[[[(Thienylcarbonyl)alkyl]oxy]phenyl]- and [[[(Pyrrylcarbonyl)alkyl]oxy]phenyl]oxazoline Derivatives with Potent and Selective Antihuman Rhinovirus Activity[1]

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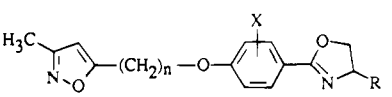
Received December 28, 1993[⊗]

As an approach to more extensive structural modifications of [(oxazolylphenoxy)alkyl]isoxazoles, we synthesized new compounds characterized by the replacement of the isoxazole nucleus with furan, pyrrole, and thiophene rings and by the presence of a ketocarbonyl group in the aliphatic chain connecting these pentatomic heterocycles to the 4-(4,5-dihydro-2-oxazolyl)phenoxy, 4-(ethoxycarbonyl)phenoxy, and 4-carboxyphenoxy moieties. Some pentamethylene derivatives were also prepared, and their antirhinovirus activity was compared to that of the corresponding ketomethylene derivatives. Syntheses were carried out by Friedel–Crafts acylation of the above pentatomic heterocycles and subsequent reaction of chloroalkyl ketones with the proper 4-substituted phenol. Reduction of the ketone function afforded the related polymethylene derivatives. The new compounds were tested for antirhinovirus activity and cytotoxicity in comparison with WIN 51711, used as reference drug. Inspection of the structure–activity relationships revealed that the thiophene ring and the carbonyl group are the structural components which to a large extent contribute to the positive biological profile in terms of both wideness of spectrum and low cytotoxicity. Among the various derivatives, compounds **8e,d** showed *in vitro* the same potency of WIN 51711 but a cytotoxicity at least 10 times lower.

Rhinoviruses (HRVs) are the most frequent etiological agents of common cold and mild localized infections of the upper respiratory tract in humans (about 40% of cases). Considering their worldwide diffusion, these syndromes have relevant socioeconomic importance and many efforts have been directed toward the identification of agents useful in the prophylaxis and therapy of HRV infections. Nevertheless, the inherent heterogeneity of this group of human pathogens, demonstrated by the isolation so far of over 100 different HRV serotypes,[2] has hampered the development of vaccines and made troublesome the design of effective, broad-spectrum drugs.

A valuable contribution to the synthesis of antirhinovirus agents has been provided by Diana and co-workers.[3–8] Chemical elaboration of the prototypical structure of arildone has led to the development of [(oxazolylphenoxy)alkyl]isoxazoles **1**, a class of potent, broad-spectrum antipicornavirus agents, which differ in the length of the aliphatic chain connecting the oxazolylphenoxy and isoxazole moieties and present a variety of substituents at different positions in the phenyl and/or oxazoline rings. Disoxaril (**1**, $n = 7$, X = R = H), in particular, has been found active *in vitro* against several entero and rhinovirus serotypes. X-ray crystallographic studies of drug–human rhinovirion complexes have shown that [(oxazolylphenoxy)alkyl]-isoxazoles bind in a hydrophobic pocket beneath the canyon floor and either block the uncoating process by stabilizing the virion structure or lead to conformational changes which affect the putative viral receptor binding site, thus preventing virion adsorption to cell receptors.[9–11]



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Although extensive structure–activity studies have been carried out on this class of antipicornavirus agents by Diana and co-workers, to the best of our knowledge no attempt has been made to ascertain whether the isoxazole nucleus really assures the best interaction of the above compounds with the specific amino acids of the viral capsid protein 1 (VP1) hydrophobic pocket. This aspect seemed to us to be of prominent interest, since each of the rhinovirus serotypes differs to varying degree in the VP1 amino acid sequence. Accordingly, as an approach to more extensive structural modifications of [(oxazolylphenoxy)alkyl]isoxazoles, we synthesized derivatives **2** characterized by the replacement of the isoxazole nucleus with pyrrole, thiophene, and furan rings and by the presence of a ketocarbonyl group in the aliphatic chain connecting these pentatomic heterocycles to the 4-(4,5-dihydro-2-oxazolyl)phenoxy, 4-(ethoxycarbonyl)phenoxy, or 4-carboxyphenoxy moieties. Some pentamethylene analogues having the formula **3** were also prepared and tested for a comparison with the

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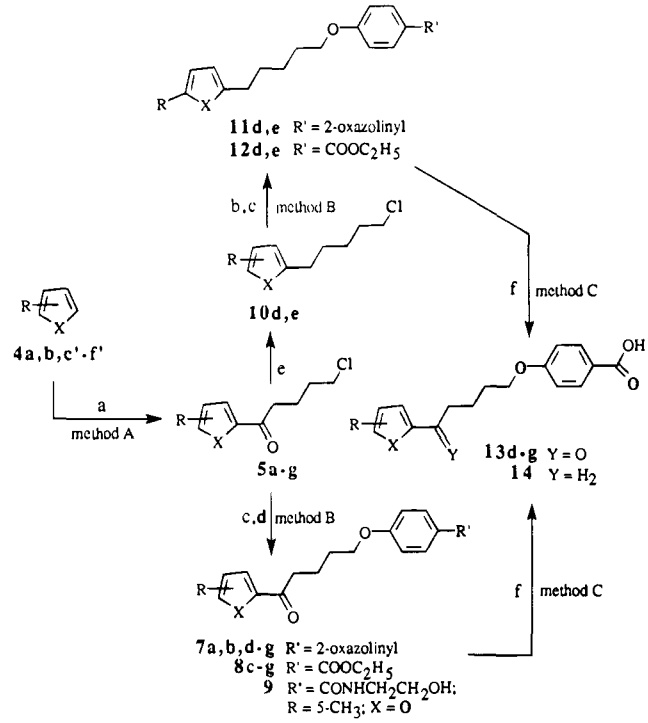
[⊗] Abstract published in *Advance ACS Abstracts*, January 1, 1995.

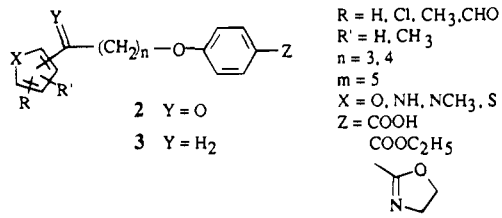
0022-2623/95/1838-0803$09.00/0

Scheme 1[a]



[a] Compounds: **a**, X = NH, R = H; **b**, X = NCH_3, R = H; **c**, X = O, R = 5-CH_3; **c'**, X = O, R = 2-CH_3; **d**, X = S, R = 5-Cl; **d'**, X = S, R = 2-Cl; **e**, X = S, R = 5-CH_3; **e'**, X = S, R = 2-CH_3; **f**, X = S, R = 3-CH_3; **f'**, X = S, R = 3-CH_3; **g**, X = S, R = 4-CH_3. Reagents: (a) $Cl(CH_2)_4COCl$, $AlCl_3$; (b) **6a**, K_2CO_3, NaI, CH_3CN; (c) **6b**, K_2CO_3, NaI, CH_3CN; (d) **6c**, K_2CO_3, NaI, CH_3CN; (e) $LiAlH_4$, $AlCl_3$; (f) KOH, C_2H_5OH.

corresponding ketomethylene analogues **2**.

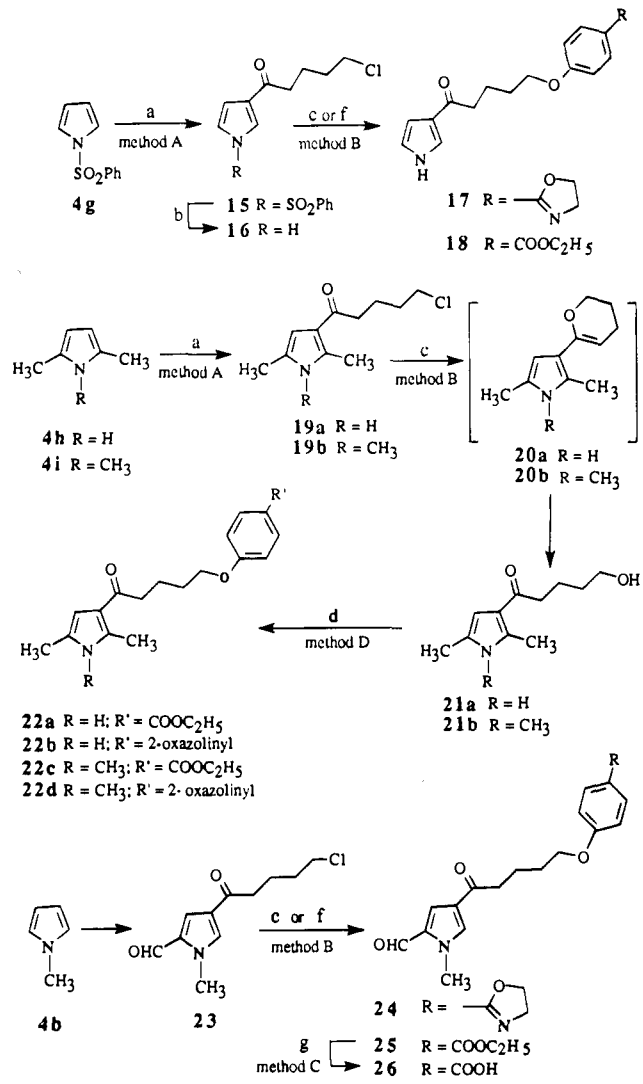


In a preliminary communication[12] we have reported on the potent and selective anti-HRV-14 activity of some compounds of formula **2**. When tested for antiviral activity, some thiophene, pyrrole, and furan derivatives were found inactive against coxsackie B1 and marginally active against polio but showed potent and selective activity against HRV-14. In the present paper we give full account on the synthesis of a larger group of non-isoxazole analogues **2** and **3**, as well as on their activity against a wider spectrum of HRV serotypes, and highlight some aspects of their structure–activity relationships.

Chemistry

Friedel–Crafts acylation (Scheme 1) of the electron-rich heteroaromatics **4a–f** with 5-chlorovaleryl chloride afforded the heteroaryl ketones **5a–g**, which readily underwent nucleophilic displacement by 4-(4,5-dihydro-2-oxazolyl)phenol (**6a**),[6] ethyl 4-hydroxybenzoate (**6b**), and *N*-(4-hydroxybenzoyl)ethanolamine (**6c**)[6] in the presence of potassium carbonate and sodium iodide to give compounds **7a,b,d–g**, **8c–g**, and **9**, respectively. Similarly, reaction of **6a,b** with the haloalkyl derivatives **10d,e**, in turn obtained by reduction of **5d,e** with lithium aluminum hydride/aluminum trichloride, provided compounds **11d,e** and **12d,e**, respectively. Finally, esters **8d–g** and **12d** were hydrolyzed to the corresponding carboxylic acids **13d–g** and **14**.

Scheme 2[a]

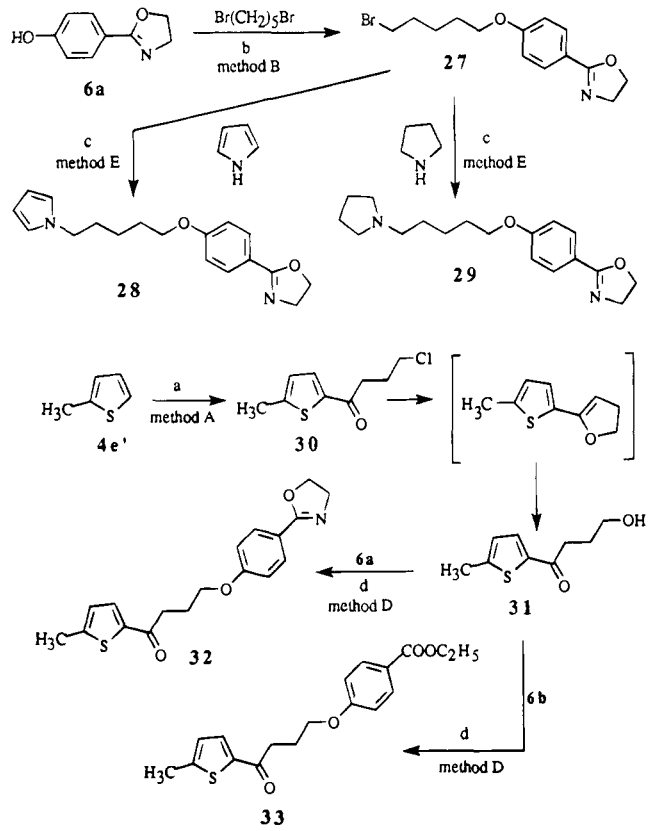


[a] (a) $Cl(CH_2)_4COCl$, $AlCl_3$; (b) NaOH, H_2O, dioxane; (c) **6a**, K_2CO_3, NaI, CH_3CN; (d) **6a** or **6b**, DEAD, Ph_3P; (e) $(COCl)_2$, DMF, then $Cl(CH_2)_4COCl$, $AlCl_3$; (f) **6b**, K_2CO_3, NaI, CH_3CN; (g) KOH, C_2H_5OH.

The synthesis of the compounds bearing the 3-pyrrolyl moiety as the heterocyclic head was performed following the procedures outlined in Scheme 2. 1-(Phenylsulfonyl)-1*H*-pyrrole (**4g**)[13] was acylated with 5-chlorovaleryl chloride, essentially as reported by Kakushima and coworkers[14] for similar cases, to give the intermediate **15**, which on treatment with sodium hydroxide in 50% aqueous 1,4-dioxane lost the benzenesulfonyl group to provide 3-(5-chloropentanoyl)-1*H*-pyrrole (**16**). Subsequent reaction with **6a** or **6b** led to compounds **17** and **18**, respectively.

When the intermediates **19a,b**, obtained by acylation of 2,5-dimethyl-1*H*-pyrrole (**4h**) and 1,2,5-trimethyl-1*H*-pyrrole (**4i**), were reacted with **6a** or **6b**, only minor amounts of the expected compounds **22a–d** were isolated, the main reaction products being derivatives **21a,b**. These are likely to arise during the aqueous workup of the reaction from the hydrolysis of dienol

Scheme 3[a]



[a] (a) $Cl(CH_2)_3COCl$, $AlCl_3$; (b) K_2CO_3, NaI, CH_3CN; (c) NaH, anhydrous dioxane; (d) **6b** or **6a**, DEAD, Ph_3P.

ether intermediates **20a,b**, whose formation can be explained by a base-mediated abstraction of a proton from the 2-methyl group of **19a,b** to generate a dienolate anion which undergoes intramolecular O-alkylation. Hence the preparation of compounds **22a–d** was performed under neutral conditions by means of a Mitsunobu reaction between **21a,b** and the suitably substituted phenols **6a,b**.

When 1-methyl-1*H*-pyrrole (**4b**) was treated successively with the Vilsmeier–Haack reagent, generated from *N,N*-dimethylformamide/phosphoryl chloride, and 5-chlorovaleryl chloride/aluminum trichloride complex,[15,16] after treatment with water, 4-(5-chloropentanoyl)-1-methyl-1*H*-pyrrole-2-carboxaldehyde (**23**) was obtained, which was reacted with **6a,b** to afford derivatives **24** and **25**, respectively. Alkaline hydrolysis of the latter compound led to the carboxylic acid **26**. Alkylation of **6a** with an excess of 1,5-dibromopentane (Scheme 3) gave the intermediate **27**, which, upon treatment with pyrrole or pyrrolidine in the presence of sodium hydride, furnished compounds **28** and **29**, respectively.

Finally, acylation of 2-methylthiophene (**4e'**) with 4-chlorobutanoyl chloride followed by reaction of the intermediate **30** with **6a,b** gave only minor amounts of compounds **32** and **33**. These latter were obtained in moderate to good yield from the intermediate **31** by the Mitsunobu reaction with **6a,b**, respectively, analogously to that described above for derivatives **19a,b**. Chemical and physical data of the new compounds are reported in Table 1.

Results and Discussion

Compounds were screened for anti-HRV activity in a tetrazolium-based colorimetric assay (MTT assay) using HeLa-Ohio cells. In the antiviral assays we used the same 15 serotypes that had been chosen to test the activity of [(oxazolylphenoxy)alkyl]isoxazoles. Whenever the compounds showed a broad spectrum of anti-HRV activity, MIC_{80}s (i.e., compound doses that inhibit 80% of the serotypes tested) were the basis for SAR considerations. In the other cases, EC_{50} values were used. Tables 2–4 show the cytotoxicity and anti-HRV activity of the new compounds together with those obtained for WIN 51711, used as reference drug.

Among the furan derivatives (Table 2), compound **8c** showed activity against HRV-2 and HRV-14 (EC_{50}s = 0.9 and 2.4 μM, respectively) comparable to those of WIN 51711 (EC_{50}s = 2.3 and 1.7 μM, respectively). Unfortunately, the limited amount of this drug made it impossible to extend the assays to additional serotypes.

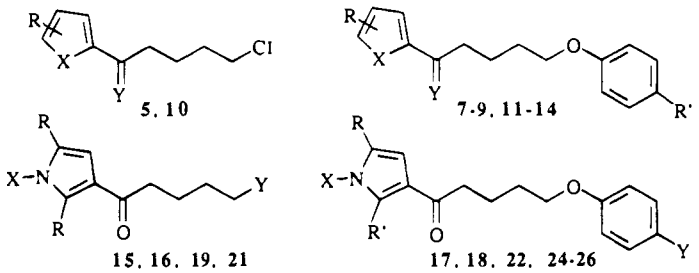
Compounds **18** and **25** were the only pyrrole derivatives with a wide spectrum of anti-HRV activity; the latter was more potent with an MIC_{80} of 8.4 μM (2.4-fold higher than that of WIN 51711). Although differently substituted, both pyrrole rings were linked to the ketomethylene chain at the 3 position and both compounds were carbethoxy derivatives. Interestingly, when the carbethoxy group was substituted by the oxazoline (see compounds **17** and **24**), a loss of antiviral activity was observed. An inactive derivative was also obtained by replacing the carbethoxy group with a carboxyl group (compare compounds **25** and **26**).

In the thiophene series, compound **8e** (Table 3) and **8d** (Table 4) were the most potent against a wide spectrum of HRV serotypes, with MIC_{80}s of 2.3 and 2.1 μM, respectively, which were slightly lower than that of WIN 51711 (3 μM).

Structure–activity relationships suggest that, as in the case of several WIN compounds, the anti-HRV activity of the thiophene derivatives is increased or lowered by the substitution of the oxazoline ring with a carbethoxy or carboxyl group, respectively (compare compounds **7d**, **8d**, and **13d**; **7e**, **8e**, and **13e**; **7f**, **8f**, and **13f**; **7g**, **8g**, and **13g**). Moreover, the potency of their anti-HRV activity depends on the position of the methyl substituent. In fact, in both the (oxazolylphenoxy)- (**7e–g**) and (carbethoxyphenoxy)alkyl (**8e–g**) derivatives, the anti-HRV activity progressively increases as the methyl group on the thiophene ring shifts from position 3 to position 5. However, the substitution of the 5-methyl for a 5-chloro in the thiophene ring leads to a loss of wide-spectrum anti-HRV activity in the (oxazolylphenoxy)alkyl derivatives (compare MIC_{80}s of compounds **7d,e**) but not in the (carbethoxyphenoxy)-alkyl derivatives (compare MIC_{80}s of compounds **8d,e**). When the MIC_{80} of **7e** is compared to that of **32** and that of **8e** is compared to the MIC_{80} of **33**, it can be concluded that thiophene derivatives with a five-carbon chain are more potent than their counterparts with a four-carbon chain.

Chlorothiophene derivatives bearing either the ethoxycarbonyl group or the oxazoline moiety were found to be equipotent. Conversely, the corresponding carboxyl derivatives always were inactive. Finally, the substitution of the carbonyl group with a further methylene group led in the thiophene series to a decrease of antiviral activity, in terms of EC_{50} and/or MIC_{80}, and to enhanced cytotoxicity (compare compounds **7d,e** with **11d,e** and **8d,e** with **12d,e**).

Table 1. Chemical and Physical Data of the New Compounds



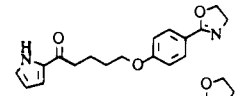
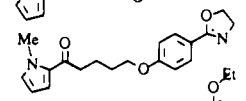
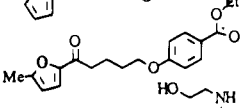
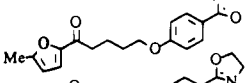
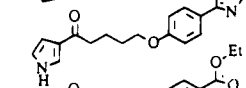
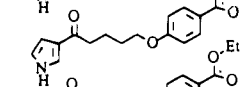
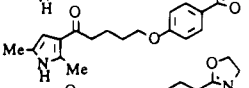
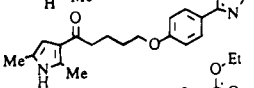
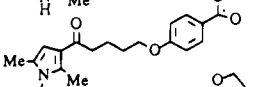
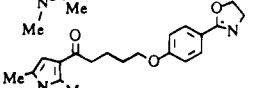
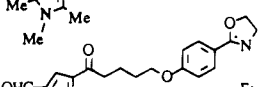
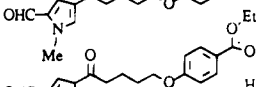
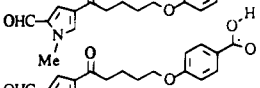
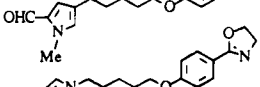
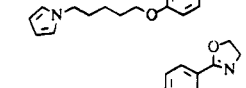
compd	X	R	Y	R'	formula	mp, °C	recryst solvent[a]	yield, %
5a	NH	H	O	–	$C_9H_{12}ClNO$	59–61	A	41
5b	NCH_3	H	O	–	$C_{10}H_{14}ClNO$	oil	–	50
5c	O	5-CH_3	O	–	$C_{10}H_{13}ClO_2$	oil	–	45
5d	S	5-Cl	O	–	$C_9H_{10}Cl_2OS$	37–38	B	95
5e	S	5-CH_3	O	–	$C_{10}H_{13}ClOS$	45–47	C	80
5f	S	3-CH_3	O	–	$C_{10}H_{13}ClOS$	32–33	A	42
5g	S	4-CH_3	O	–	$C_{10}H_{13}ClOS$	oil	–	8
7a	NH	H	O	2-oxazolinyl	$C_{18}H_{20}N_2O_3$	155–156	D	70
7b	NCH_3	H	O	2-oxazolinyl	$C_{19}H_{22}N_2O_3$	133–134	D	69
7d	S	5-Cl	O	2-oxazolinyl	$C_{18}H_{18}ClNO_3S$	143–144	E	97
7e	S	5-CH_3	O	2-oxazolinyl	$C_{19}H_{21}NO_3S$	129–130	E	25
7f	S	3-CH_3	O	2-oxazolinyl	$C_{19}H_{21}NO_3S$	108–109	C	13
7g	S	4-CH_3	O	2-oxazolinyl	$C_{19}H_{21}NO_3S$	118–119	E	16
8c	O	5-CH_3	O	COOEt	$C_{19}H_{22}O_5$	66–67	C	30
8d	S	5-Cl	O	COOEt	$C_{18}H_{19}ClO_4S$	87–89	C	76
8e	S	5-CH_3	O	COOEt	$C_{19}H_{22}O_4S$	94–95	C	71
8f	S	4-CH_3	O	COOEt	$C_{19}H_{22}O_4S$	67–69	C	38
8g	S	3-CH_3	O	COOEt	$C_{19}H_{22}O_4S$	56–57	E	100
9	O	5-CH_3	O	$CONHCH_2CH_2OH$	$C_{19}H_{23}NO_5$	125–127	F	44
10d	S	5-Cl	H_2	–	$C_9H_{12}Cl_2S$	oil	–	89
10e	S	5-CH_3	H_2	–	$C_{10}H_{15}ClS$	oil	–	44
11d	S	5-Cl	H_2	2-oxazolinyl	$C_{18}H_{20}ClNO_2S$	99–100	C	52
11e	S	5-CH_3	H_2	2-oxazolinyl	$C_{19}H_{23}NO_2S$	96–98	D	59
12d	S	5-Cl	H_2	COOEt	$C_{18}H_{21}ClO_3S$	oil	–	71
12e	S	5-CH_3	H_2	COOEt	$C_{19}H_{24}O_3S$	36–38	G	52
13d	S	5-Cl	O	COOH	$C_{16}H_{15}ClO_4S$	169–171	H	66
13e	S	5-CH_3	O	COOH	$C_{17}H_{18}O_4S$	187–189	H	93
13f	S	4-CH_3	O	COOH	$C_{17}H_{18}O_4S$	153–155	I	86
13g	S	3-CH_3	O	COOH	$C_{17}H_{18}O_4S$	155–157	H	75
14	S	5-Cl	H_2	COOH	$C_{16}H_{17}ClO_3S$	160–161	H	72
15	SO_2Ph	H	Cl	–	$C_{15}H_{16}ClNO_3S$	70–72	C	81
16	H	H	Cl	–	$C_9H_{12}ClNO$	101–103	J	85
17	H	H	2-oxazolinyl	H	$C_{18}H_{20}N_2O_3$	157–159	D	45
18	H	H	COOEt	H	$C_{18}H_{21}NO_4$	105–107	E	88
19a	H	CH_3	Cl	–	$C_{11}H_{16}ClNO$	93–95	K	55
19b	CH_3	CH_3	Cl	–	$C_{12}H_{18}ClNO$	oil	–	42
21a	H	CH_3	OH	–	$C_{11}H_{17}NO_2$	70–71	C	39
21b	CH_3	CH_3	OH	–	$C_{12}H_{19}NO_2$	72–73	I	54
22a	H	CH_3	COOEt	CH_3	$C_{21}H_{27}NO_4$	117–119	E	78 (7)[b]
22b	H	CH_3	2-oxazolinyl	CH_3	$C_{21}H_{26}N_2O_3$	118–120	E	84 (6)[b]
22c	CH_3	CH_3	COOEt	CH_3	$C_{22}H_{29}NO_4$	63–65	E	85 (13)[b]
22d	CH_3	CH_3	2-oxazolinyl	CH_3	$C_{22}H_{28}N_2O_3$	106–109	E	92 (9)[b]
23	–	–	–	–	$C_{11}H_{14}ClNO_2$	43–46	G	80
24	CH_3	CHO	2-oxazolinyl	H	$C_{20}H_{22}N_2O_4$	173–174	H	42
25	CH_3	CHO	COOEt	H	$C_{20}H_{23}NO_5$	117–118	H	78
26	CH_3	CHO	COOH	H	$C_{18}H_{19}NO_5$	181–183	L	75
27	–	–	–	–	$C_{14}H_{18}BrNO_2$	70–72	A	70
28	–	–	–	–	$C_{18}H_{22}N_2O_2$	105–107	A	53
29	–	–	–	–	$C_{18}H_{26}N_2O_2$	69–71	A	72
30	–	–	–	–	$C_9H_{11}ClOS$	oil	–	82
31	–	–	–	–	$C_9H_{12}O_2S$	oil	–	21
32	–	–	–	–	$C_{18}H_{19}NO_3S$	144–145	D	93 (12)[b]
33	–	–	–	–	$C_{18}H_{20}O_4S$	85–86	C	30 (8)[b]

[a] A = *n*-hexane. B = ethyl ether. C = cyclohexane. D = acetonitrile. E = benzene–cyclohexane. F = ethyl acetate. G = petroleum ether. H = ethanol. I = benzene. J = dichloromethane. K = carbon tetrachloride. L = toluene. [b] Data without parentheses refer to method D; data within parentheses refer to method B.

In summary, considering the general formula **2** of the new anti-HRV agents here described, the structural features which ensure the best biological profile seem to be (i) a thiophene ring as heterocyclic terminus, (ii) a chlorine atom or a methyl group in the α position of thiophene, (iii) a carbonyl group conjugated with thiophene, (iv) a tetramethylene chain connecting the carbonyl group to the phenoxy moiety, and (v) an ethoxycarbonyl substituent on the benzene ring.

Compounds **8d,e**, which best meet these structural requirements, are the most potent among the newly synthesized derivatives against a wide range of HRV

Table 2. *In Vitro* Antirhinoviral Activity of Furan and Pyrrole Derivatives

Compd	Structure	EC_{50}[a] HRV-2	EC_{50}[a] HRV-14	MIC_{80}[b]	CC_{50}[c]
7a		3.20	8.01	>32.05	>320
7b		>30.67	3.68	>30.67	>306
8c		0.91	2.42	ND	65
9		>30.58	>30.58	>30.58	>305
17		>32.05	>32.05	>32.05	>320
18		10.95	13.30	19.47	>317
22a		1.17	18.95	>29.01	204
22b		>29.41	>29.41	>29.41	205
22c		2.28	16.81	>28.01	56
22d		>28.25	11.30	>28.25	141
24		>28.25	>28.25	>28.25	>282
25		0.67	1.96	8.40	56
26		>30.90	>30.90	>30.90	>303
28		1.43	>31.85	15.92	159
29		>33.11	>33.11	>33.11	165
WIN 51711		2.35	1.76	3.51	37

[a] EC_{50}: compound concentration (μM) required to achieve 50% protection of HeLa cells from HRV-induced cytopathic effect. [b] MIC_{80}: compound concentration (μM) which inhibits 80% of the serotypes tested. The serotypes used were HRV-1A, -1B, -2, -6, -14, -15, -21, -22, -25, -30, -41, -50, -67, -86, and -89. [c] CC_{50}: compound concentration (μM) required to reduce the viability of mock-infected HeLa cells by 50%.

serotypes, showing MIC_{80} values of 2.2 and 2.3 μM, respectively, which compare well with that of WIN 51711 (3.5 μM). It is worth pointing out that contrary to WIN 51711, which is cytotoxic at a concentration of 36.7 μM, most of the new compounds are far less toxic and in some cases exhibit more than a 10-fold more favorable selectivity index (SI, defined as the ratio between CC_{50} and MIC_{80}).

Experimental Section

Chemistry. Melting points (Büchi 530 melting point apparatus) are uncorrected. IR spectra (Nujol mulls) were recorded on a Perkin-Elmer 297 instrument. ^1H NMR spectra were recorded at 90 MHz on a Varian EM-390 spectrometer. Tetramethylsilane was used as an internal reference standard. All compounds were routinely checked by TLC and ^1H NMR. NMR data were consistent with the indicated structures. TLC was performed with C. Erba silica gel Stratocrom SIF-254

Table 3. *In Vitro* Antirhinoviral Activity of Methylthiophene Derivatives

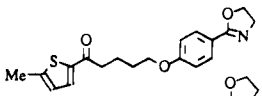
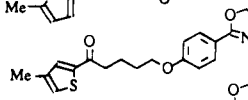
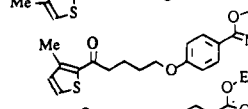
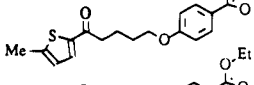
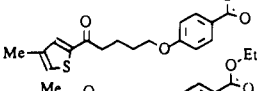
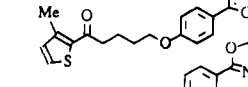
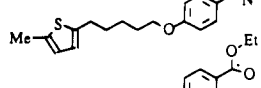
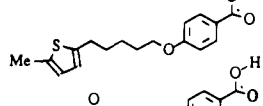
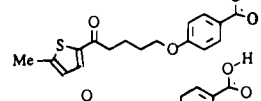
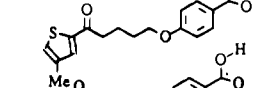
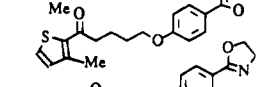
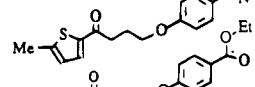
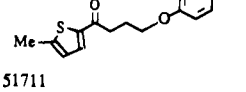
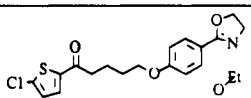
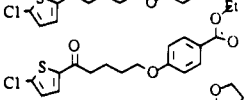
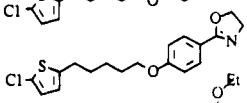
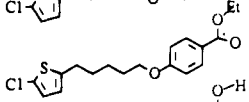
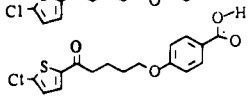
Compd	Structure	EC_{50}[a] HRV-2	EC_{50}[a] HRV-14	MIC_{80}[b]	CC_{50}[c]
7e		1.31	0.14	9.04	146
7f		2.91	0.87	14.58	73
7g		29.15	1.46	>29.15	291
8e		0.06	0.29	2.31	>289
8f		2.89	5.78	ND	>289
8g		6.94	>28.90	20.80	202
11e		2.58	11.50	>30.39	>303
12e		1.59	7.16	14.33	301
13e		>31.44	>31.44	>31.44	>314
13f		>31.44	>31.44	>31.44	>314
13g		>31.44	>31.44	>31.44	>314
32		15.15	22.73	>30.30	ND
33		10.60	>30.12	>30.12	>301
WIN 51711		2.35	1.76	3.51	37

Table 4. *In Vitro* Antirhinoviral Activity of 5-Chlorothiophene Derivatives

Compd	Structure	EC_{50}[a] HRV-2	EC_{50}[a] HRV-14	MIC_{80}[b]	CC_{50}[c]
7d		0.81	0.13	>27.10	>271
8d		0.08	0.54	2.18	>273
11d		2.57	2.85	21.43	71
12d		1.84	9.15	18.29	>284
13d		>29.50	>29.50	>29.50	>295
WIN 51711		2.35	1.76	3.51	37

precoated plates. Developed plates were visualized by UV light. Merck silica gel 60 and alumina 90 were used for chromatographic purifications. Solvents were reagent grade and, when necessary, purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of approximately 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Microanalyses (within ±0.4% of the theoretical values) were performed by the Microanalytical Laboratory of Prof. A. Pietrogrande, University of Padova, Italy. All compounds were analyzed for C, H, and N and, when present, Cl and S.

Syntheses. Specific examples presented below illustrate general synthetic methods A–E. In general, samples prepared for physical (Table 1) and biological studies (Table 2–4) were dried in high vacuum over P_2O_5 for 20 h at temperatures ranging from 25 to 110 °C, depending on the sample melting point.

Method A Example. 2-(5-Chloropentanoyl)-3-methylthiophene (5g) and 2-(5-chloropentanoyl)-4-methylthiophene (5f). A solution of 5-chlorovaleryl chloride (15.5 g, 0.1 mol) and aluminum trichloride (13.3 g, 0.1 mol) in 1,2-dichloroethane (150 mL) was added to a solution of 3-methylthiophene (**4f**) (9.8 g, 0.1 mol) in the same solvent (150 mL). The solution was stirred at room temperature for 2 h and poured onto a mixture of ice (200 g) and concentrated HCl (20 mL). Extractive workup with chloroform gave an oily residue which was chromatographed on a silica gel column eluting with 4% ethyl acetate in hexanes to yield **5g** (9.0 g): ^{1}H NMR (CCl_4) δ 1.78–1.92 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.50 (s, 3H, CH_3), 2.75–2.87 (m, 2H, $COCH_2$), 3.48–3.58 (m, 2H, CH_2Cl), 6.89 (d, J = 5.4 Hz, 1H, thiophene H-4), 7.31 (d, J = 5.4 Hz, 1H, thiophene H-5). Further elution of the above column afforded **5f** (1.9 g): ^{1}H NMR (CCl_4) δ 1.77–1.88 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.23 (s, 3H, CH_3), 2.75–2.88 (m, 2H, $COCH_2$), 3.47–3.58 (m, 2H, CH_2Cl), 7.12 (d, J = 1.5 Hz, 1H, thiophene H-3), 7.43 (d, J = 1.5 Hz, 1H, thiophene H-5).

Method B Example. 2-[5-[4-(4,5-Dihydro-2-oxazolyl)phenoxy]pentanoyl]-5-methylthiophene (7e). To a stirred solution of **5e** (1.99 g, 9.2 mmol) in dry acetonitrile (100 mL) were added sodium iodide (1.12 g, 7.5 mmol), anhydrous potassium carbonate (1.42 g, 10.3 mmol), and 4-(4,5-dihydro-2-oxazolyl)phenol (**6a**) hydrochloride (2.06 g, 10.3 mmol). The suspension was refluxed for 72 h and then filtered while hot, and the solution was evaporated. The residue was partitioned between ethyl acetate and water. The organic layer was washed successively with 5% aqueous NaOH, 5% aqueous sodium thiosulfate, water, and brine. Evaporation of the solvent left a solid residue, which was chromatographed on silica gel eluting with $CHCl_3$:ethyl acetate (1:1) to give pure **7e** (0.79 g) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.83–1.95 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.50 (s, 3H, CH_3), 2.83–2.97 (m, 2H, $COCH_2$), 3.97–4.10 (superimposed signals, 4H, CH_2N and CH_2OAr), 4.30–4.40 (m, 2H, OCH_2CH_2N), 6.78 (d, 1H, thiophene H-4), 6.87 (m, 2H, benzene H-2,6), 7.53 (d, 1H, thiophene H-3), 7.88 (m, 2H, benzene H-3,5).

5-Chloro-2-(5-chloropentyl)thiophene (10d). A solution of **5d** (1.19 g, 5 mmol) and aluminum trichloride (0.66 g, 5 mmol) in a mixture of anhydrous diethyl ether (40 mL) and anhydrous THF (30 mL) was slowly added to a cooled (5 °C) suspension of lithium aluminum hydride (0.38 g, 10 mmol) and aluminum trichloride (1.33 g, 10 mmol) in diethyl ether (10 mL). The mixture was stirred at 30–40 °C for 2 h, and then the reaction was cautiously quenched with water (20 mL) and 6 N H_2SO_4 (20 mL). The organic layer was separated, washed twice with brine, dried, and evaporated to furnish **10d** (0.99 g) as an oil homogeneous by TLC analysis (SiO_2/benzene).

Method C Example. 2-[5-(4-Carboxyphenoxy)pentanoyl]-5-methylthiophene (13e). A mixture of **8e** (1.6 g, 4.6 mmol), 1.5 N NaOH (15.3 mL, 23 mmol), and EtOH (15 mL) was heated at 90 °C for 2.5 h with stirring. After cooling, the solution was diluted with water (100 mL) and made acidic by adding 2 N HCl. Extraction with ethyl acetate followed by usual workup gave a solid, which was recrystallized to afford pure **13e** (1.4 g).

3-(5-Chloropentanoyl)-1*H*-pyrrole (16). A solution of **15** (6.52 g, 0.02 mol) in dioxane (75 mL) and 5 N NaOH (70 mL, 0.35 mol) was stirred at room temperature for 17 h. The organic layer was separated and the aqueous one extracted with ethyl acetate. The combined organic solution was washed with brine, dried, and evaporated to leave a brown solid, which was recrystallized to give **16** (3.16 g).

Method D Example. 3-[5-[4-(4,5-Dihydro-2-oxazolyl)phenoxy]pentanoyl]-1,2,5-trimethyl-1*H*-pyrrole (22d). A solution of diethyl azodicarboxylate (DEAD) (1.25 g, 7.2 mmol) in anhydrous THF (20 mL) was added over a period of 10 min to a solution of **21b** (1.51 g, 7.2 mmol), **6a** (1.17 g, 7.2 mmol), and triphenylphosphine (1.89 g, 7.2 mmol) in anhydrous THF (100 mL). After stirring at room temperature for 18 h, the solvent was removed under reduced pressure, and the residue was partitioned between water and $CHCl_3$. The organic layer was washed twice with 2 N KOH and then with brine, dried, and evaporated. Column chromatography on silica gel (ethyl acetate as eluent) provided pure **22d** (2.35 g).

4-(5-Chloropentanoyl)-1-methyl-1*H*-pyrrole-2-carboxaldehyde (23). To a cooled (0–5 °C) solution of DMF (0.78 mL, 10 mmol) in 1,2-dichloroethane (20 mL) was added over a period of 5–10 min a solution of oxalyl chloride (1.27 g, 10 mmol) in 1,2-dichloroethane (20 mL). After stirring at room temperature for 15 min, the suspension was cooled (0–5 °C) again and treated with a solution of 1-methyl-1*H*-pyrrole (**4b**) (0.81 g, 10 mmol) in 1,2-dichloroethane (20 mL). The mixture was stirred at room temperature for 15 min and then treated with aluminum trichloride (2.92 g, 22 mmol) and 5-chlorovaleryl chloride (1.55 g, 10 mmol). After 3 h, the reaction mixture was poured onto crushed ice (100 g) containing 50% NaOH (10 mL) and stirred for 10 min. The pH of the solution was adjusted to 4 with concentrated HCl, the organic layer was separated, and the aqueous one was extracted with $CHCl_3$. The combined organic solution was washed with water, dried, and evaporated to dryness. The brown oily residue was purified by chromatography on silica gel ($CHCl_3$) to afford **23** (1.81 g).

Method E Example. 1-[5-[4-(4,5-Dihydro-2-oxazolyl)phenoxy]pentyl]-1*H*-pyrrole (28). A solution of 1*H*-pyrrole (2.01 g, 30 mmol) in anhydrous dioxane (20 mL) was added dropwise to a suspension of 97% NaH (0.82 g, 33 mmol) in 20 mL of the same solvent. The mixture was refluxed for 15 min and then cooled again to room temperature. A solution of **27** (2.34 g, 7.5 mmol) was added dropwise to the above mixture, which was then refluxed for 5 h. After cooling, the reaction was quenched with a saturated solution of NH_4Cl (50 mL) and the mixture concentrated and extracted with ethyl acetate. The organic solution was dried and evaporated to give a residue, which was chromatographed on alumina eluting with $CHCl_3$ and recrystallized to afford pure **28** (1.19 g).

Antiviral Assays. HeLa-Ohio cells were grown at 37 °C in a 5% CO_2 atmosphere in Dulbecco's modified medium (D-MEM) supplemented with 10% fetal calf serum (FCS), 100 UI/mL penicillin G, and 100 μg/mL streptomycin. Cultures were checked periodically for the absence of mycoplasma contamination with a MicoTect kit (Gibco). Compounds were solubilized in DMSO as 200× stock solutions and then serially diluted in maintenance medium to achieve the desired final concentrations.

Anti-HRV assays were based on the inhibition of virus-induced cytopathogenicity. Briefly, 20 000 HeLa cells were seeded in each well of flat-bottomed microtiter trays in 50 mg/mL D-MEM containing 2% fetal calf serum, $MgCl_2$ (30 mM), and DEAE-dextran (15 μg/mL). The cells were then allowed to form a subconfluent monolayer by incubating overnight at 37 °C in a humidified incubator under an atmosphere of 5% CO_2. Maintenence medium (50 mL) containing various concentrations of the test compounds was then added followed by 20 mL of an HRV suspension calibrated to produce complete cytopathic effect within 72 h after infection [200–2000 infectious virus particles (PFU)/monolayer]. Following incubation at 33 °C, viability of the HeLa cells was determined by the 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.

Cytotoxicity of test compounds was evaluated in parallel with their antiviral activity. It was based on the viability of mock-infected cells, as monitored by the MTT method after a 72 h incubation at 37 °C.

Acknowledgment. This work has been supported by Cenci Bolognetti-Institute Pasteur Foundation (in part) and Italian MURST (60% fund, Dpt Studi Farmaceutici), Italian CNR (Dpt Farmaco Chimico Tecnologico), and Regione Autonoma della Sardegna (Dpt Biologia Sperimentale, Sezione Microbiologia).

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JM930828E