	O	O	73 ± 23	>100	>100	57 ± 9.4	>100	>100
58	OMe	H	H	NO_2	H	O	O	7.0 ± 1.4	1.7 ± 0.8	22 ± 2	17 ± 0.0	>100	>100
59	H	NO_2	Cl	H	H	O	O	1.5 ± 0.7	5.0 ± 2.3	>50	4.3 ± 1.3	60 ± 0	56 ± 2
60	CN	H	H	H	H	O	O	7.0 ± 1.4	3.3 ± 0.9	>30	15 ± 1.7	61 ± 1	67 ± 15
61	CN	H	H	H	H	O	–	15 ± 7	>20	>100	≥100	>100	>100
62	H	H	CN	H	H	O	O	9.0 ± 4.2	1.5 ± 1.1	≥100	12 ± 0.3	73	>100
63	H	H	COOH	H	H	O	O	>100	8.1 ± 1.2	>100	>100	>100	>100
64	H	H	Phe	H	H	O	O	≥4	1.4 ± 0.4	>50	4.6 ± 0.4	57	61 ± 4
65	OPhe	H	H	H	H	O	O	5.3 ± 2.3	57 ± 44	63 ± 28	10 ± 0.3	>100	>100
66	H	OMe	OBz	H	H	O	O	>4	3.6 ± 0.4	37 ± 0	8.0 ± 0.6	>100	>100
67	H	CF_3	H	H	H	O	–	13 ± 1.4	>100	>100	54 ± 8	>100	>100
68	OH	H	H	NO_2	H	–	–	10 ± 0.0	>20	>20	37 ± 0.9	58 ± 4	61 ± 8

[a]EC_{50}, 50% effective concentration required to inhibit HIV-induced giant cell formation in CEM cell cultures. Data are taken from ref. (16).
[b]IC_{50}, 50% inhibitory (cytostatic) concentration required to inhibit CEM cell proliferation by 50%. Data were obtained by counting the number of CEM cells using a Coulter Particle Counter (Coulter Electronics, Miami, FL).
[c]CC_{50}, cytotoxic concentration required to cause a decreased viability of the CRFK and Vero cell cultures by 50%. Data were obtained by a spectrophotometric analysis using the MTT dye exclusion method.
[d]Abbreviations: Me, methyl; Et, ethyl; Prop, propyl; iProp, isopropyl; But, butyl; Pent, pentyl; Phe, phenyl; Bz, benzyl.

(EC_{50}: 0.3–0.9 mg/L for Fe-CoV and ~17–20 mg/L for SARS-CoV) virtually completely lacking cytotoxic activity against the CRFK and Vero cell cultures (MIC: ≥100 mg/L). Only a few compounds were found to be markedly active (EC_{50}: <5 mg/L) against FIPV but not active against SARS-CoV at subtoxic concentrations (**14**, **20**, **23**, **26**, **28**, **43**, **54**, **55**, **59**, **60**, **64**). Instead, compounds that were more active against SARS-CoV than Fe-CoV were not found.

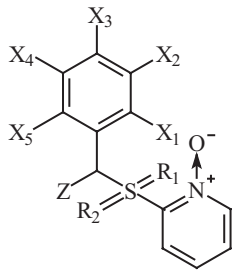
Antiviral activity of pyridine N-oxide derivatives modified in the bridge (Z) part of the molecule

A series of compounds were made that contain a substitution (*Z*) at the CH_2 position linking the phenyl group to the other part of the molecule (Table 2). Several alkyl/halogeno-substituted compounds were markedly inhibitory against SARS-CoV (EC_{50}: 2.1–2.7 mg/L) being not measurably active against FIPV (i.e. **75**–**78**). However, it should be noticed that these compounds proved markedly cytotoxic to both CRFK and Vero cell cultures, and therefore potential anti-FIPV activity may have been masked by the cellular toxicity or, alternatively, the observed anti-SARS-CoV activity has to be interpreted as a toxic rather than a specific antiviral effect. For all other antivirally active compounds—as already noted for the unsubstituted series of compounds discussed above—anti-FIPV activity was more pronounced than the anti-SARS-CoV activity. Compound **72** represents the only exception where anti-SARS-CoV activity was noticed (EC_{50}: 13 mg/L) without a trace of anti-FIPV activity. Intriguingly, this compound was toxic for CRFK cells (CC_{50}: 1.3 mg/L) but not for Vero cells (CC_{50}: >100 mg/L).

Antiviral activity of pyridine N-oxide derivatives containing a cyano-substituted ethylene bridge between the thioether and the phenyl moiety

While the **116**- and **122**-sulphide derivatives were markedly more active against FIPV than SARS-CoV, the dihalogen-substituted pyridine *N*-oxide derivative **120** was 4-fold more effective against SARS-CoV than FIPV (Table 3). Also, **117** and **118** showed antiviral activity against SARS-CoV but not FIPV. Intriguingly, the active compounds in this series of pyridine *N*-oxide derivatives contained exclusively a sulphide moiety whereas the sulphones **119** and **121** were entirely devoid of antiviral activity. An opposite trend of (in)activity with respect to sulphones versus sulphides and sulphoxides was previously found for the first and second series of compounds depicted in Tables 1 and 2.

Table 2. Antiviral activity of pyridine *N*-oxide derivatives modified in the Z part of the molecule



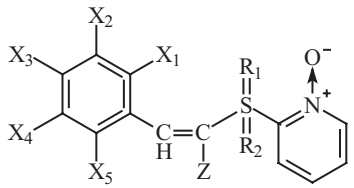
									EC_{50}[a] (mg/L)			IC_{50}[b] (mg/L)	CC_{50}[c] (mg/L)	
Code	Z[d]	X_1	X_2	X_3	X_4	X_5	R_1	R_2	HIV-1	FIPV	SARS-CoV	CEM	CRFK	Vero
69	Me[e]	H	H	H	H	H	O	–	>0.8	>0.8	5.9 ± 5.0	0.94 ± 0.69	1.4 ± 1.4	>100
70	Me	H	H	Me	H	H	O	–	>0.032	>0.8	>0.8	0.59 ± 0.09	1.0 ± 0.6	2.3 ± 0.3
71	Me	Me	H	H	Me	H	O	O	>100	35 ± 3	>100	>100	>100	>100
72	Me	Me	H	H	Me	H	O	–	>0.8	>0.8	13 ± 12	1.2 ± 0.7	1.3 ± 1.2	≥100
73	Me	Me	H	H	Me	H	–	–	6 ± 3	>100	>100	74 ± 1	>100	>100
74	Me	Me	Me	Me	Me	H	O	–	>0.16	>0.8	14 ± 0	0.45 ± 0.37	2.9 ± 0.3	31 ± 5
75	Me	H	H	F	H	H	O	–	>0.16	>0.8	2.6 ± 1.2	0.33 ± 0.03	1.9 ± 0.7	5.1 ± 1.3
76	Me	H	H	Cl	H	H	O	–	>0.16	>0.8	2.7 ± 1.3	0.64 ± 0.39	2.0 ± 1.0	5.4 ± 2.5
77	Me	Cl	Me	Cl	H	H	O	–	>0.16	>0.8	2.6 ± 0.7	0.59 ± 0.44	1.8 ± 1.2	6.0 ± 3.0
78	Me	Cl	H	Me	H	H	O	–	>0.16	>0.8	2.1 ± 0.5	0.28 ± 0.11	2.3 ± 0.6	5.3 ± 2.4
79	Me	Cl	H	H	Me	H	O	–	>0.16	>0.16	>0.8	0.19 ± 0.09	0.33 ± 0.17	1.6 ± 1.0
80	Me	H	H	SO_2CH_3	H	H	O	–	>0.8	>4	18 ± 4	3.8 ± 2.4	12 ± 4	38 ± 5
81	Me	Me	H	H	NH_2	H	O	O	20 ± 15	48 ± 4	>40	≥100	>100	91 ± 9
82	Et	H	H	H	H	H	O	O	35 ± 7	8.3 ± 0.5	82 ± 30	61 ± 0.6	>100	>100
83	Et	Me	H	H	Me	H	O	O	>20	41 ± 16	>100	80 ± 34	>100	>100
84	Prop	H	H	H	H	H	O	O	>20	>20	66 ± 30	43 ± 0.7	73 ± 23	>100
85	Prop	H	H	H	H	H	–	–	>20	>100	>100	62 ± 1.1	>100	>100
86	Prop	Me	H	H	Me	H	–	–	>4	>4	>20	9.2 ± 2.3	37 ± 26	52 ± 4
87	Hept	H	Me	Me	Me	H	–	–	>20	>4	>20	20 ± 5	11 ± 0	25 ± 16
88	Hept	Me	H	H	Me	H	O	O	>0.8	3.2 ± 0.4	>10	3.4 ± 0.5	68 ± 3	12 ± 1
89	Hept	Me	H	H	Me	H	–	–	>4	>4	>4	26 ± 15	10 ± 1	14 ± 5
90	Undec	Me	H	H	Me	H	O	O	>20	60 ± 38	>100	88 ± 22	>100	>100
91	Undec	Me	H	H	Me	H	–	–	>4	>20	>20	25 ± 17	38 ± 4	54 ± 9
92	Isobut	Me	H	H	Me	H	O	O	>4	>4	>20	6.2 ± 1.1	14 ± 3	26 ± 11
93	$–CH_2–CH=CH_2$	Me	H	H	Me	H	O	O	10 ± 3	54 ± 45	>20	38 ± 2	>100	58 ± 8
94	$–C_6H_5$	Me	H	H	H	H	O	O	>20	14 ± 1	64 ± 20	47 ± 3	≥100	>100
95	$–C_6H_5$	Me	H	H	Me	H	O	O	>20	>20	>20	43 ± 1	60 ± 1	54 ± 2
96	$–C_6H_5$	Me	H	H	Me	H	–	–	≥20	>20	>20	34 ± 2	48 ± 3	43 ± 0
97	$–CH_2(C_6H_5)$	Me	H	H	Me	H	O	O	>4	>100	>100	30 ± 19	>100	>100
98	$–CH_2(C_6H_5)$	Me	H	H	Me	H	–	–	>0.8	>4	>4	2.6 ± 0.7	9.7 ± 2.6	9.8 ± 0.2
99	–CN	Me	H	H	H	H	O	–	>0.16	>0.8	>0.8	0.67 ± 0.49	2.0 ± 0.4	2.8 ± 1.1
100	–CN	H	Me	H	H	H	O	–	>0.16	>0.8	>4	0.74 ± 0.49	4.4 ± 2.8	9.5 ± 1.4
101	–CN	Me	H	H	Me	H	–	–	>4	>4	>20	34 ± 4	19 ± 11	63 ± 11
102	–CN	H	H	F	H	H	O	–	>0.16	>0.8	>10	0.72 ± 0.41	5.0 ± 3.5	11 ± 2
103	$–CO–NH_2$	Me	H	H	H	H	O	–	>100	81 ± 12	>100	>100	>100	>100
104	$–CO–NH_2$	Me	H	H	H	H	–	–	>100	>100	>100	>100	>100	>100
105	$–CO–NH_2$	Me	H	H	Me	H	O	O	>20	66 ± 36	66 ± 12	>100	>100	≥100
106	$–CH_2COOH$	Me	H	H	Me	H	O	O	>20	14 ± 6	>100	87 ± 22	>100	>100
107	Hex-6-Br	Me	H	H	Me	H	O	O	>0.8	4.4 ± 2.0	69 ± 29	6.7 ± 0.4	64 ± 0	≥100
108	–Br	Me	H	H	Me	H	O	O	20.7 ± 9.0	24 ± 15	>100	81 ± 31	>100	>100
109	$–COOCH_3$	Me	H	H	H	H	O	–	>20	>100	>100	52 ± 7	>100	>100
110	$–COOCH_3$	Me	H	H	Me	H	O	O	2.2 ± 1.5	50 ± 37	36 ± 3	36 ± 2.1	>100	>100
111	$–COOCH_3$	H	$O(C_6H_5)$	H	H	H	O	O	>4	>20	>20	26 ± 10	58 ± 2	49 ± 2
112	$–CF_3$	Me	H	H	Me	H	O	O	12 ± 0.0	>20	>20	41 ± 0.4	76 ± 21	79 ± 12

Table 2. (*Continued*)

Code	Z[d]	X_1	X_2	X_3	X_4	X_5	R_1	R_2	EC_{50}[a] (mg/L) HIV-1	FIPV	SARS-CoV	IC_{50}[b] (mg/L) CEM	CC_{50}[c] (mg/L) CRFK	Vero
113	—CH_2— 	Me	H	H	Me	H	O	O	16 ± 6	>20	45 ± 3	24 ± 0.7	60 ± 0	>100
114	Me, Cl	H	H	H	H	H	O	O	>4	9.2 ± 3.2	>20	10 ± 0.3	58 ± 5	94 ± 3
115	Me, Cl	Me	H	H	Me	H	O	O	>4	44 ± 27	>100	23 ± 10	>100	>100

[a]EC_{50}, 50% effective concentration required to inhibit HIV-induced giant cell formation in CEM cell cultures. Data taken from ref. (16).
[b]IC_{50}, 50% inhibitory (cytostatic) concentration required to inhibit CEM cell proliferation by 50%.
[c]CC_{50}, cytotoxic concentration required to cause a decreased viability of the CRFK and Vero cell cultures by 50%.
[d]Introduction of a Z entity introduces chirality in the molecules. The compounds represent racemic mixtures.
[e]Abbreviations: Me, methyl; Et, ethyl; Prop, propyl; Isobut, isobutyl; Hept, heptyl; Undec, undecyl.

Table 3. Antiviral activity of pyridine *N*-oxide derivatives containing an ethylene bridge between the thioether and the phenyl moiety



	X_1	X_2	X_3	X_4	X_5	Z	R_1	R_2	EC_{50}[a] (mg/L) HIV-1	FIPV	SARS-CoV	IC_{50}[b] (mg/L) CEM	CC_{50}[c] (mg/L) CRFK	Vero
116	H	H	H	H	H	CN	–	–	>4	3.3 ± 0.2	>100	8.7 ± 0.2	44 ± 22	>100
117	H	H	OMe	H	H	CN	–	–	>20	>20	27 ± 22	35 ± 5.0	62 ± 5	55 ± 1
118	H	OMe	OMe	OMe	H	CN	–	–	>20	>20	14 ± 5	3.3 ± 1.0	57 ± 6	48 ± 1
119	Cl	H	Cl	H	H	CN	O	O	>4	>20	>20	8.1 ± 5.0	52 ± 9	59 ± 2
120	Cl	H	Cl	H	H	CN	–	–	>0.8	31 ± 27	7.3 ± 5.5	5.9 ± 3.8	55 ± 9	54 ± 2
121	Cl	H	H	H	Cl	CN	O	O	>4	>100	>100	11 ± 0.8	>100	>100
122	Cl	H	H	H	Cl	CN	–	–	12 ± 8	5.7 ± 1.9	59 ± 36	30 ± 19	58 ± 3	>100
123	H	H	OOct	H	H	CN	–	–	>20	>100	>100	≥100	>100	>100

Abbreviations: Me, methyl; Oct, octyl.
[a]EC_{50}, 50% effective concentration required to inhibit HIV-induced giant cell formation in CEM cell cultures. Data are taken from ref. (16).
[b]IC_{50}, 50% inhibitory (cytostatic) concentration required to inhibit CEM cell proliferation by 50%.
[c]CC_{50}, cytotoxic concentration required to cause a decreased viability of the CRFK and Vero cell cultures by 50%.

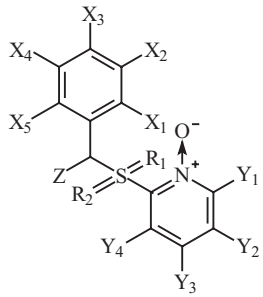
Anti-coronavirus activity of pyridine N-oxide derivatives modified at the pyridine oxide and phenyl and Z part of the molecule

Among the pyridine *N*-oxide derivatives that contain alkyl, alkoxy, halogen or nitro substituents on the pyridine moiety in addition to substituents on the phenyl and the bridge between the pyridine thioether and the phenyl, a few compounds were endowed with a pronounced anti-FIPV selectivity (Table 4). Indeed, **155** had an EC_{50} of 0.49 mg/L against FIPV being inactive against SARS-CoV. This compound was not cytotoxic at 100 mg/L, and therefore represents the most selective anti-FIPV compound among all pyridine *N*-oxide derivatives tested. The location of the chloro substituent on the pyridine moiety seems crucial, since moving the chloro from Y_1 to the Y_2, Y_3 or Y_4 position resulted in completely inactive compounds (**158**, **161**, **165**). Besides **155**, also **132**, **133**, **142** and **146** can be regarded as highly selective anti-Fe-CoV compounds. As observed before, none of the compounds was more inhibitory against SARS-CoV than against Fe-CoV. Compounds **157** and **159** were the most interesting compounds that had comparable (potent) activity against both SARS-CoV and FIPV (EC_{50}: 1.7–4.2 mg/L), being poorly cytotoxic (**157**; CC_{50}: 62 mg/L for CRFK and >100 mg/L for Vero cell cultures) to moderately cytotoxic (**159**; CC_{50}: 13 mg/L for CRFK and 71 mg/L for Vero cell cultures) (Table 4).

Anti-coronavirus activity of reduced pyridine N-oxide derivatives

A total of 23 compounds that lacked the oxygen at the N-atom of the pyridine moiety were evaluated for antiviral activity (Table 5). As a rule, lack of the oxide moiety proved detrimental for anti-SARS-CoV and anti-FIPV activity. Indeed, virtually none of the test compounds was antivirally active at subtoxic concentrations.

Table 4. Antiviral activity of pyridine *N*-oxide derivatives modified in the pyridine oxide and phenyl and Z part of the molecule



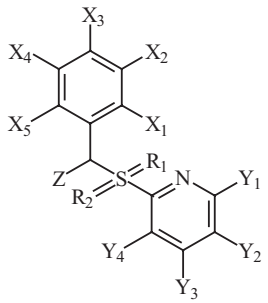
													EC$_{50}$[a] (mg/L)			IC$_{50}$[b] (mg/L)	CC$_{50}$[c] (mg/L)	
Code	X_1	X_2	X_3	X_4	X_5	Z[d]	R_1	R_2	Y_1	Y_2	Y_3	Y_4	HIV-1	FIPV	SARS-CoV	CEM	CRFK	Vero
124	Me[e]	H	H	Me	H	H	O	O	Me	H	H	H	4.0 ± 3.5	45 ± 40	>100	≥100	>100	>100
125	Me	H	H	Me	H	Cl	O	O	Me	H	H	H	2.8 ± 1.3	17 ± 10	53 ± 5.0	≥100	>100	>100
126	Me	H	H	Me	H	Me	O	O	H	Me	H	H	5.5 ± 2.1	>100	>100	≥100	>100	>100
127	H	H	Cl	H	H	H	O	O	H	H	Me	H	>4	10 ± 2	51 ± 16	20 ± 12	96 ± 1	>100
128	Me	H	H	Me	H	Me	O	O	H	H	Me	H	0.75 ± 0.35	>0.8	>10	25 ± 8	2.3 ± 0.1	13 ± 3
129	Me	H	H	Me	H	Cl	O	O	H	H	Me	H	0.42 ± 0.34	>20	61 ± 9	>100	60 ± 0	>100
130	H	H	H	H	H	H	O	O	H	H	H	Me	15 ± 5	4.0 ± 1.6	62 ± 27	28 ± 4	>100	>100
131	Me	H	H	Me	H	Me	O	O	H	H	H	Me	0.05 ± 0.0	>20	>40	38 ± 0.7	62 ± 3	47 ± 1
132	Me	H	H	Me	H	H	O	–	H	H	H	Me	12 ± 8	0.83 ± 0.48	>40	42 ± 16	18 ± 10	45 ± 1
133	Me	H	H	H	H	H	O	O	H	H	H	Me	9.3 ± 2.3	1.5 ± 1.3	>50	17 ± 5	60 ± 0	75 ± 23
134	Me	H	Me	H	H	H	O	O	H	H	H	Me	6 ± 2.0	12 ± 2	>50	11 ± 0.7	58 ± 3	60 ± 2
135	Me	H	Me	H	H	Me	O	O	H	H	H	Me	1.4 ± 0.2	79 ± 14	>40	≥100	≥100	80 ± 19
136	Me	H	H	Me	H	Et	O	O	H	H	H	Me	1.4 ± 0.2	12 ± 7	>10	20 ± 3	65 ± 5	10 ± 0
137	Me	H	H	Me	H	Cl	O	O	H	H	H	Me	>100	>100	>100	>100	>100	>100
138	Cl	H	H	H	Cl	H	O	O	H	H	H	Me	>20	11 ± 11	47 ± 16	70 ± 42	>100	>100
139	Cl	H	H	H	Cl	H	O	–	H	H	H	Me	>100	>100	≥100	4 → 100	>100	>100
140	Cl	H	H	H	Cl	H	–	–	H	H	H	Me	>20	2.8 ± 0.6	69 ± 11	31 ± 14	≥100	80 ± 2
141	H	H	H	H	H	Me	O	O	H	H	H	Me	>20	3.9 ± 0.2	>50	60 ± 27	>100	>100
142	Cl	H	H	H	H	H	O	O	H	H	H	Me	3.4 ± 0.9	0.78 ± 0.03	>20	21 ± 1	60	66 ± 7
143	Me	NO_2	H	H	H	H	O	O	H	H	H	Me	≥4	6.9 ± 5.7	16 ± 1	9.6 ± 0.4	48 ± 24	60 ± 6
144	Me	H	Me	H	H	Me	O	O	H	H	H	Me	2.4 ± 0.2	55 ± 13	29 ± 4	45 ± 2	>100	≥100
145	Cl	H	H	H	H	Me	O	O	H	H	H	Me	3.3 ± 1.1	≥100	41 ± 15	38 ± 9	>100	64 ± 11
146	Me	NO_2	H	H	H	Me	O	O	H	H	H	Me	11 ± 1	3.6 ± 1.7	>100	96 ± 5	>100	≥100
147	Me	H	H	Me	H	H	O	O	H	H	H	OMe	0.70 ± 0.14	15 ± 0	11 ± 7	8.0 ± 1.0	55 ± 3.0	66 ± 14
148	Me	H	H	Me	H	H	O	–	H	H	H	OMe	>20	>20	>100	62 ± 10	61 ± 1.0	>100
149	Me	H	H	Me	H	H	–	–	H	H	H	OMe	1.4 ± 0.2	48 ± 3	>4	70 ± 12	54 ± 8.0	15 ± 1
150	Me	H	H	Me	H	H	O	O	H	H	H	OH	45 ± 7	>100	>100	>100	>100	>100
151	Me	H	H	Me	H	H	–	–	H	H	H	OH	>20	>4	>4	65 ± 3	13 ± 1	21 ± 8
152	Me	H	H	Me	H	Me	O	O	Me	H	Me	H	≥20	>100	>100	>100	>100	>100
153	H	H	OMe	H	H	H	O	O	H	H	t-Bu	H	>20	10 ± 2	52 ± 13	38 ± 5	60 ± 0	≥100
154	H	H	OMe	H	H	H	–	–	H	H	t-Bu	H	>20	>20	>20	48 ± 12	85 ± 18	≥100
155	H	H	H	H	H	H	–	–	Cl	H	H	H	9.0 ± 7.1	0.49 ± 0.03	>100	48 ± 0.3	>100	>100
156	Me	H	H	Me	H	H	O	O	Cl	H	H	H	0.7 ± 0.1	9.7 ± 2.2	11 ± 5	4.0 ± 3.4	60 ± 0	>100
157	Me	H	H	Me	H	Me	O	O	Cl	H	H	H	0.9 ± 0.1	2.3 ± 0.5	4.2 ± 2.6	4.5 ± 0.3	62 ± 1	>100
158	H	H	H	H	H	H	–	–	H	Cl	H	H	>20	>100	>100	66 ± 14	>100	>100
159	Me	H	H	Me	H	H	O	O	H	Cl	H	H	1.9 ± 0.5	1.7 ± 0.7	4.2 ± 3.9	6.1 ± 2.3	13 ± 1	71 ± 0
160	Me	H	H	Me	H	H	O	–	H	Cl	H	H	2.1 ± 0.1	14 ± 8	69 ± 9	14 ± 13	>100	>100
161	Me	H	H	Me	H	H	–	–	H	Cl	H	H	>100	>100	>100	>100	>100	>100
162	H	H	H	H	H	Cl	O	O	H	Cl	H	H	>0.8	1.8 ± 0.7	6.5 ± 4.6	2.3 ± 0.7	12 ± 0	39 ± 26
163	H	H	H	H	H	H	O	O	H	H	H	Cl	>0.8	>4	>10	1.5 ± 0.1	10 ± 2	30 ± 28
164	H	H	H	H	H	H	O	–	H	H	H	Cl	1.5	3.0 ± 1.0	6.9 ± 2.5	3.7 ± 1.0	15 ± 0	42 ± 17
165	H	H	H	H	H	H	–	–	H	H	H	Cl	2.4 ± 0.2	>100	>100	>100	>100	>100
166	Me	H	H	Me	H	H	–	–	H	H	H	Cl	0.14 ± 0.1	1.2 ± 0.2	58 ± 7	42 ± 2	60 ± 0	62 ± 1

Table 4. (*Continued*)

														EC_{50}[a] (mg/L)			IC_{50}[b] (mg/L)	CC_{50}[c] (mg/L)	
Code	X_1	X_2	X_3	X_4	X_5	Z[d]	R_1	R_2	Y_1	Y_2	Y_3	Y_4		HIV-1	FIPV	SARS-CoV	CEM	CRFK	Vero
167	Me	H	H	Me	H	Me	O	O	H	H	H	Cl		>0.8	>4	>10	1.7 ± 0.1	11 ± 1	10 ± 0
168	Me	H	H	Me	H	Cl	O	O	H	H	H	Cl		>4	4.7 ± 1.0	29 ± 20	15 ± 7	61 ± 0	80 ± 7
169	H	H	H	H	H	H	–	–	H	H	H	NO_2		2.4 ± 0.2	5.9 ± 0.6	>20	31 ± 2	>100	69 ± 27

[a]EC_{50}, 50% effective concentration required to inhibit HIV-induced giant cell formation in CEM cell cultures. Data are taken from ref. (16).
[b]IC_{50}, 50% inhibitory (cytostatic) concentration required to inhibit CEM cell proliferation by 50%.
[c]CC_{50}, cytotoxic concentration required to cause a decreased viability of the CRFK and Vero cell cultures by 50%.
[d]Introduction of a Z entity introduces chirality in the molecules. The compounds represent racemic mixtures.
[e]Abbreviations: Me, methyl; Et, ethyl; t-bu, tertiary butyl.

Table 5. Antiviral activity of pyridine derivatives



													EC_{50}[a] (mg/L)			IC_{50}[b] (mg/L)	CC_{50}[c] (mg/L)	
Code	X_1	X_2	X_3	X_4	X_5	Z[d]	R_1	R_2	Y_1	Y_2	Y_3	Y_4	HIV-1	FIPV	SARS-CoV	CEM	CRFK	Vero
170	H	H	H	H	H	H	O	O	H	H	H	H	30 ± 14	>100	>100	68 ± 45	>100	>100
171	H	H	H	H	H	H	–	–	H	H	H	H	>20	>100	>100	51 ± 7	>100	>100
172	H	H	H	H	H	H	O	O	H	H	H	OH	60 ± 0.0	>100	>100	>100	>100	>100
173	H	H	H	H	H	H	–	–	H	H	H	OH	2.0 ± 0.7	12 ± 6	>50	35 ± 3	46 ± 19	53 ± 5
174	H	H	H	H	H	H	–	–	H	H	H	OCH_3	21 ± 13	>100	>100	87 ± 22	>100	≥100
175	H	H	H	H	H	H	–	–	H	H	H	OC_2H_5	33 ± 12	>20	>20	90 ± 17	80 ± 13	≥100
176	H	H	H	H	H	H	–	–	H	H	H	OC_4H_9	17 ± 6	>20	>20	67 ± 3.9	33 ± 29	78 ± 18
177	H	H	H	H	H	H	–	–	H	H	H	Bn	>4	>20	>20	9.7 ± 1.8	25 ± 8	61 ± 35
178	H	H	H	H	H	H	–	–	H	H	H	CN	12 ± 11	>20	>20	33 ± 6	58 ± 2	68 ± 29
179	H	H	H	H	H	H	–	–	H	NO_2	H	H	>20	>100	>100	46 ± 8	>100	>100
180	Me[e]	H	H	Me	H	H	O	O	H	H	H	H	3.2 ± 1.1	>100	>100	51 ± 7	>100	>100
181	Me	H	H	Me	H	H	O	–	H	H	H	H	3.2 ± 1.1	>100	>100	88 ± 3	>100	>100
182	Me	H	H	Me	H	H	O	O	H	H	H	OH	24 ± 10	>100	>100	>100	>100	>100
183	Me	H	H	Me	H	H	O	–	H	H	H	OH	40 ± 0.0	>100	>100	>100	>100	>100
184	Me	H	H	Me	H	H	–	–	H	H	H	OH	0.22 ± 0.17	>4	8.7 ± 3.6	39 ± 12	11 ± 1	13 ± 1
185	Me	H	H	Me	H	H	–	–	H	H	H	OCH_3	1.7 ± 1.2	>20	35 ± 5	78 ± 39	28 ± 24	94 ± 3
186	Me	H	H	Me	H	Me	O	O	H	H	H	H	9.5 ± 3.5	>100	>60	3.1 ± 1.2	>100	75 ± 21
187	H	Me	Me	H	H	H	–	–	H	H	H	H	>4	>20	>100	7.1 ± 0.1	59 ± 4	>100
188	Me	H	H	Me	H	CH_2OH	O	O	H	H	H	H	>100	≥100	>100	≥100	>100	>100
189	Me	H	H	Me	H	CH_2OCH_3	O	O	H	H	H	H	60 ± 0	≥100	>100	≥100	>100	>100
190	Me	H	H	Me	H	Cl	O	O	H	H	H	H	0.9 ± 0.1	>20	>100	38 ± 2	72 ± 24	>100
191	H	H	Cl	H	H	H	–	–	H	H	H	H	≥1	>4	>20	1.2 ± 0.1	14 ± 3	25 ± 7
192	H	H	Cl	H	H	H	–	–	H	H	$N(CH_3)_2$	CN	>20	>100	>100	>100	>100	>100

[a]EC_{50}, 50% effective concentration required to inhibit HIV-induced giant cell formation in CEM cell cultures. Data are taken from ref. (16).
[b]IC_{50}, 50% inhibitory (cytostatic) concentration required to inhibit CEM cell proliferation by 50%.
[c]CC_{50}, cytotoxic concentration required to cause a decreased viability of the CRFK and Vero cell cultures by 50%.
[d]Introduction of a Z entity introduces chirality in the molecules. The compounds represent racemic mixtures.
[e]Abbreviation: Me, methyl.

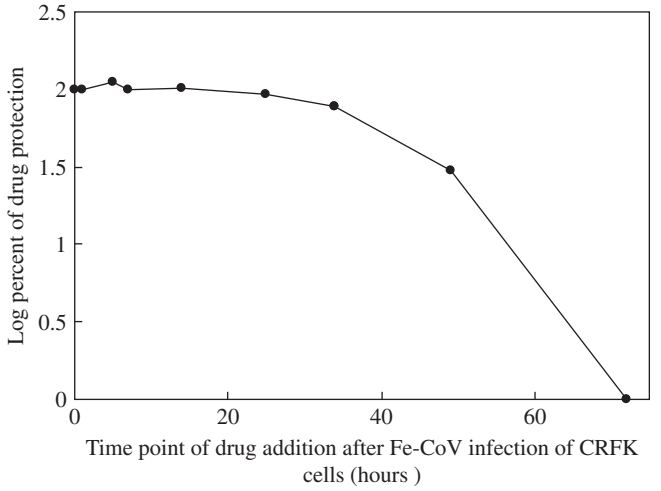


Figure 2. Effect of different time points of addition of compound **45** to Fe-CoV-infected CRFK cell cultures on protection against virus-induced cytopathicity.

Only **173** (FIPV), **184** (SARS-CoV) and **185** (SARS-CoV) showed antiviral activity, although their EC_{50}s were rather close to their CC_{50}s.

Discussion

The pyridine *N*-oxide derivatives represent a unique class of antivirals. Several members have previously been found to be active against HIV-1 and/or HIV-2, and human cytomegalovirus (HCMV), but not against several other DNA viruses including herpes simplex virus type 1 and type 2, varicella-zoster virus and vaccinia virus or against RNA viruses including vesicular stomatitis virus, reovirus-1, polio virus, Coxsackie virus B4, Semliki forest virus and parainfluenza virus.[16] Surprisingly, the pyridine *N*-oxides were now also found inhibitory against coronaviruses, in particular against the feline coronavirus type II strain of FIPV and the SARS coronavirus strain Frankfurt-1. Such an antiviral selectivity spectrum is rather unusual. Interestingly, as previously noted between HIV-1, HIV-2 and HCMV, there is no close correlation for the antiviral activity of the pyridine *N*-oxides against HIV-1 on the one hand, and FIPV and SARS-CoV on the other hand ($r = 0.29$ and 0.47, respectively) (compare the antiviral activities of the test compounds against HIV-1 with those found against the coronaviruses given in Tables 1–5). Also, there is no correlation for the EC_{50}s between FIPV and SARS-CoV in cell culture ($r = 0.06$). Indeed, there were a number of compounds that proved exclusively inhibitory to FIPV and not to SARS-CoV. Among these, only **63** and **116** were not active against HIV-1. The other compounds that discriminated between FIPV and SARS-CoV showed also activity against HIV-1.

The pyridine *N*-oxide derivatives have a peculiar mechanism of antiviral action. It has previously been shown that pyridine *N*-oxide derivatives such as **11**, **17**, **45** and **160** act at a step in the HIV-1 and HIV-2 replication cycle that follows proviral integration, i.e. at the HIV gene expression level.[18] In this respect, the pyridine *N*-oxide derivatives inhibit binding of nuclear NF-κB to DNA in the intact cell system and regeneration of IκBα after TNF-α stimulation.[18,19] Thus, targeting of a cellular protein in the transactivation process is the most likely explanation for the anti-HIV/HCMV activity of the pyridine *N*-oxide derivatives. The molecular target for the anti-coronavirus activity is still unclear. Coronaviruses replicate in the cytoplasm, and not in the nucleus. However, a TOA experiment in which the pyridine *N*-oxide derivative **45** was added at different time points after Fe-CoV infection of CRFK cell cultures revealed that addition of the compound to the virus-infected cells could be substantially delayed before losing its antiviral potential (Figure 2). A similar TOA experiment against HIV in human lymphocyte CEM cell cultures revealed also a post-integrational event as a target of **45** in the replication cycle of HIV.[18] Thus, compound **45** inhibits virus replication at an event that is clearly located after viral entry and acts most likely during the transcription process of the virus replication cycle. However, not only the differences in antiviral activity but also cytotoxicity depending on the virus type (HIV, Fe-CoV, SARS-CoV) or cell type (CEM, CRFK, Vero) is indicative for a rather specific interaction with a cellular and/or a viral factor to explain the rather unpredictable antiviral and cytostatic properties of the pyridine *N*-oxide derivatives. In this respect, while the oxide form of the compounds seems to be crucial to maintain anti-coronavirus activity, this requirement was much less stringent to keep anti-HIV-1 activity (compare anti-HIV and anti-coronavirus activity for the reduced pyridine *N*-oxides in Table 5).

Despite the pronounced cytostatic activity noticed in proliferating human CEM lymphocyte cell culture, pyridine *N*-oxide derivatives are often poorly cytotoxic in confluent CRFK and Vero cell cultures, resulting in pronounced selectivity indices for several compounds (exceeding two orders of magnitude). Moreover, compound **45**, which represents one of the most inhibitory and selective anti-coronavirus compounds in this study (lacking any pronounced selectivity against HIV-1 in CEM cell cultures), has been administered to mice at 15 mg/kg/day for 10 days (upon continuous release through Alzet pumps) or at 100 mg/kg/day intraperitoneally for 10 consecutive days. Under these experimental conditions, **45** did not result in any visible signs of toxicity or side effects in the drug-exposed animals. These observations may justify more in-depth studies on the pharmacokinetics of **45** and related compounds and on the potential of pyridine *N*-oxide derivatives as anti-coronavirus drugs *in vivo*.

In conclusion, the selective anti-coronavirus activity found for several pyridine *N*-oxide derivatives in cell culture may warrant further pre-clinical investigations to reveal the potential of this class of antiviral drugs as selective inhibitors of coronaviruses, including SARS-CoV infection in humans and Fe-CoV infection in cats.

Acknowledgements

Mrs Lizette van Berckelaer is gratefully acknowledged for excellent technical assistance and Mrs Christiane Callebaut for dedicated editorial help. The compounds were kindly provided by Dr John Lacadie and Dr Jim Pierce at Crompton Corporation (Middlebury, CT, USA). The research was supported by the René Descartes Prize-2001 of the European Commission (no. HPAW-2002-90001) and by the European Research Project SARS-DTV (contract no. SP22-CT-2004).

Transparency declarations

The authors have no financial or any other conflict of interest regarding the contents of the investigations.

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