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Identification of Novel Thiocarboxanilide Derivatives That Suppress a Variety of Drug-Resistant Mutant Human Immunodeficiency Virus Type 1 Strains at a Potency Similar to That for Wild-Type Virus

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Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium,[1] *and Uniroyal Chemical Company, Ltd., Guelph, Ontario, Canada N1E 5L7*[2]

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A large variety of carboxanilide and thiocarboxanilide derivatives in which the original oxathiin or aliphatic moieties present in the prototype compounds UC84 and UC38 were replaced by an (un)substituted furanyl, thienyl, phenyl, or pyrrole entity have been evaluated for activity against wild-type human immunodeficiency virus type 1 strain III_B [HIV-1(III_B)] and a series of mutant virus strains derived thereof. The mutant viruses contained either the Leu-100→Ile, Lys-103→Asn, Val-106→Ala, Glu-138→Lys, Tyr-181→Cys, or Tyr-188→Leu mutation in their reverse transcriptase. Several 3-(2-methylfuranyl)- and 3-(2-methylthienyl)-thiocarboxanilide ester, (thio)ether, and oxime ether derivatives showed exquisitely potent antiviral activity against wild-type HIV-1 (50% effective concentration, 0.009 to 0.021 μM). The pentenylethers of the 2-methylfuranyl and 2-methylthienyl derivatives (i.e., 313, *N*-[4-chloro-3-(3-methyl-2-butenyloxy)phenyl]-2-methyl-3-furancarbothioamide or UC-781, and 314, *N*-[4-chloro-3-(3-methyl-2-butenyloxy)phenyl]-2-methyl-3-thiophenecarbothioamide or UC-82) proved virtually equally inhibitory for wild-type and the Ile-100, Ala-106, and Lys-138 mutant virus strains (50% effective concentration, 0.015 to 0.021 μM). Their inhibitory effect against the Asn-103 and Cys-181 reverse transcriptase mutant virus strains was decreased only four- to sevenfold compared with wild-type virus. UC-781 and UC-82 should be considered potential candidate drugs for the treatment of HIV-1-infected individuals.

Some 5 years ago, the first specific inhibitors of human immunodeficiency virus type 1 (HIV-1) were identified: HEPT [1-(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (2, 19) and TIBO derivatives (tetrahydroimidazo-[4,5,1-*jk*][1,4]-benzodiazepin-2-(1*H*)-one and -thione) (23). Later, when various other structural classes of HIV-1-specific inhibitors were described, they were termed "nonnucleoside reverse transcriptase inhibitors" (NNRTIs) (12), and some NNRTIs have been subjected to clinical trials (1, 3, 9, 13, 14, 16, 18, 22, 25). All these structurally different classes of HIV-1-specific inhibitors are targeted at a nonsubstrate binding site of the reverse transcriptase (RT) of HIV-1 (11, 15, 17, 20, 24, 26).

The potent and selective anti-HIV-1 activity of oxathiin (thio)carboxanilides (3, 4, 5, 10) has also been reported. The prototype of this class of compounds, designated UC84, consists of an oxathiin moiety linked through a carboxamide group to the isopropyl ester of 2-chlorobenzoic acid (Fig. 1). We recently reported on the structure-activity relationship of a series of novel oxathiin (thio)carboxanilides in which the oxathiin moiety was either kept unchanged (5) or replaced by an aliphatic entity (4). These investigations resulted in the identification of novel thiocarboxamide derivatives that proved superior to the prototype compound UC84 in their activity against wild-type and several mutant viruses. We now report on a variety of (thio)carboxamide derivatives in which the oxathiin moiety was replaced by (un)substituted thienyl, furanyl, phenyl, or pyrrole groups and which contain an ester, ether, thioether, or oxime ether function at the 2-chlorobenzoic acid moiety of the molecule. From these studies emerged a series of highly potent and selective inhibitors of HIV-1 replication that are among the most potent NNRTIs described so far (50% effective concentration in cell culture [EC_{50}], 0.009 to 0.027 μM). These novel compounds have the unique property of equally suppressing wild-type and several mutant virus strains that contain in their RT those mutations (Leu-100→Ile, Lys-103→Asn, Val-106→Ala, Glu-138→Lys, and Tyr-181→Cys) that are known to engender resistance to NNRTIs.

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MATERIALS AND METHODS

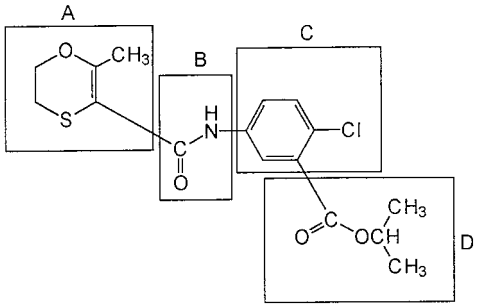
Test compounds. The synthesis of the substituted carboxanilide and thiocarboxanilide derivatives will be published elsewhere.

Cells. Human lymphocyte CEM cells were obtained from the American Type Culture Collection and grown in RPMI 1640 medium supplemented with 10% (vol/vol) inactivated fetal calf serum (Gibco), 2 mM L-glutamine (Flow Laboratories), and 0.075% (vol/vol) $NaHCO_3$ (Flow Laboratories). Cells were subcultured every 3 to 4 days.

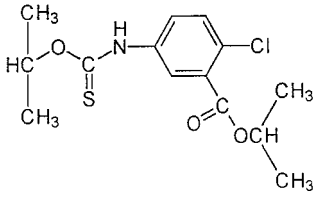
Cytostatic activity of test compounds in cell culture. All assays were performed in 96-well microtiter plates (Falcon 3072; Becton Dickinson, Paramus, N.J.). To each well were added $\sim 6 \times 10^4$ CEM cells (100 μl) and a given amount of the test compound (100 μl). The cells were allowed to proliferate for 96 h at 37°C 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 (National Cancer Institute, Bethesda, Md.). The selection and characterization of the mutant HIV-1 strains have been published previously (6–9). The following virus strains were included in this study: HIV-1/Ile-100, selected for resistance to TIBO R82150 (8); HIV-1/Asn-103, selected for resistance to TIBO R82913 (8); HIV-1/Ala-106, selected for resistance to nevirapine (7); HIV-1/Lys-138, selected for resistance to TSAO-m^3T ([2′,5′,-bis-*O*-(tert-butyldimethylsilyl)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)]-β-D-pentofuranosyl-N^3-methylthymine) (8, 9); HIV-1/Cys-181, selected for resistance to pyridinone L-697,661 (8); and HIV-1/His-188, selected for resistance to HEPT (6). The latter mutant virus strain converted to HIV-1/Leu-188 upon further passage in the absence of the test compound.

Antiviral activity of test compounds in cell cultures. CEM cells were suspended at ≈300,000 cells per ml of culture medium and infected with approximately 100 50% cell culture infective doses of HIV-1(III_B) or mutant HIV-1 strains. Then, 100 μl of the infected cell suspensions was added to 200-μl



UC-84



UC-38

FIG. 1. Structural formula of UC84 and UC38.

microtiter plate wells containing 100 μl of appropriate serial (fivefold) dilutions of the test compounds or human serum (5, 12.5, 25, and 50% final concentration). The inhibitory effect of the test compounds or human serum on HIV-1-induced syncytium formation in CEM cells was examined microscopically on day 4 postinfection. The EC_{50} was defined as the compound concentration that inhibits syncytium formation in the HIV-1-infected cell cultures by 50%.

Stability of thiocarboxanilides in human serum. The thiocarboxanilides 4 (UC-10), 313 (UC-781), and 314 (UC-82) were added to human serum at a concentration of 60 μM and incubated for 24 h at 37°C. Then, methanol was added to the serum samples to reach a final concentration of 66%, and samples were kept on ice for 20 min and centrifuged at 13,000 rpm (Zentrifuge 5412; Eppendorf) during 10 min to precipitate serum proteins. The supernatants were then analyzed by high-pressure liquid chromatography on a reverse-phase C_8 column, with a gradient from 5% acetonitrile in water to 90% acetonitrile in water. Under our experimental conditions, compounds 4, 313, and 314 eluted between 21 and 22 min.

RESULTS

Effect of replacement of the carbonyl oxygen by a sulfur atom in the carboxamide (B) part of the molecule on the antiviral potency of the test compounds. Replacement of the carbonyl oxygen atom (part B) by a sulfur atom generally resulted in an increase in antiviral activity (Table 1). The extent by which the antiviral activity increased differed between the wild-type and mutant HIV-1 strains and was clearly dependent on the nature of the A part of the molecule. The most striking effects were observed with a 2-methylfuranyl substituent in the A part of the molecule, where replacement of the carbonyl oxygen by a sulfur atom resulted in a 25- to 70-fold increase in antiviral potency of the test compound against wild-type virus (compare 282 with 281, 63 with 31, 217 with 219, 214 with 224, 243 with 242, and 263 with 257) (Table 1).

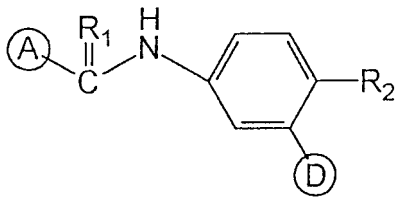
Also, if the heterocycle (A) part of the molecule contained a 2- or 3-methyl-substituted thienyl moiety, the antiviral potency was increased by 10- to 20-fold in most cases (i.e., compare 222 with 223, 284 with 283, 287 with 286, 306 with 303, and 331 with 330), although only a twofold difference in antiviral activity was noted between compounds 115 and 90. However, the increase in activity of the thioamides was the least pronounced when the A part of the molecule consisted of an (un)substituted phenyl moiety. Indeed, the increase in antiviral potency was 8- to 15-fold for compounds 168 (compare with 167), 94 (compare with 93), and 57 (compare with 28) and 4-fold for compound 149 (compare with 148), and no difference in antiviral potency was observed for the 2-aminophenyl derivatives 172 and 173 (Table 1).

The thiocarboxanilides containing 2-methylfuranyl or 2- and 3-methylthienyl or 2-fluoro- or 2-methoxy-substituted phenyl moieties showed a comparable increase in antiviral potency against wild-type and mutant viruses. This increase in activity against the mutant viruses was less pronounced for the compounds containing an unsubstituted phenyl or 2-amino- or 2-methylphenyl substituent (compare compounds 57 with 28, 173 with 172, and 149 with 148). The invariably higher antiviral activity of the thiocarboxamide derivatives over the carboxamide derivatives led us to further investigate the structure-activity relationship of substituents in the A, C (phenyl), and D (ester, ether, or oxime ether) moieties (Fig. 1), for only thiocarboxamide derivatives.

Effects of different substituents at the phenyl moiety of the phenyl (C) part of the molecule on the antiviral potency of the test compounds. The 2-chloro substituent at the phenyl moiety of the C part of the molecule was replaced by a methoxy, cyano, or methyl group in a limited number of test compounds. Replacement of 2-chloro by 2-methoxy (compound 282) increased the antiviral potency of the furanyl derivative 177 against wild-type virus by 4.5-fold but did not markedly alter the activity against the mutant viruses (except for the Ile-100 RT mutant virus that acquired a 10-fold higher sensitivity to the inhibitory effect of the compound). In contrast, the antiviral activity of the 3-methylthienyl derivatives 233, 232, and 268 remained mainly unchanged, both against wild-type virus and against mutant viruses when the chloro entity was replaced by cyano or methoxy. Also, a cyano substitution at the 2 position of the phenyl moiety did not alter the antiviral potency of the test compounds compared with the chloro-substituted derivative (compare compound 269 with 227) (Table 2). These data are in agreement with our previous observations in which replacement of 2-chloro by 2-SCH_3 or 2-C≡N or 2-OCH_3 did not markedly improve the antiviral activity of the test compounds or even diminished the antiviral potency of the test compounds in some cases (4, 5).

Effect of the nature of the heterocycle (A) part of the molecule on the antiviral potency of the test compounds. While keeping the B and C parts of the molecule unchanged (i.e., all compounds being 2-chloro-thiocarboxanilide derivatives), various substituents were introduced in the A part of the molecule. Irrespective of the nature of the D part of the molecule (i.e., cyclohexyl ester, isopropyl ester, butenyl ether or thiobutenyl ether, *t*-butyl oxime, or branched pentenyl or pentyl ether), the 3-(2-methylfuranyl) and 3-(2-methylthienyl) derivatives proved consistently superior to all other derivatives with substituents in the A part of the molecule (EC_{50}, 0.009 to 0.027 μM) [i.e., compounds 2, 162, 63, 309, 322, 313, and 315 (furanyl derivatives) and compounds 310, 331, 314, and 316 (thienyl derivatives), except for the *t*-butyl oxime and the branched heptyl ester derivatives of the 3-(2-methylfuranyl) analogs, which showed an EC_{50} of 0.142 μM (compound 4) and 0.076 μM (compound 226), respectively]. The 3-(2-methyl-4,5-dihydrofuranyl) derivative 304 of the 3-(2-methylfuranyl)thiocarboxanilide derivative 4 proved eightfold more inhibitory to wild-type HIV-1 than its unsaturated parent, 4. Also, the

TABLE 1. Comparative antiviral activity of thiocarboxanilide and carboxanilide derivatives



Code	Structure of the compound[a]				EC_{50}[b,c] (μM)							CC_{50}[b,d] (μM)
	A	R_1	R_2	D	WT[e]	Ile-100	Asn-103	Ala-106	Lys-138	Cys-181	Leu-188	
281	3-(2-me-Fur)-	O	-OMe	$-CH=N-OCH_3$	0.805	≥35	≥35	≥35	≥35	≥35	≥35	>347
282	3-(2-me-Fur)-	S	-OMe	$-CH=N-OCH_3$	0.023	0.495	3.9	0.46	0.132	1.9	3.1	>329
31	3-(2-me-Fur)-	O	-Cl	$-C(=O)-O-CH(CH_3)_2$	1.4	20		20	11	19		12
63	3-(2-me-Fur)-	S	-Cl	$-C(=O)-O-CH(CH_3)_2$	0.020	1.7		1.7	1.7	0.580		15
219	3-(2-me-Fur)-	O	-Cl	$-C(=O)-O-CH_2-C(CH_3)=CH_2$	0.45	15		3.0	3.0	5.7		11
217	3-(2-me-Fur)-	S	-Cl	$-C(=O)-O-CH_2-C(CH_3)=CH_2$	0.017	2.2		0.084	0.084	0.286		19
224	3-(2-me-Fur)-	O	-Cl	$-CH_2O-C(CH_3)_2-CH_3$	2.2	26		17	12	≥31		19
214	3-(2-me-Fur)-	S	-Cl	$-CH_2O-C(CH_3)_2-CH_3$	0.087	2.5		0.84	0.232	1.6		52
242	3-(2-me-Fur)-	O	-Cl	$-C(=O)-O-C(CH_3)$-cyclopropyl	1.7	29		14	>29	2.9		128
243	3-(2-me-Fur)-	S	-Cl	$-C(=O)-O-C(CH_3)$-cyclopropyl	0.024	1.7		0.216	0.486	0.297		46

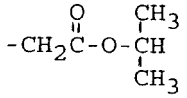
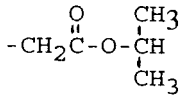
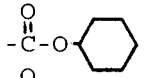
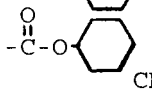
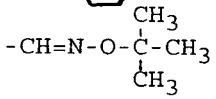
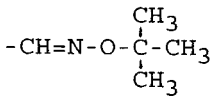
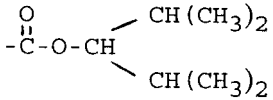
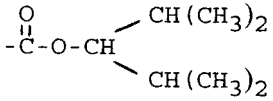
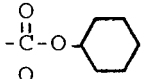
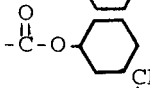
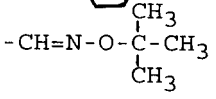
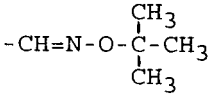
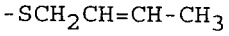
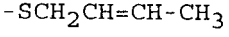
257	3-(2-me-Fur)-	O	-OMe		1.2	>30		19	>30	≥30		156
263	3-(2-me-Fur)-	S	-OMe		0.026	3.8		0.580	0.232	1.4		>288
90	2-(3-me-Thie)-	O	-Cl		0.494	14		18	16	13		17
115	2-(3-me-Thie)-	S	-Cl		0.225	1.6		1.5	1.0	0.87		37
223	2-(3-me-Thie)-	O	-Cl		0.644	13		4.5	4.4	8.4		39
222	2-(3-me-Thie)-	S	-Cl		0.027	0.540		0.540	0.135	0.540		32
283	3-(2-me-Thie)-	O	-Cl		0.400	≥5.0	≥5.0	≥5.0	≥5.0	2.4	≥5.0	10
284	3-(2-me-Thie)-	S	-Cl		0.019	0.72	1.5	0.552	0.26	0.408	>4.8	14
286	3-(2-me-Thie)-	O	-Cl		0.078	1.8	≥5.2	0.572	1.9	0.702	>5.2	15
287	3-(2-me-Thie)-	S	-Cl		0.007	0.085	0.475	0.060	0.050	0.075	>5.0	15
303	3-(2-me-DHPyr)-	O	-Cl		0.364	1.3	1.7	1.4	1.4	0.92	>5.6	27
306	3-(2-me-DHPyr)-	S	-Cl		0.035	0.148	0.216	0.067	0.108	0.067	>5.4	27
330	3-(2-me-Thie)-	O	-Cl		0.232	1.9	2.3	0.812	1.5		>5.9	14
331	3-(2-me-Thie)-	S	-Cl		0.017	0.090	0.22	0.078	0.070	0.070	≥5.6	14

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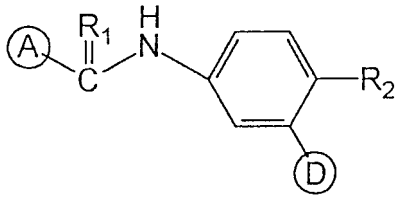
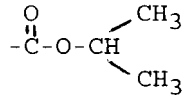
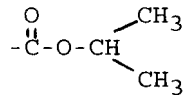
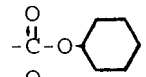
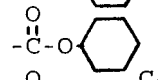
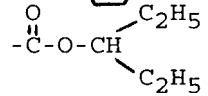
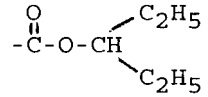
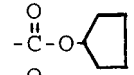
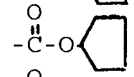
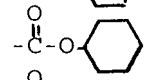
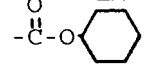
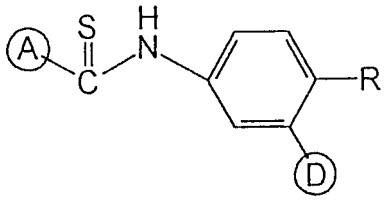
TABLE 1—*Continued*



Code	Structure of the compound[a]				EC_{50}[b,c] (μM)							CC_{50}[b,d] (μM)
	A	R_1	R_2	D	WT[e]	Ile-100	Asn-103	Ala-106	Lys-138	Cys-181	Leu-188	
28	Phe	O	-Cl		1.3	11		16	16	17		19
57	Phe	S	-Cl		0.15	13		15	6.6	15		13
167	2-F-Phe	O	-Cl		1.6	≥27		5.3	11	13		84
168	2-F-Phe	S	-Cl		0.102	0.765		0.663	0.229	0.637		48
172	2-NH_2-Phe	O	-Cl		1.7	>28		25	>28	≥28		69
173	2-NH_2-Phe	S	-Cl		1.9	≥26		≥26	15	>26		75
93	(2-MeO-Phe)-	O	-Cl		1.2	13		23	15	23		>267
94	(2-MeO-Phe)-	S	-Cl		0.102	1.7		1.7	1.5	8.9		26
148	(2-me-Phe)-	O	-Cl		0.511	6.7		17	15	6.7		14
149	(2-me-Phe)-	S	-Cl		0.124	1.5		7.7	7.7	4.1		10

[a] Abbreviations: me-Fur, methylfuranyl; me-Thie, methylthienyl; Phe, phenyl; F-Phe, fluorophenyl; NH_2-Phe, aminophenyl; MeO-Phe, methoxyphenyl; me-Phe, methylphenyl; OMe, methoxy; DHPyr, 6-methyl-3,4-dihydro-2H-pyran-5-yl.
[b] Data are means of two to three independent experiments.
[c] Compound concentration required to inhibit virus-induced cytopathicity by 50%.
[d] Compound concentration required to inhibit CEM cell proliferation by 50%.
[e] WT, wild type.

TABLE 2. Antiviral activity of thiocarboxanilide derivatives with modifications in the phenyl (C) part of the molecule



Code	Structure of the compound[a]			EC_{50}[b,c] (μM)							CC_{50}[b,d] (μM)
	A	R	D	WT[e]	Ile-100	Asn-103	Ala-106	Lys-138	Cys-181	Leu-188	
177	3-(2-me-Fur)-	-Cl	-C(H)=N-O-CH_3	0.096	7.4		1.6	0.272	5.1		77
282	3-(2-me-Fur)-	-OCH_3	-C(H)=N-O-CH_3	0.023	0.66	3.9	0.462	0.129	1.9	3.1	>329
269	3-(2-me-Fur)-	-OCH_3	-O-CH_2-CH=CH_2	0.056	9.6		2.6	0.297	2.8		>330
227	3-(2-me-Fur)-	-C≡N	-O-CH_2-CH=CH_2	0.050	20		1.5	0.302	10		>336
157	3-(2-me-Fur)-	-CH_3	-C(=O)-O-CH_2CH_3	0.030	2.9		2.3	1.2	1.9		53
233	2-(3-me-Thie)	-Cl	-O-CH_2-CH=CH_2	0.108	8.4		≥3.1	2.3	>3.1		16
232	2-(3-me-Thie)	-C≡N	-O-CH_2-CH=CH_2	0.144	≥3.2		≥3.2	1.8	>3.2		11
268	2-(3-me-Thie)	-OCH_3	-O-CH_2-CH=CH_2	0.139	15		6.2	3.5	10		22

[a] Abbreviations: me-Fur, methylfuranyl; me-Thie, methylthienyl.
[b] Data are means of at least two to three independent experiments.
[c] Compound concentration required to inhibit virus-induced cytopathicity by 50%.
[d] Compound concentration required to inhibit CEM cell proliferation by 50%.
[e] WT, wild type.

6-methyl-3,4-dihydro-2H-pyran-5-yl derivative 306 was fourfold more potent an anti-HIV-1 compound than 4 (Table 3).

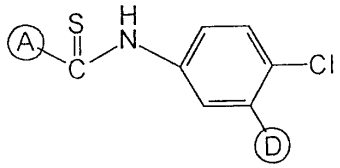
Whereas for the furanyl- and thienylthiocarboxanilide derivatives antiviral potency against the mutant virus strains was 4- to 10-fold lower than that against wild-type virus, the branched pentenyl ether derivatives 313 (UC-781) and 314 (UC-82) remained fully active against the Ile-100, Ala-106, and Lys-138 RT mutant viruses. Their EC_{50} values ranged between 0.015 and 0.021 μM for all mutant virus strains. Interestingly, UC-781 and UC-82 lost only four- to sevenfold antiviral potency against Asn-103 and Cys-181 RT mutant viruses. The saturated branched pentyl ether derivatives 315 and 316 were 5- to 10-fold less active against all these mutant virus strains than against wild-type virus (Table 3).

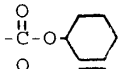
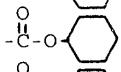
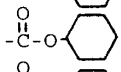
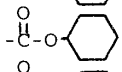
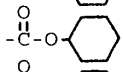
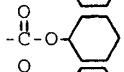
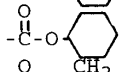
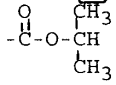
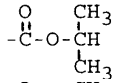
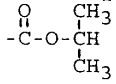
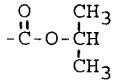
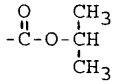
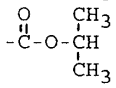
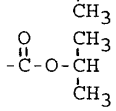
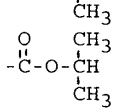
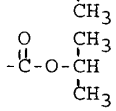
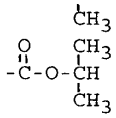
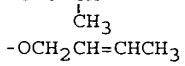
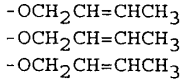
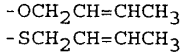
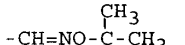
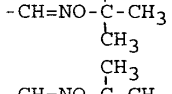
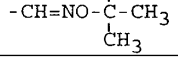
Although several (un)substituted phenyl derivatives showed an anti-HIV-1 activity similar to that of compound 4 (EC_{50}, ~0.140 μM), they were (by at least 1 to 2 orders of magnitude) less active against the mutant viruses (i.e., compounds 106, 92, 168, 57, 225, 262, and 244). Several of the other substituted phenyl derivatives had an even more decreased antiviral activity against wild-type virus and were consequently poorly active against the mutant viruses. Other substituents in the A part of the molecule [i.e., the 2-(1-methylpyrrole) derivative 299] proved clearly inferior in inhibiting mutant viruses compared with the furanyl (4) derivative. Also, the 2-(1-methylpyrrole) derivative 249 and the 2-pyrrole derivative 247 were markedly less active against several or all mutant viruses, compared with the corresponding 2-methylfuranyl thiocarboxanilide derivative 226 (Table 3).

None of the compounds showed a marked antiviral activity against the Leu-188 RT mutant virus. Only those compounds that were among the most active derivatives against wild-type HIV-1 (i.e., EC_{50} in the 0.009 to 0.027 μM range) (i.e., compounds 309, 310, 313, and 314) showed activity against the Leu-188 RT mutant virus at an EC_{50} that was around 2 μM (Table 3).

Effect of the ester, ether, or oxime ether (D) part of the molecule on the antiviral potency of the test compounds. The most efficient substituents in the A, B, and C parts of the molecule were retained, when evaluating the role of the D part of the molecule in the antiviral effectiveness of the thiocarboxanilide derivatives. Thus, in the A part, either the 3-(2-methylfuranyl)- or 3-(2-methylthienyl) substituent was retained, whereas in the B part the thiocarboxamide and in the C part the 2-chlorophenyl remained unchanged (Table 4). Three different types of D substituents were considered: (i) oxime derivatives, (ii) ether derivatives, and (iii) ester derivatives. Among the oxime derivatives (bearing a 2-methylfuranyl moiety in the A part of the molecule), several compounds proved highly inhibitory to wild-type HIV-1 (Table 5). In particular, the propenyl (237) and isopropyl (2) derivatives proved exquisitely inhibitory to HIV-1 (EC_{50}, 0.021 to 0.027 μM). Surprisingly, compound 237 was 10- to 50-fold more inhibitory to the mutant viruses than compound 2. The methyl (177), propenyl (239), cyclohexyl (266), and *t*-butyl (4) thiocarboxanilide derivatives were inhibitory to wild-type HIV-1 at an EC_{50} of 0.075 to 0.142 μM, followed by the benzyl derivative 241 (EC_{50}, 0.780 μM). However, when the activity of the test compounds against the different mutant virus strains was taken into account to assess the overall antiviral potential, compound 4 proved to be the most effective (its EC_{50} for the mutant viruses ranged between 0.214 and 0.370 μM; that is an EC_{50} value which is very close to its EC_{50} for wild-type virus). It is striking that compound 4 was 5-fold less active against wild-type virus than compound 2,

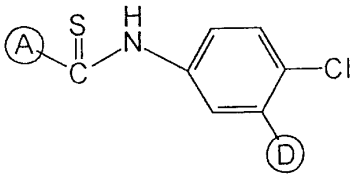
TABLE 3. Antiviral activity of thiocarboxanilide derivatives with modifications in the heterocycle (A) part of the molecule

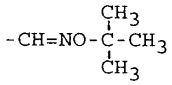
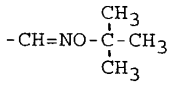
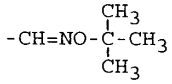
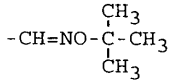
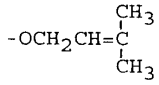
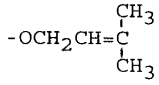
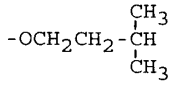
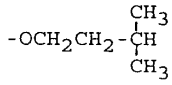
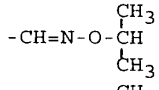
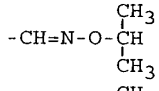
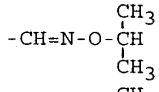
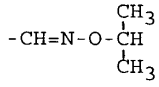
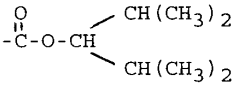
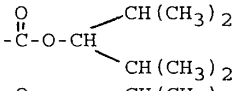
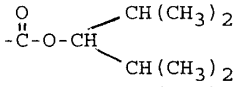
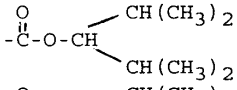
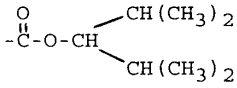


Code	Structure of the compound[a]		EC_{50}[b,c] (μM)							CC_{50}[b,d] (μM)
	A	D	WT[e]	Ile-100	Asn-103	Ala-106	Lys-138	Cys-181	Leu-188	
252	3-Thie		0.078	2.2		0.182	0.182	0.208		11
168	2-F-Phe		0.102	0.765		0.650	0.234	0.650		49
112	2-Cl-Phe		1.2	9.8		12	9.8	7.4		20
92	2-MeO-Phe		0.100	1.8		1.6	1.5	11		30
106	Phe		0.081	5.3		2.7	1.5	2.1		14
162	3-(2-me-Fur)		0.010	0.442		0.148	0.090	0.182		25
149	2-me-Phe		0.127	1.6		7.8	7.8	4.2		10
57	Phe		0.15	13		15	6.6	15		13
121	2-NH_2-Phe		1.4	17		21	15	15		19
122	2-Fur		0.310	16		16	12	16		23
123	2-(3-me-Fur)		0.06	9.0		3.0	1.6	1.6		45
124	2-(3-me-Thie)		0.028	2.0		1.7	0.840	0.756		15
126	2-Thie		0.261	8.7		12	5.3	12		14
71	2-MeO-Phe		0.513	9.6		16	1.9	15		73
81	2-Me-Phe		1.3	13		25	14	22		17
63	3-(2-me-Fur)		0.020	1.7		1.7	1.7	0.580		15
138	3-Thie		0.058	1.2		2.2	1.5	1.1		12
309	3-(2-me-Fur)		0.018	0.174	0.297	0.161	0.056	0.232	2.0	28
310	3-(2-me-Thie)		0.018	0.141	0.570	0.072	0.054	0.090	1.9	18
305	3-(1,2-dime-Pyr)		0.510	2.5	≥6	1.2	1.8	0.390	>6	15
322	3-(2-me-Fur)		0.012	0.111	0.240	0.075	0.090	0.135	3.0	14
331	3-(2-me-Thie)		0.017	0.090	0.224	0.081	0.070	0.070	≥5.6	14
4	3-(2-me-Fur)		0.14	0.238		0.364	0.210	0.210		16
304	3-(2-me-4,5-dihydro-Fur)		0.017	0.17	0.169	0.081	0.112	0.126	3.3	31

Continued on following page

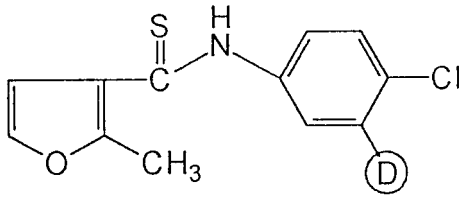
TABLE 3—*Continued*

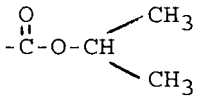
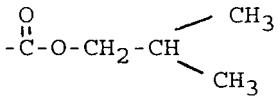
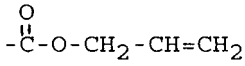
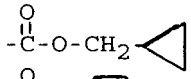
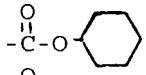
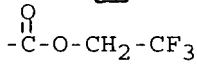
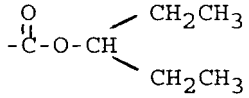
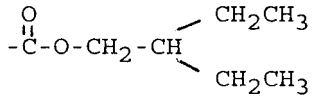
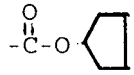
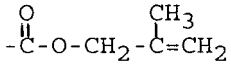
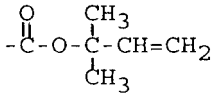
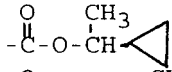
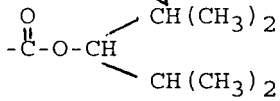


Code	Structure of the compound[a]		EC_{50}[b,c] (μM)							CC_{50}[b,d] (μM)
	A	D	WT[e]	Ile-100	Asn-103	Ala-106	Lys-138	Cys-181	Leu-188	
306	3-(2-me-DHPyr)		0.035	0.148	0.216	0.067	0.108	0.067	2.7	27
299	2-(1-me-Pyr)		0.081	0.364	0.476	0.280	0.364	0.126	>5.6	36
222	2-(3-me-Thie)		0.027	1.4		0.540	0.135	0.54		33
225	Phe		0.086	5.8		0.288	0.230	2.0		28
313	3-(2-me-Fur)		0.009	0.021	0.069	0.015	0.015	0.045	1.5	>300
314	3-(2-me-Thie)		0.009	0.017	0.057	0.014	0.017	0.037	1.9	16
315	3-(2-me-Fur)		0.021	0.180	0.450	0.291	0.090	0.240	>30	>300
316	3-(2-me-Thie)		0.020	0.082	0.262	0.169	0.099	0.197	>5.6	>282
267	2-(3-me-Fur)		0.029	2.2		1.5	0.450	1.8		9.3
240	2-(3-me-Thie)		0.022	1.8		0.224	0.448	1.4		36
262	Phe		0.150	>3		2.2	0.510	>3		27
2	3-(2-me-Fur)		0.027	10		≥12	6.0	9.9		9.9
244	2-F-Phe		0.147	>2.5		2.1	1.7	>2.5		18
247	2-Pyr		0.264	>2.6		>26	>26	>26		>264
249	2-(1-me-Pyr)		0.075	10		2.0	1.0	1.7		>250
256	2-(3-me-Thie)		0.110	7.3		0.488	0.805	1.3		≥244
226	3-(2-me-Fur)		0.076	2.2		2.2	0.202	1.6		9.4

[a] Abbreviations: Thie, thienyl; Phe, phenyl; MeO, methoxy; me-Fur, methylfuranyl; dime-Pyr, dimethylpyrrole; Pyr, pyrrole; DHPyr, 6-methyl-3,4-dihydro-2H-pyran-5-yl.
[b] Data are means of at least two to three independent experiments.
[c] Compound concentration required to inhibit virus-induced cytopathicity by 50%.
[d] Compound concentration required to inhibit CEM cell proliferation by 50%.
[e] WT, wild type.

TABLE 4. Antiviral activity of thiocarboxanilide ester derivatives with modifications in the D part of the molecule



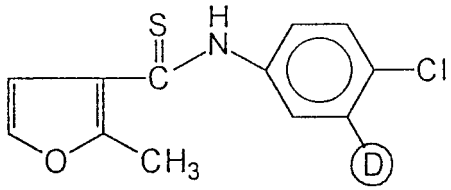
Code	Structure of the compound (D)	EC_{50}[a,b] (μM)							CC_{50}[b,c] (μM)
		WT[d]	Ile-100	Asn-103	Ala-106	Lys-138	Cys-181	Leu-188	
63		0.021	1.8		1.8	1.8	0.600		15
140		0.028	0.539		0.213	0.170	0.199		24
141		0.03	1.9		1.2	0.600	1.1		33
142		0.043	1.1		0.570	0.242	0.199		19
162		0.011	0.450		0.151	0.093	0.186		26
161		0.018	1.3		0.212	0.172	0.225		>265
163		0.109	1.6		0.819	0.437	0.819		44
164		0.018	0.710		0.210	0.184	0.210		18
183		0.013	0.467		0.110	0.096	0.124		12
217		0.017	2.3		0.086	0.086	0.286		19
216		0.110	11		0.550	0.275	1.4		69
243		0.025	1.8		0.220	0.495	0.302		47
226		0.076	2.2		2.2	0.202	1.6		9.4

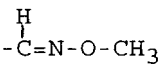
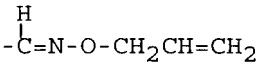
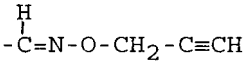
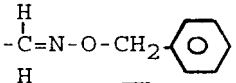
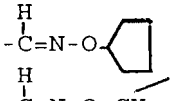
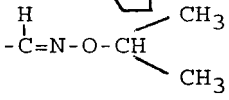
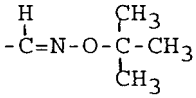
[a] Compound concentration required to inhibit virus-induced cytopathicity by 50%.
[b] Data are means of at least two to three independent experiments.
[c] Compound concentration required to inhibit CEM cell proliferation by 50%.
[d] WT, wild type.

but ~30- to 50-fold more effective against the mutant viruses than compound 2, as compounds 4 and 2 differ only by an isopropyl (2) versus a *t*-butyl (4) group in the D part of the molecule (Table 5).

The majority of the ester derivatives of the 3-(2-methylfuranyl)thiocarboxanilides proved highly inhibitory to wild-type HIV-1. The EC_{50} of the most active derivatives ranged from 0.011 to 0.025 μM (compounds 162, 183, 217, 63, 161, 164, and 243) (Table 4). The optimal ester substituents were cyclopentyl (183) and cyclohexyl (162), a branched hexyl (164) or butenyl (217) or isopropyl (63), or a fluoro-substituted ethyl group (161). However, the potent activity of the ester derivatives against the wild-type virus did not necessarily guarantee a pronounced activity against the different mutant virus strains. For example, the isopropyl ester derivative 63 proved 30- to 100-fold less efficient in inhibiting the mutant viruses than wild-type virus,

TABLE 5. Antiviral activity of thiocarboxanilide oxime ether derivatives, with modifications in the D part of the molecule



Code	Structure of the compound (D)	EC_{50}[a,b] (μM)							CC_{50}[b,c] (μM)
		WT[d]	Ile-100	Asn-103	Ala-106	Lys-138	Cys-181	Leu-188	
177		0.070	5.4		1.2	0.199	3.7		56
237		0.021	1.5		0.255	0.231	0.900		36
239		0.075	2.2		1.0	1.5	2.4		30
241		0.780	>2.6		1.6	≥2.6	2.3		>259
266		0.063	0.742		0.234	0.412	0.357		15
2		0.027	10		≥12	6.0	9.9		9.9
4		0.142	0.242		0.370	0.214	0.214		16

[a] Compound concentration required to inhibit virus-induced cytopathicity by 50%.
[b] Data are means of at least two to three independent experiments.
[c] Compound concentration required to inhibit CEM cell proliferation by 50%.
[d] WT, wild type.

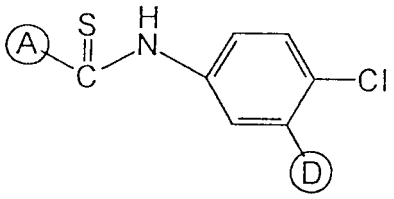
whereas the cyclopentyl and cyclohexyl derivatives 183 and 162 were approximately 10-fold less effective. Also, the ester derivatives generally lost more antiviral activity against Ile-100 RT mutant virus than against the other mutant viruses (Table 4). The unbranched propenyl 141 and the branched pentyl (163) and pentenyl (216) esters had EC_{50} values around 0.110 μM and proved, in general, inhibitory to the mutant virus strains within the 0.437 to 1.6 μM concentration range. All data being taken together, the thiocarboxanilide cyclohexyl and cyclopentyl ester derivatives showed the most favorable activity spectrum against wild-type and mutant virus strains.

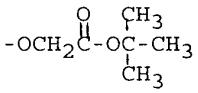
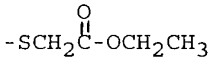
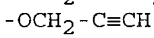
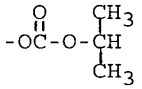
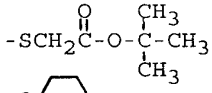
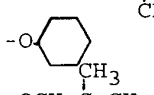
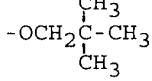
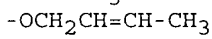
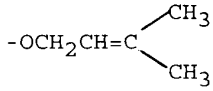
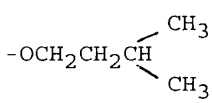
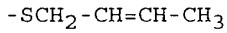
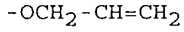
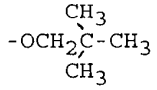
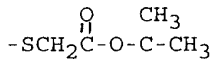
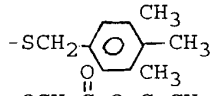
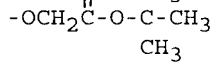
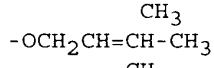
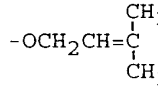
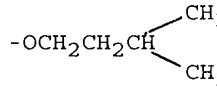
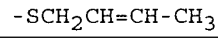
A series of exquisitely potent inhibitors of HIV-1 replication were found among several ether and thioether derivatives of the 2-methylfuranyl- and 2-methylthienylthiocarboxanilide analogs. In general, there was no marked difference in the antiviral potencies of the ether and thioether derivatives (compare 318 with 326, 310 with 331, 309 with 322, and 254 with 324). The highest antiviral potency was found with unbranched and branched pentyl and unbranched butenyl derivatives (EC_{50}, 0.009 to 0.021 μM). As mentioned earlier, the branched pentenyl derivatives 313 and 314 retained remarkably high activity against the Ile-100, Asn-103, Ala-106, Lys-138, and Cys-181 RT mutant virus strains, whereas the branched pentyl and unbranched butenyl derivatives partially showed a 10-fold reduced activity against the mutant viruses (Table 6). Thus, the branched pentenyl derivatives 313 and 314 turned out to be the most potent HIV-1-specific inhibitors among the 124 thiocarboxanilide analogs investigated in this study. The ether derivatives that contained an additional carboxyester function (i.e., 254, 213, 319, 324, 318, and 326) as well as the unbranched propenyl (233) and propynyl (215) ethers proved markedly less efficient inhibitors of both wild-type and mutant virus strains than the pure alkyl and alkenyl ether derivatives. As a rule, and as also observed for the other thiocarboxanilides, all ether derivatives were markedly less inhibitory to the Leu-188 RT mutant virus. Only those compounds that were most active against wild-type virus (i.e., 309, 313, 314, 322, 310, and 331) still demonstrated activity against Leu-188 RT-mutated virus (i.e., at an EC_{50} close to 2 μM) (Table 6).

Stability of thiocarboxanilide derivatives in human serum. The thiocarboxanilide oxime *t*-butyl derivative 4 and the thiocarboxanilide pentenyl ether derivatives 313 and 314 and their closely related pentyl derivatives 315 and 316 have been examined for their stability in fresh human serum. Upon incubation of 200 μM test compound in the serum for 24 h at 37°C, no marked conversion of the test compounds to more polar derivatives, no significant hydrolysis of the oxime or ether linkage at the phenyl moiety of the test compounds, and no saturation of the branched pentenyl (D part) moiety in 313 and 314 could be observed. These data point to the high stability of the novel thiocarboxanilide ether derivatives in serum and are in sharp contrast with the thiocarboxanilide ester derivatives as previously reported (10).

Anti-HIV-1 activity of thiocarboxanilide derivatives in the presence of different human serum concentrations. The antiviral activity of the thiocarboxanilides 4, 313, and 314 and the

TABLE 6. Antiviral activity of thiocarboxanilide ether and thioether derivatives, with modifications in the D part of the molecule



Code	Structure of the compound[a]		EC_{50}[b,c] (μM)							CC_{50}[b,d] (μM)
	A	D	WT[e]	Ile-100	Asn-103	Ala-106	Lys-138	Cys-181	Leu-188	
254	3-(2-me-Fur)		0.757	1.6		0.520	2.0	1.3		11
213	3-(2-me-Fur)		0.229	4.9		15	1.6	5.4		≥270
215	3-(2-me-Fur)		0.196	16		2.4	1.1	13		58
317	3-(2-me-Fur)		0.286	0.800	1.2	0.429	0.944	1.7	>5.7	223
319	3-(2-me-Fur)		1.1	13	12	9.0	1.8	0.30	>6.0	75
324	3-(2-me-Fur)		0.55	1.2	1.8	0.75	0.450	1.1	>5.0	17
327	3-(2-me-Fur)		0.094	0.940	0.940	0.256	1.4	≥5.7	>5.7	15
298	3-(2-me-Fur)		0.45	4.2	2.1	6.0	1.5	≥6	>6	12
309	3-(2-me-Fur)		0.018	0.171	0.298	0.156	0.054	0.225	1.9	27
313	3-(2-me-Fur)		0.009	0.024	0.069	0.015	0.015	0.033	1.5	>297
315	3-(2-me-Fur)		0.021	0.172	0.450	0.144	0.120	0.240	>30	>296
322	3-(2-me-Fur)		0.012	0.111	0.285	0.075	0.090	0.135	2.9	14
233	2-(3-me-Thie)		0.108	8.3		≥3.1	2.2	>3.1		16
311	2-(2-me-Thie)		0.395	1.4	1.2	1.4	0.141	1.6	>5.6	17
318	2-(2-me-Thie)		0.312	0.816	1.1	0.360	1.0	0.336	>4.8	13
321	2-(2-me-Thie)		0.200	1.4	1.1	1.4	1.3	1.5	>5.0	27
326	2-(2-me-Thie)		0.168	0.402	0.301	0.251	0.602	0.218	>5.0	5.3
310	2-(2-me-Thie)		0.018	0.131	0.539	0.068	0.051	0.085	1.8	17
314	2-(2-me-Thie)		0.009	0.017	0.054	0.017	0.014	0.014	1.7	16
316	2-(2-me-Thie)		0.019	0.082	0.254	0.155	0.099	0.197	>5.6	>282
331	2-(2-me-Thie)		0.017	0.090	0.226	0.082	0.070	0.071	≥5.8	14

[a] Abbreviations: me-Fur, methylfuranyl; me-Thie, methylthienyl.
[b] Data are means of at least two to three independent experiments.
[c] Compound concentration required to inhibit virus-induced cytopathicity by 50%.
[d] Compound concentration required to inhibit CEM cell proliferation by 50%.
[e] WT, wild type.

TABLE 7. Effect of human serum concentrations on the anti-HIV-1 activity of thiocarboxanilides

Compound	EC_{50} (μM)[a,b] at concn of human serum (%):				
	0	5	12.5	25	50
4	0.057	0.114	0.128	0.199	0.213
313	0.009	0.021	0.030	0.051	0.042
314	0.014	0.026	0.057	0.085	0.071
MKC 442	0.010	0.023	0.026	0.049	0.046

[a] Compound concentration required to reduce the HIV-1-induced cytopathicity by 50%.
[b] Data are the means of two independent experiments.

HEPT derivative MKC 442 has been examined in the presence of increasing concentrations of human serum (i.e., 5, 12.5, 25, and 50%) (Table 7). The antiviral potency of the test compounds gradually decreased in the presence of higher serum concentrations. However, the decreased anti-HIV-1 activity was only 3.5-fold for 4, 4- to 5-fold for 313, and 5-fold for 314 in the presence of the highest human serum concentration tested (i.e., 50%). The HEPT derivative lost approximately four- to fivefold antiviral activity in the presence of 50% human serum (Table 7).

DISCUSSION

Whereas the oxathiin carboxanilide prototype compound UC84 proved markedly active against wild-type HIV-1 (EC_{50}, ~0.05 μM), it was virtually inactive against a series of mutant virus strains including the Ile-100, Ala-106, Lys-138, Cys-181 mutant virus strains (EC_{50}, ≥50 μM) (4, 5). In contrast, the isopropoxythiocarboxanilide derivative UC38, which was only twofold more potent against wild-type HIV-1 (EC_{50}, ~0.03 μM), inhibited the Ile-100, Ala-106, Lys-138, and Cys-181 mutant virus strains at an EC_{50} ranging between 1.2 and 2.0 μM (5). These observations point to the necessity of including as many mutant virus strains as possible in the evaluation program of novel NNRTI derivatives.

In this study, we included a series of mutant viruses containing those mutations in their RT that are considered the most relevant in conferring resistance to a variety of NNRTIs in both cell culture systems and patients (11, 12, 17). Our structure-activity relationship studies revealed the crucial role of the 2-methylfuranyl and 2-methylthienyl entities in the A part of the molecule, the thiocarboxamide function in the B part of the molecule, and the chloro substituent on the phenyl moiety of the C part of the molecule. The majority of the thiocarboxanilides proved less inhibitory to the mutant virus strains than wild-type virus. In addition, the decrease in potency against the mutant virus strains differed from one mutant virus strain to another, depending on the nature of the mutation. In no case [of the 124 different (thio)carboxanilide derivatives reported here and another 100 (thio)carboxanilide derivatives previously reported in references 4 and 5], could any compound that showed a higher antiviral activity against one of the mutant virus strains than against wild-type virus be identified.

However, two novel compounds (313 [designated UC-781] and 314 [designated UC-82]) were discovered that had extremely low and almost similar EC_{50} values against wild-type HIV-1 and a series of mutant viruses containing either the Leu-100→Ile, Lys-103→Asn, Val-106→Ala, Glu-138→Lys, or Tyr-181→Cys mutation in their RT. These findings are of particular importance since the EC_{50} values of these compounds against the mutant virus strains were as low as 0.015 to 0.07 μM. Such an exquisite activity against RT mutant virus strains has not been observed for the NNRTIs that have been or are currently subjects of clinical trials (i.e., TIBO R82913, α-APA R89439, BHAP U-87204 and U90152, nevirapine [BI-RG-587], and pyridinone L-697,661). Therefore, these novel thiocarboxanilide derivatives (i.e., 313 or 314) should be considered candidate drugs to be included in future clinical trials. Since these compounds efficiently suppress wild-type virus as well as mutant virus strains, containing either the Ile-100, Asn-103, Ala-106, Lys-138, or Cys-181 mutation in their RT, they may also be expected to inhibit mutant virus strains containing more than one of these mutations (i.e., the Asn-103 and Cys-181 mutations combined, as has been observed to appear in HIV-1-infected cell cultures that were treated with pyridinone L-697,661 [20]). Another favorable property of the thiocarboxanilides 4, 313, and 314 is the marginal decrease of antiviral potency in the presence of human serum. Anti-HIV-1 activity was decreased by not more than 3.5- to 5-fold in the presence of 50% human serum and was still in the middle nanomolar range (EC_{50}, 0.042 to 0.213 μM). Our data indicate that the test compounds may still be efficiently taken up by the HIV-1-infected cells and display a marked antiviral activity in the presence of high serum concentrations.

It is interesting to note that the previously reported (thio) carboxanilide derivatives having an oxathiin or aliphatic entity (4, 5) or a thienyl or furanyl entity (10) in the A part of the molecule invariably showed a decreased inhibitory effect against the Asn-103 and Lys-138 RT mutant virus strains and, to a lesser extent, the Ile-100 RT mutant virus strain. Moreover, when monitored for development of resistance to the (thio)carboxanilide derivatives, the Asn-103 and Ile-100 mutant virus strains (and in a few cases also the Lys-138 mutant virus) predominantly appeared in the drug-treated cell cultures (10). In striking contrast, the novel thiocarboxanilide derivatives reported here potently suppress the Ile-100, Asn-103, and Lys-138 RT mutant virus strains, making it unlikely that these mutant viruses would arise in cell culture in the presence of higher nanomolar concentrations of the particular test compounds. Indeed, 313 and 314 selected for virus mutants (i.e., Leu-100→Ile, Val-106→Ala, and Glu-138→Lys) that were only marginally resistant to the thiocarboxanilides (less than 10- to 20-fold), at fixed initial concentrations of 0.03 to 0.075 μM. At higher initial concentrations (i.e., 0.30 and 0.75 μM), 313 and 314 were able to completely suppress resistant virus breakthrough in cell culture (data not shown). These data are in agreement with our findings that lower to middle nanomolar concentrations of 313 and 314 potently suppress the Ile-100, Asn-103, Ala-106, Lys-138, and Cys-181 RT mutant virus strains, obtained in the presence of other NNRTIs. The only exception to the exquisitely potent activity of the test compounds against a broad range of NNRTI-resistant mutant virus strains is their markedly lower activity against the Leu-188 RT mutant virus strain. Leucine represents the consensus amino acid at position 188 of the RT from HIV-2 strains, which may explain the poor inhibitory activity of the majority of NNRTIs against this mutant virus strain. The most potent inhibitors (i.e., 313 and 314) inhibited this mutant virus at an EC_{50} (~1.5 to 1.8 μM) that was 100- to 200-fold higher than the EC_{50} for the other virus strains.

Although no information is currently available on the sites of molecular interaction of the thiocarboxanilides with the amino acid residues of the hydrophobic pocket (15, 20, 24, 26) of the RT of HIV-1, one may speculate that the Tyr-188 residue, as present in wild-type RT, represents a point of crucial interaction with a lipophilic pharmacophore of the thiocarboxanilide

molecule. Efforts are currently under way to design novel derivatives that efficiently inhibit the Leu-188 RT mutant virus. Also, on the basis of our previous and most recent observations that BHAP U-90152 and, in particular, quinoxaline HBY 097 are inhibitory to Leu-188 RT mutant virus (data not shown), combinations of compounds 313 and 314 with these NNRTIs should be pursued to investigate the feasibility of paired drug combinations to compensate for the decreased inhibitory effect of compounds 313 and 314 against Leu-188 RT mutant virus.

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