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Activity of various thiocarboxanilide derivatives against wild-type and several mutant human immunodeficiency virus type 1 strains ☆

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Abstract

A large variety of carboxanilide derivatives in which the original oxathiin moiety present in the prototype compound UC84 was replaced by a non-cyclic lipophilic entity has been evaluated for their inhibitory effect against wild-type human immunodeficiency virus type 1 (HIV-1/III_B) and several mutant viruses derived thereof (i.e. HIV-1/138-Lys, HIV-1/181-Cys, HIV-1/106-Ala and HIV-1/100-Ile). Isopropoxy was the most favorable substituent resulting in molecules that were markedly inhibitory to the wild-type (EC_{50} 0.004–0.04 μg/ml) as well as the mutant HIV-1 strains (EC_{50} 0.06–0.75 μg/ml). In this respect, they proved superior to several other HIV-1-specific non-nucleoside reverse transcriptase inhibitors (NNRTIs) that are currently the subject of clinical trials. One of the most potent HIV-1 inhibitors among the thiocarboxanilide derivatives, namely UC38, selected for a mutant virus strain in which Lys at position 101 and Gly at position 190 of the reverse transcriptase was replaced by Glu.

Keywords: Oxathiin carboxanilide, HIV, Resistance, AIDS

☆ Dedicated to the memory of Dr. Ethel Felauer.

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[1] Deceased April 20, 1994.

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1. Introduction

Numerous classes of human immunodeficiency virus type 1 (HIV-1)-specific inhibitors have been described (Baba et al., 1989; Merluzzi et al., 1990; Pauwels et al., 1990, 1992; Goldman et al., 1991; Romero et al., 1991; Balzarini et al., 1992; Kleim et al., 1993; for an overview, see De Clercq, 1993). They are characterized by a high specificity towards HIV-1 and a relatively low toxicity in cell culture and in patients, and they are targeted at the HIV-1 reverse transcriptase (RT) (De Clercq, 1993). Although they are highly potent and selective as anti-HIV-1 agents, they rapidly lead to the emergence of drug-resistant virus strains, both in vitro and in vivo (Nunberg et al., 1991; Richman et al., 1991; Mellors et al., 1992; Balzarini et al., 1993; for an overview, see De Clercq, 1994). The mutations leading to virus-drug resistance are located in the reverse transcriptase gene and have been well described and characterized (Schinazi et al., 1994). They are located around a hydrophobic pocket of the p66 subunit of HIV-1 RT, and result in resistance of the virus strain to the particular compound in which presence it was selected. Although resistance against the compound concerned may exceed several orders of magnitude, the drug-resistant mutant virus strains may still show either sensitivity or resistance towards various other classes of HIV-1-specific RT inhibitors depending the nature of the amino acid change in their reverse transcriptase (Balzarini et al., 1993a,b).

Oxathiin carboxanilide represents a structurally well-defined class of HIV-1-specific RT inhibitors (Bader et al., 1991). We have evaluated for anti-HIV activity a series of carboxanilide derivatives in which the oxathiin moiety was replaced by a non-cyclic lipophilic entity. The structure–activity relationships (SAR) of the carboxanilide derivatives against wild-type HIV-1 have been previously performed by W.A. Harrison and colleagues from Guelph (Ontario, Canada) and J. Bader from the National Cancer Institute at the National Institutes of Health (Bethesda, MD, USA) (unpublished data). Based on the SAR, a number of test compounds have been selected for investigation against wild-type HIV-1/III_B and several mutant viruses, including HIV-1/138-Lys, HIV-1/181-Cys, HIV-1/106-Ala and HIV-1/100-Ile. The latter mutant virus strains had emerged in the presence of either TSAO-m^3T (HIV-1/138-Lys), pyridinone (HIV-1/181-Cys), nevirapine (HIV-1/106-Ala), or TIBO R82150 (HIV-1/100-Ile) (Balzarini et al., 1993a–c).

We found that several newly developed carboxanilide derivatives were exquisitely inhibitory to wild-type HIV-1/III_B, and also proved markedly inhibitory to the mutant virus strains included in this study.

2. Materials and methods

2.1. Test compounds

The synthesis of the substituted carboxanilide derivatives will be published elsewhere.

2.2. 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 3–4 days.

2.3. Antiviral activity of the test compounds

CEM cells were suspended at 250,000 cells per ml of culture medium and infected with 100 $CCID_{50}$ (1 $CCID_{50}$ being the 50% cell culture infective dose) of HIV-1(III_B) or mutant HIV-1 strains selected for resistance against TIBO R82150 (HIV-1/100-Ile) (Balzarini et al., 1993a), TSAO-m^3T (HIV-1/138-Lys) (Balzarini et al., 1994), nevirapine (HIV-1/106-Ala) (Balzarini et al., 1993b) or pyridinone L 697,661 (HIV-1/181-Cys) (Balzarini et al., 1993a). Then, 100 μl of the infected cell suspensions was added to 200-μl microtiter plate wells containing 100 μl of an appropriate dilution of the test compounds (i.e. 100, 20, 4, 0.8, 0.16, 0.032, 0.006 μg/ml). The inhibitory effect of the test compounds on HIV-1-induced syncytium formation in CEM cells was examined on day 4 post-infection as described previously (Balzarini et al., 1993a,b).

2.4. Selection of a UC38-resistant HIV-1 strain

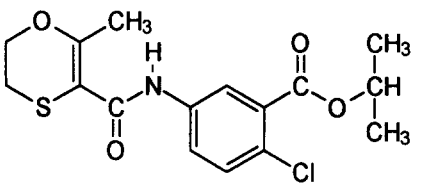
HIV-1(III_B) was subjected to 4 passages in CEM cell cultures (4×10^5 cells/ml) in the presence of 0.5 μg/ml of UC38 in 24-well plates (Falcon, Becton Dickinson). Passages were performed every 3–4 days by adding 0.1 ml of the infected cultures to 0.9 ml of 4×10^5 uninfected CEM cells per ml. As soon as virus-induced cytopathicity became fully prominent in the cell culture, subsequent subcultivations were performed in the presence of increasing amounts of inhibitor (i.e. 2.5, 10 and 20 μg/ml). The highest compound concentration at which the virus-infected CEM cells were subcultivated was 10–20 μg/ml. Virus was further passaged for an additional 9 subcultivations before the supernatant was frozen in aliquots at -70°C and the reverse transcriptase gene of the virus was characterized.

2.5. Determination of the amino acid sequence of the reverse transcriptase of the UC38-resistant HIV-1 strain

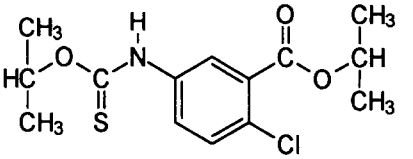
The procedure of CEM cell infection with HIV-1/UC38, preparation of the samples for PCR assays, amplification of the proviral DNA, and sequencing of the 727 bp fragment covering amino acid residues 50–270 have been reported earlier (Balzarini et al., 1993a–c).

3. Results

The prototype compound of the carboxanilide derivatives (designated UC84) consists of an oxathiin moiety (part A), linked to a carboxamide group (part B). The amide



UC 84



UC 38

Fig. 1. Structural formulae of UC84 (NSC 615985) and UC38 (NSC 629243).

function is connected to 2-chlorobenzoic acid (part C) that is esterified with an isopropyl group (part D) (Fig. 1). We have now synthesized and evaluated for anti-HIV activity a series of UC84 derivatives in which the oxathiin (A) part was replaced by various alkoxy groups, and where the D-part was also extensively modified. The B-part of the UC84 molecule was modified from C=O to C=S in virtually all derivatives, whereas the C-part of the UC84 molecule (2-chlorobenzoic acid) was kept unchanged in most instances.

3.1. Modifications of the B-part of the molecule

The C=S (B-part)-containing carboxanilide derivatives proved, as a rule, more inhibitory to HIV-1 replication than the C=O-containing derivatives. Replacing the carbonyl function of the B-part of UC84 by a C=S function (**131**) increased the anti-HIV-1 activity of UC84 by two-fold (Table 1). Also, **45** (containing C=S) in which the oxathiin group was replaced by a  -group in part A of the molecule was 6- to 7-fold more inhibitory to HIV-1 than its C=O counterpart **34**. However, compound UC38 (Fig. 1) that instead of the oxathiin moiety contained an isopropoxy group in part A of the molecule, and that also contains C=S instead of C=O in the B-part, proved almost 60-fold more inhibitory to HIV-1 replication than the corresponding carbonyl derivative **10**. Thus, as a rule, replacement of the group in the B-part (C=O by C=S) increased the antiviral activity, the magnitude of which depending on the nature of the A-part. Introduction of a substituent on the sulfur atom of the C=S moiety of the molecule resulted in a dramatic decrease of the antiviral activity. For example, com-

pounds **53** and **58** in which a methyl or a propyl group was substituted on the sulfur atom, were inhibitory only at concentrations that were 70 to > 1000-fold higher than that of the unsubstituted parent UC38 (EC_{50} 0.009 μg/ml). Thus, an unsubstituted –CS–NH-moiety in the B-part of the molecule is required for optimal anti-HIV activity.

3.2. Modification of the A-part of the molecule

The oxathiin moiety (A-part) of the molecule could be replaced by a number of alkoxy groups without loss of antiviral activity (Table 1). In particular, an isopropoxy group as the A part maintained full antiviral activity (compare UC38 with **131**) (EC_{50} 0.009 and 0.007 μg/ml, respectively). The *tert*-butoxy derivative (**184**) was 100-fold less inhibitory, whereas the *sec*-butoxy derivatives **230** and **231** were only 2- to 3-fold less active. Comparative study of the antiviral activity of a series of compounds in which the isopropoxy moiety (UC38) was replaced by a  (**45**), methoxy (**62**), ethoxy (**54**), *n*-propoxy (**61**), *n*-butoxy (**44**), or *n*-pentoxy (**56**) revealed that the optimal alkoxy chain length was between ethoxy and butoxy (EC_{50} 0.05–0.08 μg/ml). A *sec*-butoxy (**236**) or an allyloxy (**55**) group also maintained the antiviral activity (EC_{50} 0.03–0.06 μg/ml) of the compounds. In contrast, increasing or decreasing the length of the *n*-alkoxy groups or replacing one of the methylene groups by an oxygen (**64**) or sulfur (**87**) severely reduced the antiviral activity (EC_{50} 0.19–1.9 μg/ml).

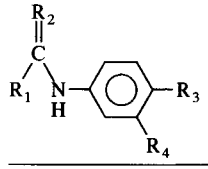
3.3. Modification of the D-part of the molecule

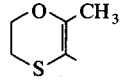
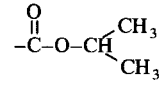
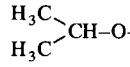
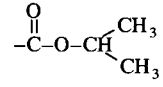
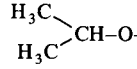
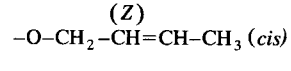
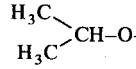
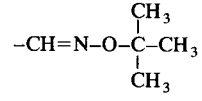
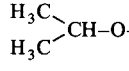
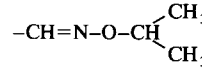
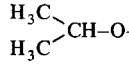
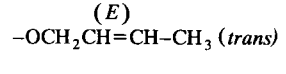
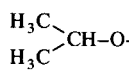
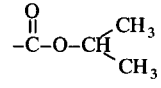
If the optimized A-part (i.e. isopropoxy) of the molecule was kept constant, and the D-part modified (acyl ester, ether or oxime), the isopropoxycarbonyl group emerged as the most efficient D-part of the molecule (EC_{50} 0.007 μg/ml for **131** and 0.009 μg/ml for UC38). Upon replacement of the acyl part by an ethoxycarbonyl (**79**), propoxycarbonyl (**73**), butoxycarbonyl (**74**), isobutoxycarbonyl (**78**) or *tert*-butoxycarbonyl (**97**), 3-pentyloxycarbonyl (**76**), *sec*-butoxycarbonyl (**75**), 2-ethylbutoxycarbonyl (**72**), 2-ethylhexyloxycarbonyl (**99**), cyclopentoxycarbonyl (**127**), cyclopropoxycarbonyl (**129**), diisopropylmethoxycarbonyl (**238**) or allyloxycarbonyl (**192**) the antiviral activity of the test compounds remained within the 0.01–0.08 μg/ml range, but if the D-part was shortened to a methoxycarbonyl (**89**) or trifluoroethoxycarbonyl group (**95**), antiviral efficacy was drastically reduced.

Among the ether derivatives, **3** proved exquisitely effective, being twice as potent an antiviral compound as the corresponding isopropoxycarbonyl derivative **131**. Interestingly, compound **7** in (*E*) (*trans*) configuration proved 10-fold less inhibitory to HIV-1 than its (*Z*) (*cis*) enantiomeric derivative **3**. The presence of a butenyloxy (**264**) or propynyloxy (**221**) function resulted in a 10- to 20-fold decreased antiviral potency, and the antiviral activity further diminished when one of the methylenes in the alkoxy moiety of the D-part of the molecule was replaced by a carbonyl or ester function (i.e. **210**, **211** and **212**).

Also the oxime derivatives **5** and **6**, containing an isopropyl or *tert*-butyl function, showed a favorable antiviral activity, which decreased with shortening the chain length (**85**).

Table 1
Antiviral activity of oxathiin carboxanilide derivatives against wild-type and mutant HIV-1 strains in CEM cells



Compound	R_1	R_2	R_3	R_4	EC_{50} [a] (μg/ml)					CC_{50} [b] (μg/ml)
					III_B	100 Leu → Ile	106 Val → Ala	138 Glu → Lys	181 Tyr → Cys	
UC84		O	Cl		0.015	≥ 40	> 20	≥ 20	15	8.8
UC38		S	Cl		0.009	0.65	0.65	0.39	0.60	12
3		S	Cl		0.004	0.5	0.6	0.09	0.6	5.8
5		S	Cl		0.01	0.60	2.0	0.50	0.50	6.2
6		S	Cl		0.01	0.21	0.45	0.07	0.35	> 100
7		S	Cl		0.04	0.40	0.50	0.10	0.55	5.9
10		O	Cl		0.55	7.5	≥ 10	≥ 10	≥ 10	> 100

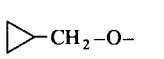
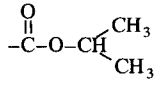
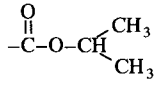
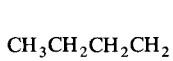
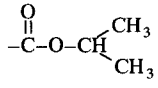
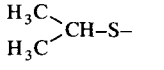
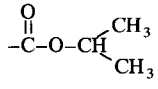
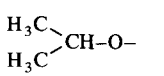
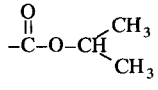
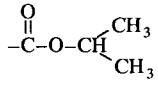
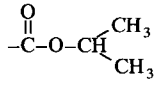
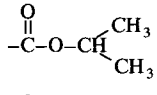
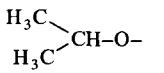
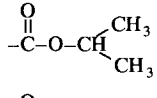
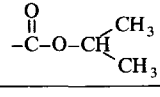
34		O	Cl		0.45	4.0	9.0	6.0	8.5	7.0
44	$CH_3CH_2CH_2CH_2-O-$	S	Cl		0.06	3.5	6.0	2.5	7.0	7.0
45		S	Cl		0.07	2.5	5.5	2.0	6.0	6.9
52		S	Cl		6.5	> 10	> 10	> 10	> 10	28
53 [c]			Cl		> 10	> 10	> 10	> 10	> 10	24
54	CH_3CH_2-O-	S	Cl		0.08	5.0	6.5	6.0	6.0	15
55	$CH_2=CHCH_2-O-$	S	Cl		0.06	4.0	6.5	2.2	5.5	17
56	$CH_3CH_2CH_2CH_2CH_2-O-$	S	Cl		0.55	5.5	10	6.0	6.0	22
58 [d]			Cl		0.65	10	> 10	8.5	> 10	47
61	$CH_3CH_2CH_2-O-$	S	Cl		0.05	2.7	5.5	0.65	5.5	7.0

Table 1 (continued)

Compound	R_1	R_2	R_3	R_4	EC_{50} [a] (μg/ml)					CC_{50} [b] (μg/ml)
					III_B	100 Leu → Ile	106 Val → Ala	138 Glu → Lys	181 Tyr → Cys	
62	CH_3–O–	S	Cl	–C(=O)–O–CH(CH$_3$)(CH$_3$)	1.9	≥ 10	≅ 10	> 10	≥ 10	35
64	$CH_3OCH_2CH_2$–O–	S	Cl	–C(=O)–O–CH(CH$_3$)(CH$_3$)	0.19	6.0	7.0	6.0	≥ 10	20
72	$(H_3C)_2CH$–O–	S	Cl	–C(=O)–O–CH_2CH(CH_2CH_3)(CH_2CH_3)	0.03	0.50	0.85	0.50	2.5	6.5
73	$(H_3C)_2CH$–O–	S	Cl	–C(=O)–O–$CH_2CH_2CH_3$	0.01	3.1	0.8	0.45	1.8	11
74	$(H_3C)_2CH$–O–	S	Cl	–C–O–$CH_2CH_2CH_2CH_3$	0.04	0.60	0.70	0.55	0.60	12
75	$(H_3C)_2CH$–O–	S	Cl	–C(=O)–O–CH(CH_2CH_3)(CH_3)	0.02	0.60	1.0	0.55	0.85	6.0
76	$(H_3C)_2CH$–O–	S	Cl	–C(=O)–O–CH(CH_2CH_3)(CH_2CH_3)	0.04	0.70	4.0	0.60	4.0	5.5
77	$(H_3C)_2CH$–O–	S	CH_3	–C(=O)–O–CH_2CH_3	0.03	7.0	≥ 10	4.0	8.5	> 100
78	$(H_3C)_2CH$–O–	S	Cl	–C(=O)–O–CH_2CH(CH_3)(CH_3)	0.01	0.90	0.85	0.55	2.0	10

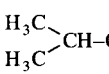
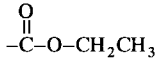
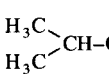
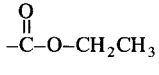
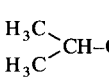
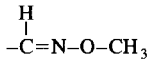
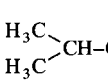
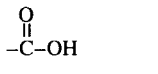
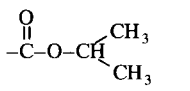
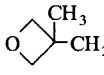
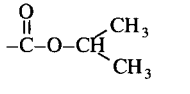
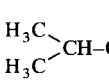
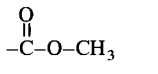
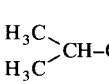
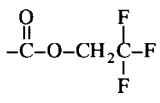
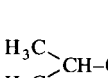
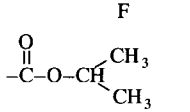
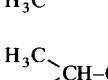
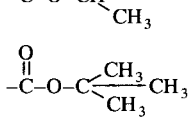
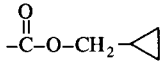
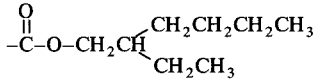
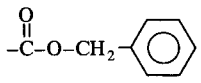
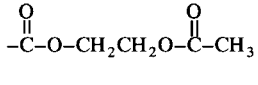
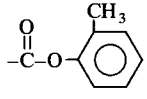
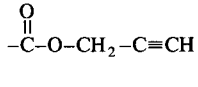
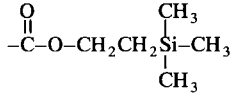
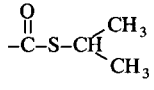
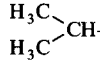
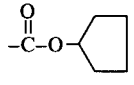
79		S	Cl		0.03	3.2	5.0	0.75	4.5	9.2
84		S	SCH_3		0.06	3.5	5.0	4.0	5.0	24
85		S	Cl		0.06	2.6	6.0	0.7	5.0	> 100
86		S	Cl		> 10	> 10	> 10	> 10	> 10	> 100
87	$CH_3SCH_2CH_2-O-$	S	Cl		0.45	5.0	5.5	5.0	6.0	84
88		S	Cl		> 10	> 10	> 10	> 10	> 10	26
89		S	Cl		0.50	6.0	≥ 10	7.5	≥ 10	42
95		S	Cl		0.35	7.5	> 10	7.0	> 10	56
96		S	SCH_3		0.04	0.85	6.0	0.75	1.7	> 100
97		S	Cl		0.04	5.0	5.0	1.4	6.0	4.9

Table 1 (continued)

Compound	R_1	R_2	R_3	R_4	EC_{50} [a] (μg/ml)					CC_{50} [b] (μg/ml)
					III_B	100 Leu → Ile	106 Val → Ala	138 Glu → Lys	181 Tyr → Cys	
98		S	Cl		0.04	0.70	2.7	0.60	1.4	6.3
99		S	Cl		0.08	0.85	10	7.5	6.5	16
100		S	Cl		0.35	5.5	8.5	0.65	5.5	> 100
101		S	Cl		1.0	≥ 10	> 10	> 10	≥ 10	17
102		S	Cl		0.40	5.5	≥ 10	7.5	≥ 10	47
103		S	Cl		0.29	6.0	≥ 10	6.0	≥ 10	16
110		S	Cl		0.20	2.5	5.5	7.0	1.8	19
113		S	Cl		0.02	0.55	1.9	0.40	0.60	9.0
127		S	Cl		0.02	0.75	0.55	0.60	0.55	4.7

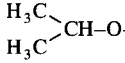
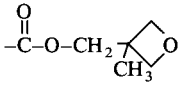
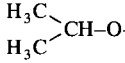
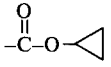
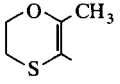
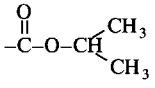
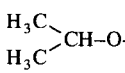
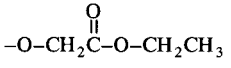
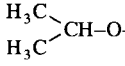
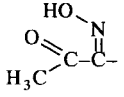
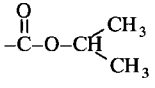
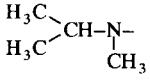
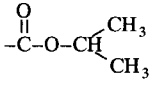
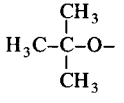
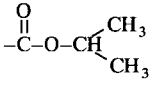
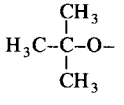
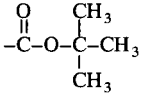
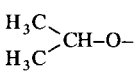
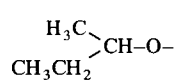
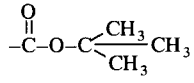
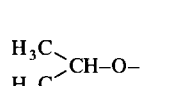
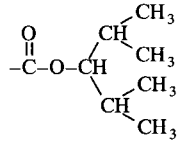
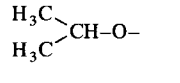
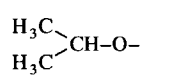
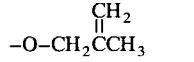
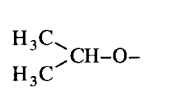
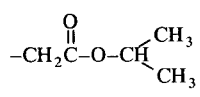
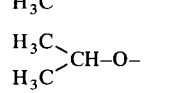
128		S	Cl		0.05	0.70	0.85	0.70	0.50	7.1
129		S	Cl		0.04	0.90	2.0	0.65	0.80	15
131		S	Cl		0.007	0.45	0.65	0.50	0.50	16
143		S	Cl		0.39	5.0	2.0	5.0	7.7	41
144		S	Cl		0.21	3.0	2.0	1.5	3.0	4.4
160		O	Cl		0.65	≥ 10	2.0	≥ 10	6.0	39
171		S	Cl		> 10	> 10	> 10	> 10	> 10	> 100
184		S	Cl		0.60	≥ 10	0.85	5.5	6.5	29
191		S	Cl		0.65	> 10	1.0	0.65	> 10	42
192		S	Cl		0.04	0.70	0.10	0.14	0.85	21

Table 1 (continued)

Compound	R_1	R_2	R_3	R_4	EC_{50} [a] (μg/ml)					CC_{50} [b] (μg/ml)
					III_B	100 Leu → Ile	106 Val → Ala	138 Glu → Lys	181 Tyr → Cys	
193	$(H_3C)_2CH-O-$	S	Cl	$-S-CH_2CH=CH_2$	0.04	0.70	0.50	0.09	4.5	21
203	$(H_3C)_2CH-O-$	S	Cl	$-CH_2O-C(CH_3)_3$	4.5	> 10	≥ 10	≥ 10	> 10	11
210	$(H_3C)_2CH-O-$	S	Cl	$-O-C(=O)-O-CH(CH_3)_2$	0.40	7.0	3.5	1.0	6.0	75
211	$(H_3C)_2CH-O-$	S	Cl	$-O-CH_2C(=O)-CH_3$	0.55	4.5	5.0	4.0	7.5	20
212	$(H_3C)_2CH-O-$	S	Cl	$-O-CH(CH_3)C(=O)-O-CH_2CH_3$	0.65	6.5	1.5	2.0	8.5	71
221	$(H_3C)_2CH-O-$	S	Cl	$-O-CH_2C\equiv CH$	0.08	4.0	0.5	0.7	3.0	4.6
228	$(H_3C)_2CH-O-$	S	Cl	$-O-CH_2C(CH_3)=N-O-CH_3$	0.07	1.0	2.0	0.50	4.0	5.6
230	$H_3C-CH(CH_2CH_3)-O-$	S	Cl	$-C(=O)-O-CH(CH_3)_2$	0.02	0.30	0.40	0.09	0.60	10
231	$H_3CCH_2-CH(CH_3)-O-$	S	Cl	$-C(=O)-O-CH(CH_3)_2$	0.03	0.70	0.40	0.07	0.65	9.8

236		S	Cl		0.03	0.65	0.35	0.12	0.50	8.0
238		S	Cl		0.07	> 1	≥ 1	≥ 1	> 1	7.6
253		S	–C≡N	–O–CH_2CH=CH_2	0.05	≥ 1	0.40	0.60	0.65	3.8
264		S	Cl		0.09	> 1	4.0	0.70	> 1	11
265		S	OCH_3		0.008	0.75	0.24	0.06	0.55	7.7
271		S	OCH_3	–O–CH_2CH=CHCH_3	0.04	3.0	2.0	1.47	3.7	7.1
Nevirapine	–	–	–	–	0.007	0.03	2.3	2.3	0.10	> 20
TIBO R82913	–	–	–	–	0.016	0.3	2.0	0.5	1.7	> 20

[a] 50% Effective concentration, or compound concentration required to reduce HIV-1-induced giant cell formation in CEM cell cultures by 50%.

[b] 50% Cytotoxic concentration, or compound concentration required to reduce viability of the host cells by 50%.

[c] The amide carbon R_1–C[S]–N– has now changed to R_1–C[S–CH_3]=N–.

[d] The amide carbon R_1–C[S]–N(H)– has now changed to R_1–C[S–C_3H_7]=N–.

3.4. Modification of the C-part of the molecule

Substitution of the chloro group in the C-part of the molecule by a methyl, methylthio, cyano or methoxy group (i.e. **77**, **84**, **96**, **253**, **265** and **271**) also gave antivirally active molecules. These data suggest that the chlorine in the C-part is not an absolute requirement for anti-HIV activity.

Compound **86**, that represents the free acid of one of the most active carboxanilide derivatives (UC38), was devoid of any antiviral activity. This observation suggests that the nature of the ester moiety may play an important role in the eventual antiviral activity of the carboxanilide derivative in vitro, but also that the ester derivatives can be inactivated upon hydrolysis in vivo and thus rapidly loose their antiviral activity. Therefore, enzymatically more stable UR compounds such as the ether (**3**) or the oxime (**5**, **6**) derivatives may turn out to be more potent HIV-1 inhibitors in vivo than the ester derivatives.

3.5. Activity against mutant HIV-1 strains

Although very active against the wild-type virus (HIV-1/III_B), UC84 proved more than 1000-fold less effective against a series of mutant HIV-1 strains containing the 138 Glu → Lys, 181 Tyr → Cys, 106 Val → Ala or 100 Leu → Ile mutation in the reverse transcriptase (Table 1). Replacing the carbonyl function in part B of the molecule by C=S (**131**) increased the activity against these mutant virus strains by at least 1–2 orders of magnitude, whereas the same modification resulted in no more than a two-fold increase in potency against the wild-type virus. Also, when the C=O moiety of part B of the molecule was replaced by C=S in the other UC derivatives (i.e. **10**), activity against the mutant viruses was markedly increased (i.e. UC38). However, the C=S function is not the only factor that may determine inhibitory activity of the compounds against the mutant virus strains, since compounds **34** and **45** showed only poor activity against these mutant virus strains. Thus, the C=S function in part B may act, at least partially, in concert with part A in determining the anti-HIV activity of the molecule. Interestingly, UC38, that is structurally similar to **131** except for the presence of an isopropoxy instead of an oxathiin moiety in part A, showed activity against the mutated viruses that was comparable to **131**.

In general, an unbranched alkoxy chain in the A-part of the compounds resulted in poor, if any, inhibitory activity against the mutant viruses (compare **54**, **55**, **56**, **61**, **62**, **64** and **87** with UC38), whereas their activity against wild-type virus was much more pronounced and highly dependent on the length of the alkoxy chain (as mentioned above).

In contrast, the nature of the ester moiety in the D-part of the molecule could be varied to a marked extent without impairing activity against the mutant viruses. For example, the butoxycarbonyl (**74**) and allyloxycarbonyl (**192**) derivative, but not the propynyloxycarbonyl (**103**) or *tert*-butoxycarbonyl (**203**) derivative of UC38, showed pronounced activity against the mutant viruses, as did the parent compound UC38. In other cases, slight changes in the ester part of the drugs (i.e. 2-ethylbutoxycarbonyl (**72**), 3-pentyloxycarbonyl (**76**) or isobutoxycarbonyl (**75** and **78**)) led to activity against some

Table 2
Activity of test compounds against wild-type and mutant HIV-1 strains in CEM cells

Compound	EC_{50} ($\mu g/ml$) [a]	
	HIV-1/III_B [b] (101-Lys)	HIV-1/UC38 [c] (101-Glu + 190-Glu)
UC84	0.015 ± 0.007	> 2.5
UC38	0.008 ± 0.002	> 2.5
3	0.010 ± 0.0	⩾ 2.5
5	0.035 ± 0.025	> 2.5
6	0.013 ± 0.004	> 2.5
7	0.004 ± 0.0	1.5 ± 0.0
Pyridinone L 697,661	0.007 ± 0.003	0.035 ± 0.015
TSAO-m^3T	0.030 ± 0.010	> 2.5
BHAP U-88204	0.009 ± 0.005	1.0 ± 0.0
Nevirapine	0.007 ± 0.0	> 2.5
TIBO R 82913	0.02 ± 0.01	> 2.5

[a] See footnote to Table 1.
[b] HIV-1/III_B containing amino acid Lys at RT position 101 (codon AAA).
[c] HIV-1/UC38 containing amino acid Glu at RT position 101 (codon GAA) and amino acid Glu at RT position 190 (codon GAA).

mutant viruses (i.e. the HIV-1 RT 138 Glu → Lys and 100 Leu → Ile mutants), but not against the others (i.e. the HIV-1 RT 181 Tyr → Cys and 106 Val → Ala mutants) (Table 1).

3.6. Development of HIV-1 resistance against UC38 in cell culture

A drug-resistant HIV-1 mutant strain was selected in the presence of escalating concentrations of UC38. In this drug-resistant HIV-1 strain, two mutation were found in the amino acid sequence between position 50 and 270 of the reverse transcriptase; namely, substitution of Lys at position 101 (codon AAA) by Glu (codon GAA) and Gly at position 190 (codon GGA) by Glu (codon GAA). This mutant HIV-1 strain proved virtually resistant to all HIV-1-specific RT inhibitors, including 6 carboxanilide derivatives, TSAO-m^3T, BHAP, nevirapine and TIBO (Table 2). Only against pyridinone L 697,661 did it retain marked sensitivity.

4. Discussion

From a series of 70 compounds structurally related to UC84, in which the oxathiin moiety had been replaced by a non-cyclic (mostly alkoxy) entity, various congeners emerged with a potency against wild-type HIV-1(III_B) that was comparable to that of UC84. Several compounds showed a markedly more pronounced efficacy against HIV-1

RT (100 Leu → Ile, 106 Val → Ala, 138 Glu → Lys or 181 Tyr → Cys) mutant virus strains than UC84. In particular, compounds UC38, **3**, **6**, **7**, **74**, **127**, **230**, **231**, **236** and **265** showed a favorable activity spectrum against both wild-type and mutant HIV-1 strains. The EC_{50} of these compounds for the wild-type virus ranged from 0.004 to 0.04 $\mu g/ml$, and for the mutant virus strains from 0.06 to 0.75 $\mu g/ml$.

In no case was activity greater against the mutant virus than against the wild-type HIV-1. Although the UC compounds were in general equally effective against wild-type HIV-1 as the current clinically investigated HIV-1-specific inhibitors, they clearly showed a considerably more favorable antiviral spectrum against the mutant viruses included in our study (Table 1). This may make this class of HIV-1-specific RT inhibitors attractive candidate compounds for further investigation as specific anti-HIV agents, particularly for their efficacy against HIV-1 mutant strains that are resistant to other drugs.

It also became clear from this study that subtle changes in the structure of the test compounds resulted in dramatic changes in their antiviral activity, the structure–activity relationship of the test compounds for wild-type virus not being identical to their structure–activity relationship for the mutant viruses.

A mutant virus strain selected for resistance in the presence of UC38 in HIV-1(III_B)-infected CEM cell cultures emerged within 3–4 subcultivations, that is at a speed which is very similar to the emergence of mutant HIV-1 strains resistant to other HIV-1-specific RT inhibitors such as nevirapine, TIBO R82150, pyridinone, BHAP and TSAO (Balzarini et al., 1993a,b). The mutant virus strain contained a 101 Lys → Glu + 190 Gly → Glu mutation in its RT. Surprisingly, this mutant virus strain remained highly sensitive to pyridinone L 697,661, while being resistant to all other HIV-1-specific RT inhibitors evaluated (Table 2). The reason for the anomalous behaviour of the pyridinone against this mutant virus strain is unclear. Also, mutations at positions 100, 101 and 103 of the reverse transcriptase were found in virus strains that had been passed in the presence of other carboxanilide derivatives (data not shown).

In conclusion, several derivatives of the oxathiin carboxanilide prototype UC84 in which the oxathiin moiety was replaced by an alkoxy group proved to be potent inhibitors of both wild-type and mutant HIV-1 strains. The structure–activity relationship of the compounds for the wild-type virus was not identical to that for the mutant virus strains. This points to the importance of extending anti-HIV assays with HIV-1-specific RT inhibitors to HIV-1 mutant strains containing drug-resistant mutations in their RT gene.

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3.3010.91), the Belgian Geconcerteerde Onderzoeksacties (project 90/94-2), the Medical Faculty of the Karolinska Institute, the Swedish Society of Medicine and the Swedish Physicians against AIDS. We also acknowledge Uniroyal Chemical (Middlebury, CT, USA) for supplying the compounds used in this study. We are grateful to Ann Absillis and Birgitta Wallström for excellent technical assistance, Dr. Jef Rozenski for drawing the figures and Christiane Callebaut for fine editorial help.

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