

Isothiazole derivatives as antiviral agents

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We recently described the synthesis and antiviral activity of the compounds 5-phenyl-3-(4-cyano-5-phenylisothiazol-3-yl) disulphanyl-4-isothiazole-carbonitrile and 5-(4-cyano-5-phenylisothiazol-3-yl)-O-ethyl thiocarbonate, which were found to be effective against both HIV-1 (III_B) and HIV-2 (ROD). We have now evaluated these compounds against both RNA and DNA viruses, obtaining high selectivity indexes for poliovirus 1 (SI: 223 and 828, respectively) and Echovirus 9 (SI: 334 and 200, respectively). In our previous studies, 3-methylthio-5-(4-OBn-phenyl)-4-isothiazolecarbonitrile was found to exhibit a broad spectrum of action against picornaviruses, we therefore

selected this compound and 5-(4-cyano-5-phenylisothiazol-3-yl)-O-ethyl thiocarbonate as the model for the synthesis of a new isothiazole derivative, 5-[4-cyano-5-(4-OBn-phenyl)isothiazol-3-yl]-O-ethyl thiocarbonate. This compound was evaluated against picornaviruses, measles virus, HIV-1 (III_B) and HIV-2 (ROD), and some DNA viruses (adenovirus type 2 and herpes simplex virus type 1). The compound was shown to be active against rhinoviruses 2, 39, 86 and 89, Coxsackie B1 and measles virus.

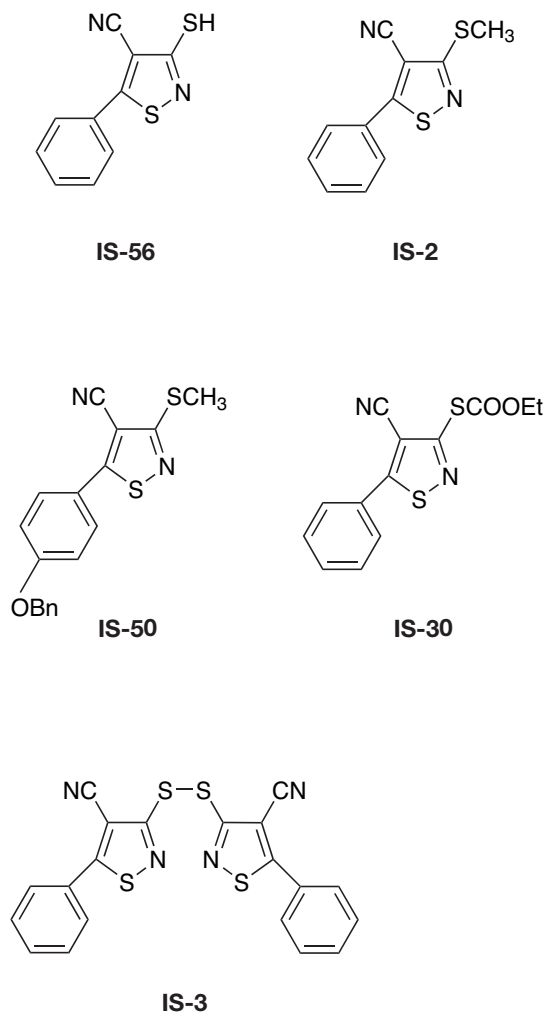
Keywords: isothiazole derivatives, HIV, picornavirus, measles

Introduction

Our attempts to find new antiviral agents have led to the identification of 3-methylthio-5-aryl-4-isothiazolecarbonitriles, which were found to be active against a number of RNA viruses. Our chemical approach was to investigate the effect of some structural modifications at different positions in the isothiazole nucleus to establish the requirements for optimum activity. Structure–activity relationship studies revealed that the presence of a short thioalkyl chain in the 3-position, a cyano or methylester group in the 4-position, and an unsubstituted phenyl ring in the 5-position are the structural features that seem to ensure the best activity profile against polio 1 and Echo 9 enteroviruses. The highest activity against the above-mentioned viruses was obtained with 3-methylthio-5-phenyl-4-isothiazolecarbonitrile [IS-2] (Cutri *et al.*, 1998, 1999), and this compound was selected as a lead compound for further synthesis of new isothiazole derivatives. Further studies demonstrated that the presence of bulky substituents at the *para* position of the phenyl ring broadened the spectrum of activity: in fact, 3-methylthio-5-(4-OBn-phenyl)-4-isothiazolecarbonitrile [IS-50] proved active against Coxsackie B1, encephalomyocarditis (EMC), rhinoviruses (HRVs) and measles virus (Cutri *et al.*, 1998; Garozzo *et al.*, 2000).

Among the previously synthesized compounds, only 3-mercapto-5-phenyl-4-isothiazolecarbonitrile [IS-56] was found to inhibit the replication of HIV-1 (III_B) and HIV-2 (ROD). Further studies led to the synthesis of 5-phenyl-3-(4-cyano-5-phenylisothiazol-3-yl) disulphanyl-4-isothiazolecarbonitrile [IS-3] and *S*-[3-(4-cyano-5-phenyl)isothiazolyl] ethyl thiocarbonate [IS-30], which showed promising activity against both HIV-1 (III_B) and HIV-2 (ROD). From these data, we postulated that [IS-56] was the active component, as most probably the disulphide bridge of compound [IS-3] is reduced and the *O*-ethylthiocarbonate of compound [IS-30] is hydrolyzed, both reactions leading to the formation of the molecule responsible for anti-HIV activity (Cutri *et al.*, 2004).

In the present study, we evaluated isothiazoles [IS-3] and [IS-30] against both RNA viruses (polio 1, Echo 9, Coxsackie B1, EMC, HRV-2, HRV-29, HRV-39, HRV-86, HRV-89 and measles) and DNA viruses (adenovirus 2 and HSV-1). Moreover, we report the antiviral activity of new isothiazole derivatives, synthesized to assess whether the introduction or combination of different chemical modifications in the isothiazole structure could improve the antiviral properties and/or broaden the antiviral spectrum.

Figure 1. Structural formulae of isothiazole derivatives

Materials and methods

Chemistry

Melting points were determined on a Büchi 510 apparatus (Büchi Laboratoriumstechnik AG, Flawil, Switzerland) and were not corrected. Elemental analyses for all new compounds were performed on a C Erba Model 1106 elemental analyzer (Carlo Erba, Milan, Italy) and the data of C, H, N and S were within $\pm 0.3\%$ of calculated values. Thin layer chromatography (TLC) was used to monitor reactions. Infra-red (IR) spectra were recorded as KBr pellets using a Perkin-Elmer 281 spectrophotometer (Perkin-Elmer, Monza, Italy). Mass spectra (MS) data were run on a C Erba/Kratos mass spectrometer.

General procedure for the synthesis of isothiazole derivatives

Figure 1 shows the structural formulae of the isothiazole derivatives [IS-56], [IS-2], [IS-50], [IS-30] and [IS-3]. The synthesis and chemical properties of these compounds were previously reported (Cutri *et al.*, 1998, 2004).

A solution of $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ (4.54 mmol) in a mixture of methanol (50 ml) and water (5 ml) was refluxed for 15 min. A solution of known 3-chloro-5-phenyl-4-isothiazole-carbonitrile (4.5 mmol) [1] (Nakagawa *et al.*, 1970) in methanol (20 ml) was added dropwise and the mixture was refluxed for 3 h. After cooling, the solution was partitioned between water and diethyl ether and the intermediate sodium salt [2] was converted to the desired 4-cyano derivatives [3] and [4] by treatment with suitable dibromoalkanes (2.25 mmol). The mixture was stirred at room temperature for 12 h and, after filtering and washing with water, the compounds were purified by crystallization (Figure 2).

A solution of sodium ethoxide, prepared from sodium (11 mmol) and absolute ethanol (5 ml), and malononitrile (11 mmol) was added to the solution of 4-(phenyl-methoxy)-benzenecarbothioic acid *O*-ethyl ester [5] (7.3 mmol) (Cutri *et al.*, 1998). The reaction mixture was stirred at room temperature for 1 h (Hartke & Peshkar, 1968). Sulphur (12 mmol) was then added and the mixture was refluxed for 6 h (James & Krebs, 1982). After cooling, the excess sulphur was removed by filtration and an excess of ethyl chloroformate was added to the filtrate. After stirring, product [7] that separated out was filtered, washed with water and purified by crystallization (Figure 3). IUPAC nomenclature and chemical data of new compounds are as follows: 1,3-bis(4-cyano-5-phenylisothiazol-3-yl)sulphanyl propane [3] (yield 37%; mp 175–178°C [ethyl acetate]; IR [KBr] 2217 [CN] cm^{-1} ; MS m/e 476 [M], 259, 218); 1,4-bis(4-cyano-5-phenylisothiazol-3-yl)sulphanyl butane [4] (yield 39%; mp 186–189°C [ethyl acetate]; IR [KBr] 2217 [CN] cm^{-1} ; MS m/e 490 [M], 273, 77); *S*-[4-cyano-5-(4-OBn-phenyl)isothiazol-3-yl]-*O*-ethyl thiocarbonate [7] (yield 70%; mp 70–74°C [cyclohexane]; IR [KBr] 2221 [CN], 1735 [C=O] cm^{-1} ; MS m/e 396 [M], 324, 91).

Compounds were dissolved in dimethyl sulphoxide (DMSO) and diluted in maintenance medium to achieve the final concentration needed. The final dilution of test compounds contained a maximum concentration of 0.01% DMSO, which was not toxic to the cells used.

Reference compounds

R 77975 (Pirodavir) (Andries *et al.*, 1992) was kindly provided by Janssen Research Foundation (Beerse, Belgium). Zidovudine (AZT) was synthesized as previously described (Horwitz *et al.*, 1964). Nevirapine (BI-RG587) was obtained from Boehringer Ingelheim (Ridgefield, CT, USA). Ritonavir (ABT538) was kindly

Figure 2. Synthesis of isothiazole derivatives [3] and [4]

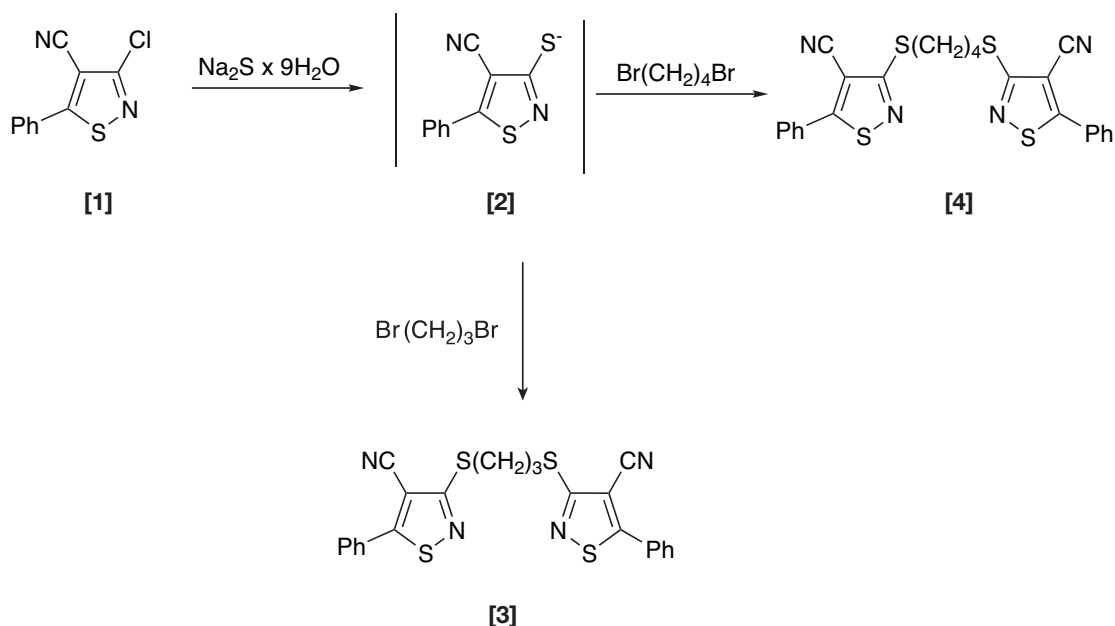
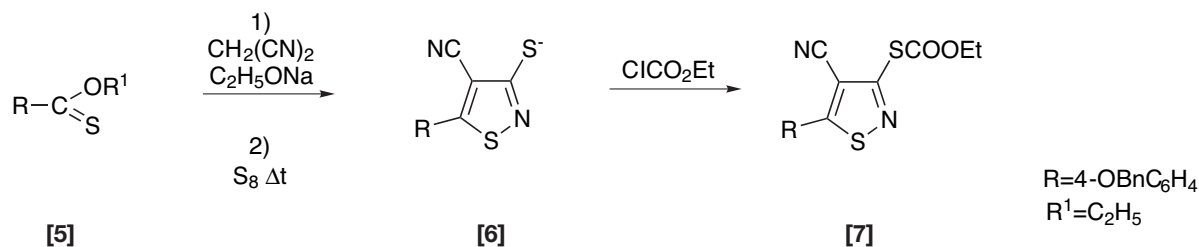


Figure 3. Synthesis of isothiazole derivative [7]



provided by JM Leonard, Abbott Laboratories (Abbott Park, IL, USA).

Virology

Viruses and cells. Poliovirus 1 (Sabin strain), Echovirus 9 (Hill strain), Coxsackievirus B1, measles (Edmonston strain) and adenovirus type 2 were purchased from the American Type Culture Collection (ATCC) and propagated in human epidermoid carcinoma larynx cells (HEp-2). Encephalomyocarditis (EMC strain) and herpes simplex type 1 (F strain) were purchased from the ATCC and propagated in mouse connective tissue cells (L-929) and African green monkey kidney cells (Vero), respectively. Human rhinoviruses (HRV-2, HRV-29, HRV-39, HRV-86 and HRV-89) were kindly supplied by Professor Paolo

La Colla (Dipartimento di Biologia Sperimentale, Sezione di Microbiologia, Università di Cagliari, Cagliari, Italy), and were propagated in human epitheloid carcinoma cervix cells (HeLa-Ohio) at 33°C.

Cell lines were kept at 37°C in a humidified atmosphere with 5% CO_2 and grown in Dulbecco's modified Eagle's Minimum Essential medium (DMEM) or Eagle's Minimum Essential medium (MEM) for HeLa cells, supplemented with 10% heat-inactivated fetal calf serum (FCS), 200 $\mu\text{g}/\text{ml}$ streptomycin and 200 units/ml penicillin G.

For all the above-mentioned viruses working stocks were prepared as cellular lysates using DMEM (MEM for HeLa cells) with 2% heat-inactivated FCS.

The origin of HIV-1 (III_B) and HIV-2 (ROD) virus stocks was previously described (Barré-Sinoussi *et al.*, 1983;

Popovic *et al.*, 1984). HIV-1 (III_B) and HIV-2 (ROD) stocks were obtained from the culture supernatant of HIV-1- or HIV-2-infected MT-4 cells, respectively. The MT-4 cells (Miyoshi *et al.*, 1982) were grown at 37°C in a humidified atmosphere with 5% CO₂ and maintained in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 0.1% sodium bicarbonate and 20 µg/ml gentamicin.

Cell viability. The cytotoxicity of the test compounds was evaluated by measuring the effect produced on cell morphology and cell growth. Cell monolayers were prepared in 24-well tissue culture plates and exposed to various concentrations of the compounds. Plates were checked by light microscopy after 24, 48 and 72 h. Cytotoxicity was scored as morphological alterations (for example, rounding up, shrinking and detachment). The viability of the cells was determined by a tetrazolium-based colorimetric method using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), as previously described (Denizot & Lang, 1986; Garozzo *et al.*, 1994). The 50% cytotoxic dose (CC₅₀) is the concentration of the compound that reduced the absorbance of the control sample by 50%.

Antiviral activity: plaque assays. The assay of antiviral activity against polio 1, Echo 9, Coxsackie B1, EMC, measles, adeno 2 and HSV-1 viruses was carried out by the 50% plaque reduction assay as previously described (Cutri *et al.*, 1998). The compound concentration required to inhibit virus plaque formation by 50% is expressed as the 50% effective concentration (EC₅₀) and calculated by dose-response curves and linear regression.

Anti-rhinovirus activity: cytopathic effect inhibition assays. Infectivity of HRV stock was determined by the MTT method: the reciprocals of viral dilution that resulted in 50% reduction of absorbance of formazan in the infected cells at 48–72 h was determined as the MTT ID₅₀ (50% infective dose).

The anti-rhinovirus assay was based on the inhibition of virus-induced cytopathogenicity on HeLa-Ohio cells, as previously described (Garozzo *et al.*, 2000). Briefly, subconfluent monolayers grown in 96-well tissue culture plates were treated with or without various concentrations of the test compounds at concentrations below the CC₅₀, and then infected with 10× the MTT ID₅₀ of the HRV stock to produce complete cytopathic effect within 48–72 h after infection. After incubation at 33°C, the viability of mock-infected and virus-infected cells was quantified by the MTT method. The compound concentration required to inhibit virus-induced cytopathogenicity by 50% was expressed as EC₅₀ and calculated by dose-response curves using linear regression.

Anti-HIV activity and cytotoxicity assays. Compounds were tested for potency (EC₅₀) to achieve 50% protection of MT-4 cells against HIV-1 (III_B) and HIV-2 (ROD) cytopathicity by measuring the viability of MT-4 cells 5 days after infection. Cytotoxicity of the compounds was determined in parallel by measuring the viability of mock-infected cells on day 5. The number of viable cells was quantified semi-automatically by the MTT method, as previously described by Pauwels *et al.* (1987, 1988).

Results

Tables 1, 2 and 3 report the *in vitro* EC₅₀ and CC₅₀ values of the test and reference compounds. In Table 4, selectivity indexes (SI, determined as the ratio CC₅₀/EC₅₀) for polio 1 and Echo 9 are reported. The data for previously published compounds are also reported.

Compounds [IS-56] and [IS-2] were particularly effective against polio 1 and Echo 9; [IS-2] showed slight activity against EMC and [IS-56] was also found to be active against HIV-1 (III_B) and HIV-2 (ROD) (Tables 1, 2 and 3). Compound [IS-50] exhibited the broadest anti-rhinovirus spectrum among all the isothiazole derivatives studied, and was weakly effective against Coxsackie B1, EMC and measles viruses (Tables 1 and 2). Finally, [IS-3] and [IS-30] were found to be slightly active against HIV-1 (III_B) and HIV-2 (ROD) (Table 3) (Cutri *et al.*, 1998, 2004; Garozzo *et al.*, 2000).

As a result of this study, [IS-3] and [IS-30] were found to exhibit a good activity against polio 1 and Echo 9 enteroviruses. In particular, [IS-30] showed the lowest EC₅₀ value against polio 1 (0.007 µg/ml) and was slightly active against Coxsackie B1, EMC and measles viruses. In regard of the anti-rhinovirus activity, these compounds were found to be active against some HRV serotypes tested: [IS-3] against HRV-39 and [IS-30] against HRV-29 and HRV-86. No inhibition of adenovirus 2 and HSV-1 was detected for these compounds (Tables 1 and 2).

The newly synthesized compound, *S*-[4-cyano-5-(4-OBn-phenyl)isothiazol-3-yl]-*O*-ethyl thiocarbonate [7], was prepared and tested in comparison with [IS-50] and [IS-30]. Its cytotoxicity was found to be greater than that of [IS-50] and [IS-30] in all the cell lines except for MT-4 cells (Tables 1, 2 and 3).

As far as the anti-picornavirus activity is concerned, [7] exhibited an interesting antiviral activity against Coxsackie B1 with an EC₅₀ value of 0.396 µg/ml and showed its best inhibitory effect (0.1 µg/ml) against HRV-39. This compound was also weakly effective against HRV-2, HRV-86 and HRV-89, whereas no activity was observed against polio 1, Echo 9 and HRV-29 (Tables 1 and 2). Finally, we observed a good antiviral activity against measles virus with an EC₅₀ value of 0.594 µg/ml, which is lower than that

Table 1. Antiviral activity of isothiazole compounds

Compound	CC ₅₀ , µg/ml*			EC ₅₀ , µg/ml [†]				
	HEp-2/L-929/Vero	Polio 1	Echo 9	Cox B1	EMC	Measles	Adeno 2	HSV-1
[IS-56]	13	0.054	0.065	>13	>13	>13	>13	>13
[IS-2]	4.64	0.01	0.058	>4.64	2.32	>4.64	>4.64	>4.64
[IS-50]	>16.92	>16.92	>16.92	3.38	6.77	3.38	>16.92	>16.92
[IS-3]	8.69	0.039	0.026	>8.69	>8.69	>8.69	>8.69	>8.69
[IS-30]	5.8	0.007	0.029	2.9	2.9	2.9	>5.8	>5.8
[3]	>4.76	>4.76	>4.76	>4.76	>4.76	>4.76	>4.76	>4.76
[4]	>4.9	>4.9	>4.9	>4.9	>4.9	>4.9	>4.9	>4.9
[7]	3.96	>3.96	>3.96	0.396	>3.96	0.594	>3.96	>3.96
R 77975 [‡]	7	0.085	0.018	0.74	ND	ND	ND	>7

All data represent means of at least three separate experiments. *CC₅₀, the concentration that inhibited HEp-2, L-929 and Vero cell growth by 50%, as compared with control cultures. HEp-2 cells were used to propagate polio 1, Echo 9, Cox B1 and measles viruses; L-929 cells were used to propagate EMC; Vero cells were used to propagate HSV-1. [†]EC₅₀, the concentration that inhibited virus plaque formation by 50%. [‡]R 77975, reference compound Pirodavir. ND, not determined.

Table 2. Anti-rhinovirus activity of isothiazole compounds

Compound	CC ₅₀ , µg/ml*	EC ₅₀ , µg/ml [†]				
	HeLa-Ohio	HRV-2	HRV-29	HRV-39	HRV-86	HRV-89
[IS-56]	15.28	>15.28	>15.28	>15.28	>15.28	>15.28
[IS-2]	18.56	>18.56	>18.56	>18.56	>18.56	>18.56
[IS-50]	>16.92	6.77	6.77	1	0.67	0.67
[IS-3]	8.69	>8.69	>8.69	2	>8.69	>8.69
[IS-30]	7.25	>7.25	2.9	>7.25	1.45	>7.25
[3]	0.7	>0.7	>0.7	>0.7	>0.7	>0.7
[4]	0.49	>0.49	>0.49	>0.49	>0.49	>0.49
[7]	1.98	1	>1.98	0.1	1	0.79
R 77975 [‡]	7	0.0036	0.007	0.007	0.032	0.0018

All data represent means of at least three separate experiments. *CC₅₀, the concentration that inhibited HeLa-Ohio cell growth by 50%, as compared with control cultures. [†]EC₅₀, the concentration that inhibited virus-induced cytopathogenicity by 50%. [‡]R 77975, reference compound Pirodavir.

obtained for [IS-50] and [IS-30] (Table 1). No inhibitory effect was observed for compound [7] against HIV-1 (III_B), HIV-2 (ROD) and the DNA viruses tested. The newly synthesized compounds [3] and [4] were inactive against all the viruses studied (Tables 1, 2 and 3).

Discussion

In the present study, we explored the antiviral activity of compounds 5-phenyl-3-(4-cyano-5-phenylisothiazol-3-yl) disulphanyl-4-isothiazolecarbonitrile [IS-3] and *S*-(4-cyano-5-phenylisothiazol-3-yl)-*O*-ethyl thiocarbonate [IS-30], which were tested against picornaviruses, measles viruses and some DNA viruses. These compounds, characterized by the presence of a cyano group in the 4-position and an unsubstituted phenyl ring in the 5-position, differ from each other with regard to the substituent in the

3-position, although we assume that, most probably, the disulphide bridge of [IS-3] is reduced and the *O*-ethylthiocarbonate group of [IS-30] is hydrolyzed, both leading to the formation of the free thiol group (Cutri *et al.*, 2004). These compounds, which could be considered as 'prodrugs' of [IS-56], were found to be very effective against polio 1 and Echo 9 (Table 1). In particular, when compared with lead compound [IS-2], for [IS-3] and [IS-30] we obtained higher SI values for Echo 9 (334 and 200, respectively) and for [IS-30] the highest SI observed for polio 1 (828) (Table 4). It is noteworthy that [IS-30] was also effective against Coxsackie B1, EMC and measles viruses (Table 1).

As far as anti-rhinovirus activity was concerned, [IS-3] and [IS-30] with an unsubstituted phenyl in the 5-position were only weakly active against some HRV serotypes tested (Table 2).

In order to establish the necessary structural requirements to obtain a broad-spectrum antiviral activity and/or to improve antiviral properties, we selected [IS-50] and [IS-30] as the starting compounds for the synthesis of the new isothiazole derivative [7]. The structural formula of this isothiazole presents a combination of different chemical groups, with an *O*-ethylthiocarbonate group in the 3-position, as in [IS-30], and a bulky substituent, the *O*-Bn group, at the *para* position of the phenyl ring in the 5-position, as in [IS-50], whereas the cyano group in the 4-position is maintained.

In particular, the simultaneous introduction of *O*-ethylthiocarbonate and of *O*-Bn groups in the structure of compound [7] led to an interesting antiviral activity against Cocksackie B1 and measles viruses with EC₅₀ values of 0.396 µg/ml and 0.594 µg/ml, respectively, which were lower than those observed for [IS-50] and [IS-30] (Table 1).

The anti-rhinovirus activity of these isothiazoles depends on the presence of bulky substituents in the 5-position: in fact, the presence of the *O*-Bn group at the *para* position of the phenyl ring seems to be responsible for good anti-HRV activity, as we observed for both compounds [IS-50] and [7] (Table 2). Mode of action studies showed that many anti-rhinovirus compounds inhibit viral replication by binding to a hydrophobic pocket on the virion surface, thereby preventing viral adsorption or uncoating depending on the viral serotype involved. As already seen for similar isothiazole compounds, we could postulate that voluminous groups introduced into the 5-position may interfere with viral replication by inducing conformational changes in the binding site, also suggesting a potential capsid-binding activity for compound [7] (Garozzo *et al.*, 2000; Cutri *et al.*, 2002).

Nevertheless, the presence of the 5-(4-Bn*O*-phenyl) group in the 5-position caused a loss of antiviral activity against polio 1 and Echo 9 for both compounds [IS-50] and [7] (Table 1).

No inhibition of HIV-1 (III_B) and HIV-2 (ROD) was detected for compound [7]. Similarly to [IS-50], the steric effect of the bulky group in the 5-position is probably predominant and results in the loss of the anti-HIV activity of compound [7] (Table 3).

Structure-activity relationship (SAR) studies revealed that the presence of the mercapto group in the 3-position of the isothiazole ring as well as its alkylation maintained antiviral activity against polio 1 and Echo 9 (Cutri *et al.*, 1998, 1999), whereas the free thiol group at position 3 seems to be responsible for anti-HIV activity (Cutri *et al.*, 2004). In this study we synthesized compounds [3] and [4] in an attempt to obtain more extensive structural modifications, wherein the monomers of [IS-56] were linked by a chemically stable alkyl bridge. As observed with the other alkylated monomers, these compounds were not able to

Table 3. Anti-HIV activity of isothiazole compounds

Compound	CC ₅₀ , µg/ml*	EC ₅₀ , µg/ml†	
	MT-4 cells	HIV-1	HIV-2
[IS-56]	34.2	7.8	9.7
[IS-2]	>125	>125	>125
[IS-50]	>125	>125	>125
[IS-3]	52.3	13.6	17.4
[IS-30]	108	15.2	13.4
[3]	>125	>125	>125
[4]	>125	>125	>125
[7]	>125	>125	>125
Zidovudine	9.82	0.0003	0.0005
Nevirapine	19.65	0.014	>20
Ritonavir	>10	0.039	0.10

All data represent means for at least three separate experiments. *CC₅₀, the concentration that inhibited MT-4 cell growth by 50%, as compared with control cultures. †EC₅₀, the concentration that inhibited virus-induced cytopathogenicity by 50%.

Table 4. Selectivity index of compounds active against polio 1 and Echo 9 viruses

Compound	Selectivity index *	
	Polio 1	Echo 9
[IS-56]	241	200
[IS-2]	464	80
[IS-3]	223	334
[IS-30]	828	200
R 77975†	82	389

*The selectivity index (SI) was determined as the ratio of CC₅₀ to EC₅₀. †R 77975, reference compound pirodavir.

inhibit HIV replication *in vitro*, as the intermediate alkyl chain did not make the conversion into an SH group possible (Table 3). Moreover, compounds [3] and [4] were ineffective against other RNA viruses, thus indicating that the presence of an alkyl chain interrupting the disulphide bridge annihilated any antiviral activity (Tables 1 and 2).

Finally, for compound [7] we obtained an antiviral spectrum similar to that shown by [IS-50], due to the presence of the same voluminous group in the 5-position, responsible for inhibitory activity against rhinoviruses.

Our results indicate that new isothiazole derivatives endowed with a broader antiviral spectrum can be obtained starting from the parent compounds. In fact, the new compound [IS-30] is effective against polio 1, Echo 9, Cocksackie B1, EMC, HRV-29, HRV-86, measles viruses, HIV-1 (III_B) and HIV-2 (ROD), often with lower EC₅₀ values than those obtained with previous isothiazole compounds. Moreover, compound [7] had broad spectrum activity, being effective against

rhinoviruses HRV-2, HRV-39, HRV-86, HRV-89, Coxsackie B1 and measles virus. The mode of action of these compounds is currently under study. Further research will focus on the mechanism of action of compound [IS-30], which represents a strong candidate for *in vivo* evaluation as a systemic agent for the treatment of picornavirus infections.

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Received 9 August 2007, accepted 2 October 2007