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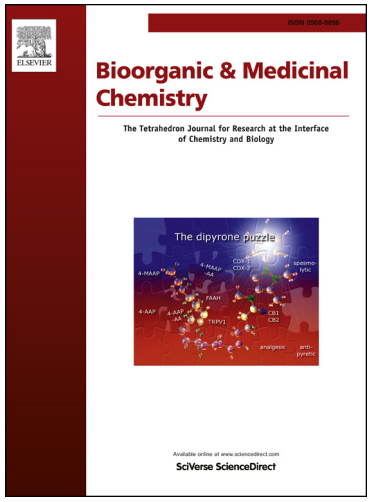

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Synthesis and antiviral properties of novel indole-based thiosemicarbazides and 4-thiazolidinones

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ABSTRACT

A novel series of indolylthiosemicarbazides (**6a**-**6g**) and their cyclization products, 4-thiazolidinones (**7a**-**7g**), have been designed, synthesized and evaluated, in vitro, for their antiviral activity against a wide range of DNA and RNA viruses. Compounds **6a**, **6b**, **6c** and **6d** exhibited notable antiviral activity against Coxsackie B4 virus, at EC_{50} values ranging from 0.4 to 2.1 µg/ml. The selectivity index (ratio of cytotoxic to antivirally effective concentration) values of these compounds were between 9 and 56. Besides, **6b**, **6c** and **6d** also inhibited the replication of two other RNA viruses, Sindbis virus and respiratory syncytial virus, although these EC_{50} values were higher compared to those noted for Coxsackie B4 virus. The SAR analysis indicated that keeping the free thiosemicarbazide moiety is crucial to obtain this antiviral activity, since the cyclization products (**7a**-**7g**) did not produce any antiviral effect.

Keywords

Indole; Thiosemicarbazide; 4-Thiazolidinone; Antiviral activity; Coxsackie B4 virus

1. Introduction

Viral diseases continue to cause serious morbidity and mortality worldwide. For several virus infections, no antiviral medications are presently available. There is a clear need for new antiviral agents with new mechanisms of action or broad-spectrum activity, to face the issues of drug-resistant mutant viruses or emerging and neglected viruses.

The *Picornaviridae* are a family of non-enveloped, single-stranded RNA-viruses for which no antiviral drugs are yet available. Among these, the Enterovirus genus is of particular medical importance.[1] It contains the Coxsackie viruses which are classified into group A (serotypes A1 to A24) and group B (serotypes B1 to B6) based on early observations of the pathogenicity in mice.[2] Coxsackie virus group B (CVB) and, to a lesser extent, Coxsackie virus group A and some other enteroviruses, are the main viral causes of myocarditis and pericarditis. CVB is also associated with a wide variety of other diseases, including diabetes, common cold, cardiomyopathy, neurological disorders and inflammation.[3] Outbreaks of CVB occur annually throughout the world. Several synthetic molecules have been reported to be selective inhibitors of enteroviruses, and some of these have entered into clinical trials.[4-7] Unfortunately, neither of these investigational compounds has been formally approved for the treatment of acute enteroviral (including Coxsackie virus) infections.

The indole core is a ubiquitous substructure in a large number of biologically active natural and synthetic molecules. Delavirdine (I), arbidol (II) and methisazone (III) (Fig. 1) are indole derived marketed drugs that have been used to treat viral diseases. Arbidol is an influenza virus inhibitor which is marketed in Russia, China and a few other countries. It was reported to have a wide spectrum of antiviral activity against a number of enveloped and non-enveloped viruses in addition to influenza viruses.[8] For instance, arbidol was found to exhibit potent inhibitory activity against CVB3 and CVB5.[3,9] Another indole compound, N-methylisatin-β-thiosemicarbazone, methisazone, was used in the preventive treatment of smallpox after 1962. Based on in vitro studies, the antiviral spectrum of methisazone appears to include diverse DNA and RNA viruses.[10,11] A series of investigations on isatin-β-thiosemicarbazones revealed their antiviral properties against various virus types, such as herpes simplex virus (HSV),[12] Moloney leukemia virus,[13] Japanese encephalitis virus[14] and human immunodeficiency virus (HIV).[15] In a recent report by Zhang et al,[16] an isatin-β-hydrazone derivative (encoded ID45) was described as a promising antiviral agent against CVB3. Broad antiviral in vitro activity that includes CVB2[17] or CVB4[18] was further reported for some 2,3,5-trisubstituted indole derivatives synthesized by Giampieri et al[17] and

isoindolylureas synthesized by Verma et al.[18] Another class are indole-2-carboxamide derivatives, some of which were revealed to have promising antiviral properties during the past decade. Compounds with this scaffold were found to inhibit the replication of neurotropic alphavirus,[19] HIV-1,[20] HIV-1 wild type or drug-resistant mutant strains, and CVB4.[21] Another relevant scaffold is the 4-thiazolidinone substructure, which is present in several small synthetic molecules endowed with in vitro antiviral characteristics.[22-24]

In view of these literature reports, we have focused our work on the design and synthesis of a new series of indolylthiosemicarbazides (**6a-6g**) and their cyclization products, 4-thiazolidinones (**7a-7g**). These new compounds were evaluated for in vitro antiviral activity against a wide variety of DNA and RNA viruses.

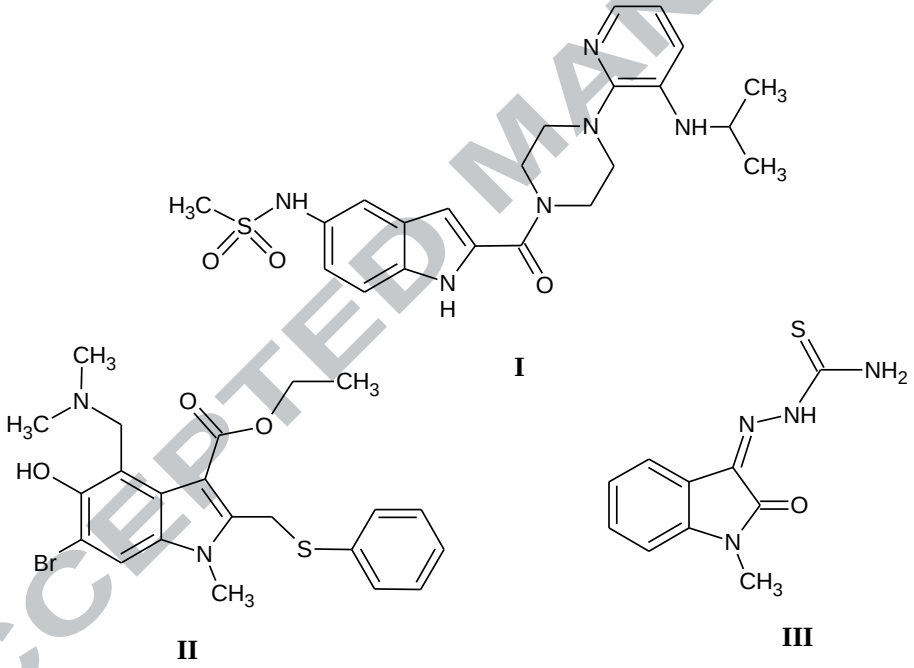


Figure 1. Structures of some indole containing antiviral drugs delavirdine (**I**), arbidol (**II**) and methisazone (**III**)

2. Results and Discussion

2.1. Chemistry and structural characterization

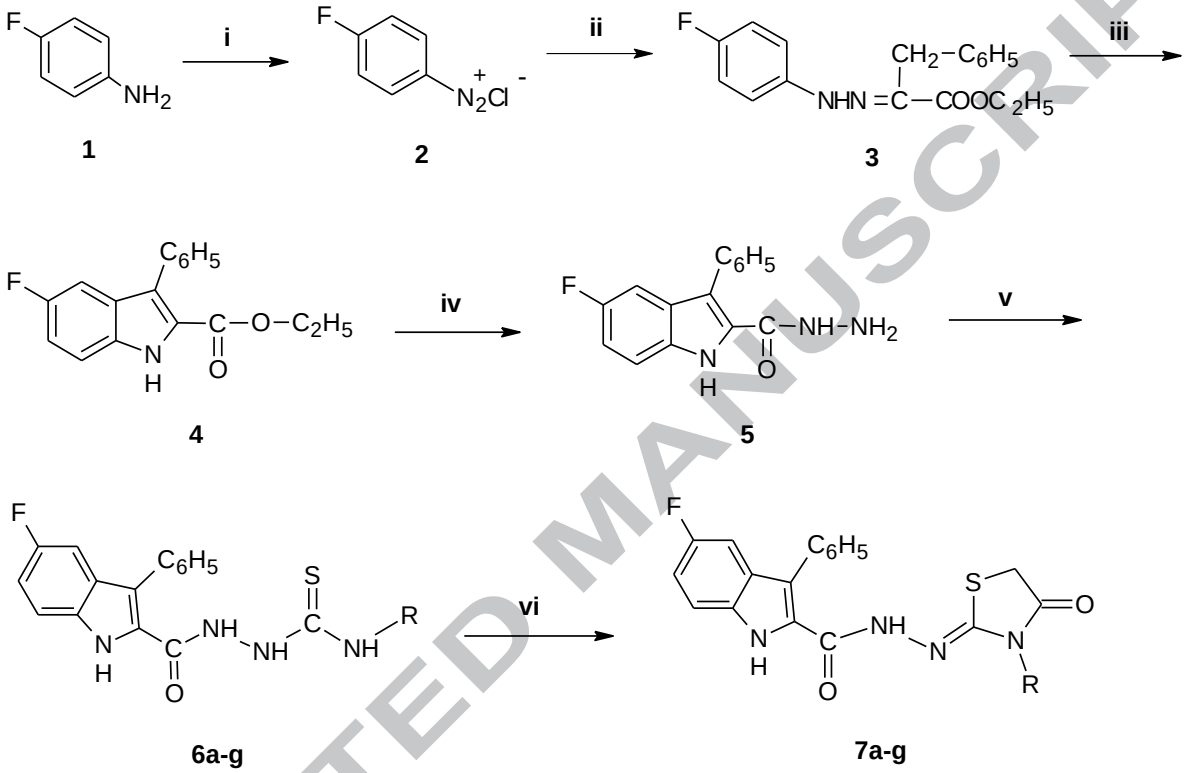
The synthetic pathways for the preparation of the target products are illustrated in Scheme 1. The key intermediate **5** was previously synthesized and patented by Ba-Maung and co-workers as an angiogenesis inhibitor.[25] The structures of the new compounds were established by microanalysis, IR, ^{1}H-NMR, ^{13}C-NMR (proton decoupled and APT), 2D-NMR (HSQC and HMBC) and electrospray ionization mass spectrometry (ESI-MS). The absolute stereochemistry of **7d** was determined by an X-ray diffraction study.[26]

The absence of the N-H_2 resonance of the intermediate hydrazide (**5**) at δ 4.48 ppm together with three new resonances located at about δ 9.68-9.82, 9.35-9.57 and 7.80-8.43 ppm assigned to the N_1H, N_2H and N_4H protons, supported the synthesis of new thiosemicarbazides (**6a-6g**). Observation of new lactam C=O bands (1710-1728 cm^{-1}) besides C=O amide bands (1636-1670 cm^{-1}) in the IR spectra of **7a-7g** provided evidence for ring closure. New singlets assigned to the methylene protons of 4-thiazolidinone ring (δ 3.95-4.05 ppm) in the ^{1}H-NMR spectra of **7a-7g** provided further confirmation. Peaks associated with the indole subunit were observed in the expected regions and were assigned on the basis of ^{1}H-^{1}H and ^{1}H-^{19}F couplings.

^{13}C-NMR experiments (proton decoupled and APT) run on **5**, **6a**, **6b**, **6e**, **6g**, **7a-c**, **7e** and 2D-NMR experiments (HSQC and HMBC) run on **6c**, **6d**, **6f**, **7d**, **7f, 7g** allowed unambiguous assingment of the proton and carbon chemical shifts. The carbocyclic indole carbons which explicitly showed the ^{13}C-^{19}F couplings of the 5-fluoro indole core were observed as separate doublets with characteristic coupling constants related to the ipso, ortho, meta and para positions and allowed definite positional assignment of the C3, C3a, C4-7 and C7a carbons of **6** and **7**. Cross peaks observed in the HMBC spectrum of compound **6c** enabled the definite assignment of C=O (δ161.93-162.04 ppm) and C=S (δ 181.96-182.93 ppm) carbons of the thiosemicarbazide analogs, **6a-6g**. The spectra of **7a-7g** displayed the typical carbonyl (δ 171.61-172.94 ppm) and methylene (δ 33.39-33.58 ppm) carbons of the 4-thiazolidinone skeleton, which further verified the aimed conversion.

Electrospray ionization mass spectrometry (ESI-MS) was employed to confirm the molecular weights of compounds **6a-6g** and **7a-7g**. All compounds were analyzed under negative-ion ESI conditions since none were responsive to the positive-ion mode.

Deprotonated [M-H]$^-$ ions observed in the ESI-MS, verified the calculated molecular weights of the new compounds.



6a,7a: R= CH_3
6b,7b: R= C_2H_5
6c,7c: R= C_3H_7
6d,7d: R= C_3H_5
6e,7e: R= C_4H_9
6f, 7f: R= $CH_2C_6H_5$
6g,7g: R= $CH_2CH_2C_6H_5$

Scheme 1. Synthesis of **6** and **7**. Reagents and conditions: (i) 7% $NaNO_2$, EtOH, conc. HCl, 0°C; (ii) ethyl 2-benzyl-3-oxo-butanoate, KOH, EtOH, 0°C; (iii) conc. HCl, reflux, 4h ; (iv) $H_2NNH_2.H_2O$, EtOH, reflux, 6h; (v) substituted isothiocyanate, EtOH, reflux, 3h; (vi) ethyl bromoacetate, fused sodium acetate, abs. EtOH, reflux, 3h.

2.2. Antiviral activity

Antiviral evaluation in cell-based assays revealed that compounds **6a**, **6b**, **6c** and **6d** have interesting activity against Coxsackie B4 virus. This effect, observed in two different cell lines (HeLa and Vero; Table 1), was quite strong since the antiviral EC_{50} values were in the range of 0.4 to 2.1 µg/ml, and the values for the selectivity index (ratio of cytotoxic to antivirally effective concentration; see values between square brackets in Table 1) were between 9 and 56. Compound **6b** (with an ethyl substituent) was slightly more potent than the analogues carrying a methyl (**6a**), propyl (**6c**) or allyl (**6d**) moiety; this is also visible from the dose-response curves shown in Figure 2. Besides, **6b**, **6c** and **6d** also inhibited the replication of two other RNA viruses, Sindbis virus and respiratory syncytial virus, although these EC_{50} values were higher compared to those noted for Coxsackie B4 virus. Of note, the analogues carrying a larger butyl (**6e**) or aromatic (**6f** and **6g**) substituent at the R position were devoid of this antiviral activity. The crucial role of the free thiosemicarbazide moiety was evident from the fact that their cyclized analogues, 4-thiazolidinones (**7a-7g**), did not produce any antiviral effect.

Compounds **6a-6g** and **7a-7g** did not display activity against any of the other RNA-viruses tested (such as HIV or influenza virus), nor against DNA-viruses (i.e. HSV-1, HSV-2, feline herpesvirus and vaccinia virus) (Table 2). On the other hand, this broad antiviral testing allowed to determine the cytotoxicity of the test compounds in diverse mammalian cell lines (Table 3). This analysis revealed that the 4-thiazolidinone derivatives (**7**) were consistently less cytotoxic than the corresponding thiosemicarbazides (**6**). Within series **6**, the compounds endowed with anti-Coxsackie B4 virus activity (**6a-6d**) tended to be less cytotoxic than the antivirally inactive counterparts **6e-6g**.

Table 1
Antiviral activity in HeLa and Vero cell cultures infected with diverse RNA viruses

Compound	Antiviral assays in HeLa cells				Antiviral assays in Vero cells					
	Antiviral EC_{50}[a] value (µg/ml)			MCC[b] (µg/ml)	Antiviral EC_{50}[a] value (µg/ml)					MCC[b] (µg/ml)
	Vesicular stomatitis virus	Coxsackie B4 virus	Respiratory syncytial virus		Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie B4 virus	Punta Toro virus	
6a	>100	2.1 ± 0.2 [9]	>100	20	>100	>100	>100	1.7 ± 0.5 [12]	>100	20
6b	>100	0.87 ± 0.47 [23]	≥4 [≥5]	20	>100	>100	3.2 ± 0.8 [6]	0.4 ± 0.0 [50]	>100	20
6c	>100	1.1 ± 0.3 [18]	≥2.3 [≥9]	20	>100	>100	6.5 ± 2.5 [15]	1.8 ± 0.0 [56]	>100	100
6d	>100	1.5 ± 0.1 [13]	≥4 [≥5]	20	>100	>100	≥4 [≥5]	2.0 ± 0.2 [10]	>100	20
6e	>100	>100	>100	4	>100	>100	>100	>100	>100	10
6f	>100	>100	>100	4	>100	>100	>100	>100	>100	10
6g	>100	>100	>100	4	>100	>100	>100	>100	>100	10
7a	>100	>100	>100	100	>100	>100	>100	>100	>100	20
7b	>100	>100	>100	>100	>100	>100	>100	>100	>100	20
7c	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
7d	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
7e	>100	>100	>100	>100	>100	>100	>100	>100	>100	>10
7f	>100	>100	>100	>100	>100	>100	>100	>100	>100	≥10
7g	>100	>100	>100	100	>100	>100	>100	>100	>100	10
DS-5000[c]	8.0 ± 3.1 [>12]	≥4 [≥25]	5.3 ± 3.3 [>19]	>100	>100	>100	68 ± 32 [>1.5]	40 ± 10 [>2.5]	20 ± 0 [>5]	>100
Ribavirin[d]	23 ± 6 [>11]	61 ± 32 [>4]	15 ± 7 [>17]	>250	>250	>250	250 ± 0 [>1]	>250	50 ± 0 [>5]	>250

Table 1 (continued-footnotes)

[a] EC_{50}: 50% effective concentration, producing 50% inhibition of virus-induced cytopathic effect, as determined by microscopy.
[b] MCC: minimum inhibitory concentration, or compound concentration causing minimal changes in cell morphology, as assessed by microscopy.
[c] DS-5000: dextran sulfate of MW 5000.
[d] Data for ribavirin are expressed in µM.
Values shown are the mean ± SEM of three independent tests.
In square brackets, the selectivity index (i.e. ratio of MCC to antiviral EC_{50}) is given.

Table 2

Results for the DNA- and RNA-viruses shown to be insensitive to the synthesized compounds

Compound	Assays in HEL[a] cells			Assays in MT-4[b] cells		Assays in MDCK[c] cells		Assays in CRFK[d] cells	
	HSV-1	HSV-2	Vaccinia virus	HIV-1	HIV-2	Influenza A	Influenza B	Feline coronavirus	Feline herpesvirus
6a	>100	>100	>100	>100	>100	>100	>100	>100	>100
6b	>100	>100	>100	>100	>100	>100	>100	>100	>100
6c	>100	>100	>100	>100	>100	>100	>100	>100	>100
6d	>100	>100	>100	>100	>100	>100	>100	>100	>100
6e	>100	>100	>100	>100	>100	>100	>100	>100	>100
6f	>100	>100	>100	>100	>100	>100	>100	>100	>100
6g	>100	>100	>100	>100	>100	>100	>100	>100	>100
7a	>100	>100	>100	>100	>100	>100	>100	>100	>100
7b	>100	>100	>100	>100	>100	>100	>100	>100	>100
7c	>100	>100	>100	>100	>100	>100	>100	>100	>100
7d	>100	>100	>100	>100	>100	>100	>100	>100	>100
7e	>100	>100	>100	>100	>100	>100	>100	>100	>100
7f	>100	>100	>100	>100	>100	>100	>100	>100	>100
7g	>100	>100	>100	>100	>100	>100	>100	>100	>100
Ribavirin[e]						8.9	2.3		
Ganciclovir[e]	0.5	0.2	>100					>100	7.4
Brivudin[e]	0.04	29	22						
Azidothymidine				0.0019	0.0018				

[a]HEL: human embryonic lung fibroblast cells;
[b]MT4: human T-lymphoblast cells;
[c]MDCK: Madin-Darby canine kidney cells;
[d]CRFK: Crandell-Rees feline kidney cells.
[e]For these compounds, data are expressed in µM. All other concentrations are expressed in µg/ml.

Table 3
Cytotoxic activity in diverse mammalian cell lines[a]

Compound	MCC[b] (µg/ml)				CC_{50}[c] (µg/ml)	
	HEL	HeLa	Vero	MDCK	CRFK	MT4
6a	20	20	20	20	7.5	18
6b	20	20	20	4	2.9	14
6c	20	20	100	0.8	9.7	11
6d	20	20	20	4	8.6	13
6e	>10	4	10	10	1.4	11
6f	>10	4	10	>10	1.9	11
6g	10	4	10	2	1.9	11
7a	>100	100	20	100	>100	>125
7b	>100	>100	20	100	>100	>125
7c	>100	>100	>100	>100	>100	>125
7d	>100	>100	>100	100	>100	>125
7e	>10	>100	>10	>10	>100	≥98
7f	>10	>100	≥10	>10	>100	38
7g	>10	100	10	>10	17	≥108
DS-5000[d]	ND	>100	>100	ND	ND	ND
Ribavirin[e]	>250	>250	>250	100	ND	ND
Ganciclovir[e]	>100	ND	ND	ND	>100	ND
Azidothymidine	ND	ND	ND	ND	ND	>25

[a]HEL: human embryonic lung fibroblast cells; HeLa: human cervix carcinoma cells; Vero: African green monkey kidney cells; MDCK: Madin-Darby canine kidney cells; CRFK: Crandell-Rees feline kidney cells; MT4: human T-lymphoblast cells.
[b]MCC: minimum inhibitory concentration, or compound concentration causing minimal changes in cell morphology, as assessed by microscopy.
[c]CC_{50}: 50% cytotoxic concentration, assessed by the spectroscopic MTS cell viability assay.
[d]DS-5000: dextran sulfate with MW 5000.
[e]For these compounds,data are expressed in µM.
ND: not done.

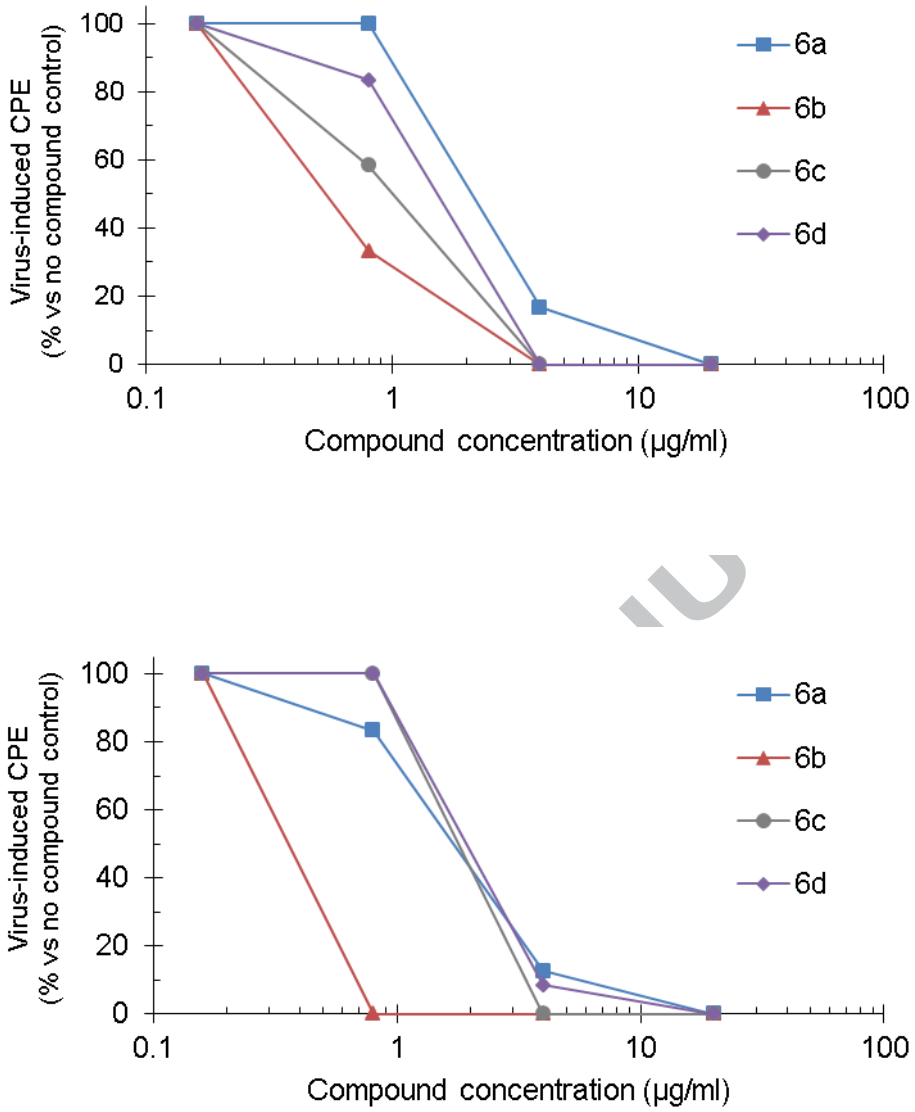


Figure 2. Dose-response curves for inhibition of Coxsackie B4 virus replication. (Upper panel: assay in HeLa cells; lower panel: assay in Vero cells)

3. Conclusion

We have efficiently synthesized novel thiosemicarbazide (**6a**-**6g**) and 4-thiazolidinone (**7a**-**7g**) derivatives with the 5-fluoro-3-phenyl-1*H*-indole scaffold, and evaluated their *in vitro* antiviral activity against a broad range of DNA and RNA viruses. Compounds **6a**, **6b**, **6c** and **6d** exhibited significant and selective inhibitory effect on the replication of Coxsackie B4 virus. Compounds **6b**, **6c** and **6d** also had weaker antiviral activity against Sindbis virus and respiratory syncytial virus. The SAR analysis indicated that keeping the free thiosemicarbazide moiety is crucial for the antiviral activity since the cyclization products (**7a**-**7g**) did not produce any antiviral effect. Also, it was observed that the presence of a bulkier butyl (**6e**) or aromatic (**6f** and **6g**) substituent at the R position led to a dramatic decrease in biological activity.

In conclusion, the promising anti-Coxsackie B4 virus activity of new indolylthiosemicarbazide derivatives (**6a**-**6d**) makes them interesting lead compounds for further antiviral drug development. Hence, we have embarked on further structural optimization, besides mechanistic antiviral experiments, to improve and understand this antiviral activity.

4. Experimental

4.1. Chemistry

4.1.1. General methods

Melting points were determined in open capillary tubes with a Buchi B-540 melting point apparatus and are uncorrected. Microanalyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. IR spectra were recorded in KBr discs (υ_{max} in cm^{-1}) on a Perkin-Elmer 1600 FTIR. ^{1}H-NMR (DMSO-d_6), ^{13}C-NMR (Proton decoupled, APT) (DMSO-d_6) and heteronuclearcorrelation ^{1}H-^{13}C (HSQC, HMBC) (DMSO-d_6) spectra were run on Bruker AC 200 (200 MHz) and Varian^{UNITY}INOVA (500 MHz) instruments. Chemical shifts are reported as δ (ppm) relative to TMS as internal standard and coupling constants (*J*) are given in hertz (Hz). MS (ESI-) were determined on a Finnigan LCQ Advantage Max mass spectrometer. (br.:broad, ind.: indole, thz.:thiazolidinone)

4.1.2 Ethyl 2-benzyl-2-(4-fluorophenylhydrazono)acetate (3)

To a solution of **1** (0.02 mol) in ethanol (10 mL), water (10 mL) and conc. HCl (6 mL), 7% aqueous $NaNO_2$ solution (10 mL) was added dropwise at 0^{o}C with stirring. The resulting solution

of diazonium salt (**2**) was poured into a cooled (0^{o}C) mixture of ethyl 2-benzyl-3-oxobutanoate (0.02 mol), ethanol (10 mL), water (10 mL) and KOH (5.4 g) while stirring. The resulting mixture was refrigerated overnight. The red oily residue thus obtained was separated, washed with water and used without further purification.

4.1.3. Ethyl 5-fluoro-3-phenyl-1H-indole-2-carboxylate (4)

A solution of **3** (0.02 mol) in conc. HCl (20 mL) was heated under reflux for 4 h. The crude product was filtered off, washed with water until tested neutral to litmus and used without further purification.

4.1.4. 5-Fluoro-3-phenyl-1*H*-indole-2-carbohydrazide (5)[25]

A mixture of **4** (0.02 mol), ethanol (20 mL) and $H_2NNH_2.H_2O$ (98%, 8 mL) was heated under reflux for 6 h. The resulting brown crystals were filtered off and recrystallized from ethanol-chloroform. Mp 222-225 °C; IR(KBr): υ_{max} 3279 (N-H), 1624 (C=O); ^{1}H-NMR (DMSO-d_6/200MHz):δ 4.48 (s, 2H, NH_2), 7.10 (td, 1H, J=9.1, 2.2, H6-ind.), 7.21 (dd, 1H, J=9.8, 2.0, H4-ind.), 7.34-7.38 (m, 1H, H7-ind.), 7.42-7.61 (m, 5H, 3-C_6H_5-ind.), 8.86 (s, 1H, CONH), 11.82 (s, 1H, NH); ^{13}C-NMR(Proton decoupled, DMSO-d_6/125MHz):δ 104.65 (d, J=23.7, C4-ind.), 112.78 (d, J=26.1, C6-ind.), 114.24 (d, J=10.0, C7-ind.), 117.50 (d, J=4.3, C3-ind.), 127.36 (d, J=8.7, C3a-ind.), 129.21 (3-C_6H_5(C4)-ind.), 129.94 (3-C_6H_5(C3,C5)-ind.), 130.26 (C2-ind.), 130.41 (3-C_6H_5(C2,C6)-ind.), 132.82 (C7a-ind.), 134.27 (3-C_6H_5(C1)-ind.), 158.28 (d, J=232.2, C5-ind.), 162.23 (C=O).

4.1.5. General procedure for the synthesis of 1-((5-fluoro-3-phenyl-1*H*-indol-2-yl)carbonyl)-4-substituted-3-thiosemicarbazides (6a-6g)

A mixture of **5** (0.005 mol) and an appropriate isothiocyanate(0.005 mol) was refluxed in 30 mL abs. ethanol for 3 h and then allowed to cool. The precipitate was filtered and purified by recrystallization from ethanol.

4.1.5.1. 1-((5-fluoro-3-phenyl-1*H*-indol-2-yl)carbonyl)-4-methyl-3-thiosemicarbazide (6a)

White powder (81.4%); mp 198-200 °C; IR(KBr): υ_{max} =3479, 3342, 3157 (O-H, N-H), 1636 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 2.90 (3H, d, J=4.4, NH-C$\underline{H}_3$), 7.15 (1H, td, J=9.3, 2.4, H6-ind.), 7.23 (1H, dd, J=10.0, 2.2, H4-ind.), 7.34 (1H, t, J=7.3, 3-C_6H_5(H4)-ind.), 7.44 (2H, t, J=7.5, 3-C_6H_5(H3,H5)-ind.), 7.56-7.52 (3H, m, H7, 3-C_6H_5(H2, H6)-ind.), 7.82 (1H, br. s, N_4H),

9.41 (1H, s, N_2H), 9.72 (1H, s, N_1H), 11.78 (1H, s, NH-ind.);^{13}C-NMR (APT) (DMSO-d_6/125MHz):δ 31.52 (NH-CH_3), 104.97 (d, J=23.5, C4-ind.), 113.65 (d, J=26.8, C6-ind.), 114.46 (d, J=9.6, C7-ind.), 119.51 (d, J=5.3, C3-ind.), 127.28 (d, J=9.9, C3a-ind.), 127.55 (3-C_6H_5(C4)-ind.), 128.60 (C2-ind.), 129.13 (3-C_6H_5(C3,C5)-ind.), 130.52 (3-C_6H_5(C2,C6)-ind.), 132.85 (C7a-ind.), 133.83 (3-C_6H_5(C1)-ind.), 158.37 (d, J=233.9, C5-ind.), 161.98 (C=O), 182.93 (C=S); MS (ESI-) m/z (%):341.1 ($M-H^-$, 100).Anal. Calcd for $C_{17}H_{15}FN_4OS.H_2O$ (342.39): C, 56.65; H, 4.75; N, 15.55. Found: C, 57.11; H, 5.12; N, 15.62.

4.1.5.2. 4-Ethyl-1-((5-fluoro-3-phenyl-1*H*-indol-2-yl)carbonyl)-3-thiosemicarbazide (6b)

White powder (76.9%); mp 189-191 °C; IR(KBr): υ_{max} 3337, 3177 (N-H), 1646 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 1.07 (3H, t, J=7.3, NH-CH_2-C$\underline{H}_3$), 3.48 (2H, m, NH-C$\underline{H}_2$-CH_3), 7.16 (1H, td, J=9.2, 2.4, H6-ind.), 7.23 (1H, dd, J=9.8, 2.4, H4-ind.), 7.35 (1H, t, J=7.3, 3-C_6H_5(H4)-ind.), 7.45 (2H, t, J=7.8, 3-C_6H_5(H3,H5)-ind.), 7.54-7.57 (3H, m, H7, 3-C_6H_5(H2, H6)-ind.), 7.81 (1H, br. t, N_4H), 9.36 (1H, s, N_2H), 9.68 (1H, s, N_1H), 11.82 (1H, s, NH-ind.);^{13}C-NMR (APT) (DMSO-d_6/125MHz):δ 15.18 (NH-CH_2-$\underline{C}H_3$), 56.73 (NH-$\underline{C}H_2$-CH_3), 104.96 (d, J=23.5, C4-ind.), 113.64 (d, J=26.4, C6-ind.), 114.47 (d, J=9.6, C7-ind.), 119.50 (d, J=5.3, C3-ind.), 127.28 (d, J=9.6, C3a-ind.), 127.56 (3-C_6H_5(C4)-ind.), 128.58 (C2-ind.), 129.14 (3-C_6H_5(C3,C5)-ind.), 130.56 (3-C_6H_5(C2,C6)-ind.), 132.88 (C7a-ind.), 133.85 (3-C_6H_5(C1)-ind.), 158.37 (d, J=233.8, C5-ind.), 161.94 (C=O), 181.96 (C=S); MS (ESI-) m/z (%):355.1 ($M-H^-$, 100). Anal. Calcd for $C_{18}H_{17}FN_4OS$ (356.42): C, 60.66; H, 4.81; N, 15.72. Found: C, 60.02; H, 5.06; N, 15.48.

4.1.5.3. 1-((5-fluoro-3-phenyl-1*H*-indol-2-yl)carbonyl)-4-propyl-3-thiosemicarbazide (6c)

White flakes (67.7%); mp 191-193 °C; IR(KBr): υ_{max} 3349, 3290 (N-H), 1656 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 0.83 (3H, t, J=7.3, NH-CH_2-CH_2-C$\underline{H}_3$), 1.51 (2H, m, NH-CH_2-C$\underline{H}_2$-CH_3), 3.41 (2H, br. q, NH-C$\underline{H}_2$-CH_2-CH_3), 7.15 (1H, td, J=9.3, 2.4, H6-ind.), 7.23 (1H, dd, J=9.8, 2.0, H4-ind.), 7.35 (1H, t, J=7.6, 3-C_6H_5(H4)-ind.), 7.45 (2H, t, J=7.6, 3-C_6H_5(H3,H5)-ind.), 7.54-7.57 (3H, m, H7, 3-C_6H_5(H2, H6)-ind.), 7.81 (1H, br. t, N_4H), 9.36 (1H, s, N_2H), 9.68 (1H, s, N_1H), 11.82 (1H, s, NH-ind.);^{13}C-NMR (HMBC) (DMSO-d_6/125MHz):δ 11.88 (NH-CH_2-CH_2-$\underline{C}H_3$), 22.72 (NH-CH_2-$\underline{C}H_2$-CH_3), 45.00 (NH-$\underline{C}H_2$-CH_2-CH_3), 104.96 (d, J=23.5, C4-ind.), 113.64 (d, J=26.4, C6-ind.), 114.49 (d, J=9.6, C7-ind.), 119.48 (d, J=5.3, C3-ind.), 127.30 (d, J=9.6, C3a-ind.), 127.57 (3-C_6H_5(C4)-ind.), 128.58 (C2-ind.), 129.14 (3-C_6H_5(C3,C5)-ind.), 130.55 (3-C_6H_5(C2,C6)-ind.), 132.88 (C7a-ind.), 133.85 (3-C_6H_5(C1)-ind.), 158.37 (d, J=233.9, C5-ind.),

161.93 (C=O), 182.24 (C=S); MS (ESI-) m/z (%):369.1 ($M-H^-$, 100). Anal. Calcd for $C_{19}H_{19}FN_4OS$ (370.44): C, 61.60; H, 5.17; N, 15.12. Found: C, 61.38; H, 5.76; N, 15.07.

4.1.5.4. 4-Allyl-1-((5-fluoro-3-phenyl-1*H*-indol-2-yl)carbonyl)-3-thiosemicarbazide (6d)

White needles (69.0%); mp 193-195 °C; IR(KBr): υ_{max} 3366, 3290 (N-H), 1669 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 4.13 (2H, t, J=5.2, NH-C$\underline{H}_2$-CH=CH_2), 5.05 (1H, dd, J=10.3, 1.5, NH-CH_2-CH=C$\underline{H}_2$), 5.13 (1H, dd, J=17.3, 1.5, N-CH_2-CH=C$\underline{H}_2$), 5.78-5.86 (1H, m, NH-CH_2-C$\underline{H}$=CH_2), 7.15 (1H, td, J=9.1, 2.4, H6-ind.), 7.23 (1H, dd, J=9.8, 2.4, H4-ind.), 7.33 (1H, t, J=7.3, 3-C_6H_5(H4)-ind.), 7.43 (2H, t, J=7.6, 3-C_6H_5(H3,H5)-ind.), 7.52-7.57 (3H, m, H7, 3-C_6H_5(H2, H6)-ind.), 7.99 (1H, t, J=5.7, N_4H), 9.47 (1H, s, N_2H), 9.75 (1H, s, N_1H), 11.80 (1H, s, NH-ind.); ^{13}C-NMR (HSQC) (DMSO-d_6/125MHz): δ 46.55 (NH-$\underline{C}H_2$-CH=CH_2), 104.96 (d, J=24.0, C4-ind.), 113.64 (d, J=26.4, C6-ind.), 114.47 (d, J=9.6, C7-ind.), 116.14 (N-CH_2-CH=$\underline{C}H_2$), 119.56 (d, J=5.2, C3-ind.), 127.31 (d, J=9.6, C3a-ind.), 127.57 (3-C_6H_5(C4)-ind.), 128.56 (C2-ind.), 129.13 (3-C_6H_5(C3,C5)-ind.), 130.51 (3-C_6H_5(C2,C6)-ind.), 132.88 (C7a-ind.), 133.84 (3-C_6H_5(C1)-ind.), 135.45 (NH-CH_2-$\underline{C}$H=CH_2), 158.38 (d, J=233.9, C5-ind.), 161.99 (C=O), 182.60 (C=S); MS (ESI-) m/z (%):367.1 ($M-H^-$, 100).Anal. Calcd for $C_{19}H_{17}FN_4OS$ (368.43): C, 61.94; H, 4.65; N, 15.21. Found: C, 61.67; H, 5.11; N, 15.16.

4.1.5.5. 4-Butyl-1-((5-fluoro-3-phenyl-1*H*-indol-2-yl)carbonyl)-3-thiosemicarbazide (6e)

White crystals (92.8%); mp 194-196 °C; IR(KBr): υ_{max} 3385, 3300, 3169 (N-H), 1670 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 0.87 (3H, t, J=7.3,CH_2-C$\underline{H}_3$), 1.23-1.30 (2H, m, C$\underline{H}_2$-CH_3), 1.44-1.50 (2H, quin., J=7.3, NH-CH_2-C$\underline{H}_2$), 3.42-3.45 (2H, m, NH-C$\underline{H}_2$-CH_2), 7.16 (1H, td, J=9.3, 2.4, H6-ind.), 7.23 (1H, dd, J=9.8, 2.0, H4-ind.), 7.35 (1H, t, J=7.3, 3-C_6H_5(H4)-ind.), 7.45 (2H, t, J=7.8, 3-C_6H_5(H3,H5)-ind.), 7.53-7.57 (3H, m, H7, 3-C_6H_5(H2, H6)-ind.), 7.80 (1H, br. t, N_4H), 9.35 (1H, s, N_2H), 9.68 (1H, s, N_1H), 11.82 (1H, s, NH-ind.);^{13}C-NMR (APT) (DMSO-d_6/125MHz): δ 14.50 (CH_2-$\underline{C}H_3$), 20.13 ($\underline{C}H_2$-CH_3), 31.62 (NH-CH_2-$\underline{C}H_2$) 44.04 (NH-$\underline{C}H_2$-CH_2), 104.96 (d, J=23.5, C4-ind.), 113.64 (d, J=26.8, C6-ind.), 114.50 (d, J=9.6, C7-ind.), 119.49 (d, J=5.5, C3-ind.), 127.31 (d, J=9.9, C3a-ind.), 127.57 (3-C_6H_5(C4)-ind.), 128.57 (C2-ind.), 129.14 (3-C_6H_5(C3,C5)-ind.), 130.55 (3-C_6H_5(C2,C6)-ind.), 132.88 (C7a-ind.), 133.86 (3-C_6H_5(C1)-ind.), 158.37 (d, J=233.9, C5-ind.), 161.94 (C=O), 182.18 (C=S); MS (ESI-) m/z (%):383.1 ($M-H^-$, 100). Anal. Calcd for $C_{20}H_{21}FN_4OS$ (384.47): C, 62.48; H, 5.51; N, 14.57. Found: C, 62.08; H, 5.68; N, 14.35.

4.1.5.6. 4-Benzyl-1-((5-fluoro-3-phenyl-1*H*-indol-2-yl)carbonyl)-3-thiosemicarbazide (6f)

White flakes (82.3%); mp 202-204 °C; IR(KBr): υ_{max} 3346, 3323, 3294, 3248 (N-H), 1670 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 4.77 (2H, d, J=5.9, NH-C$\underline{H}_2$), 7.15 (1H, td, J=9.3, 2.4, H6-ind.), 7.22-7.25 (2H, m, CH_2-$C_6\underline{H}_5$(H4), H4-ind.), 7.28-7.31 (4H, m, CH_2-$C_6\underline{H}_5$(H2,H3,H5,H6), 7.33 (1H, t, J=7.3, 3-C_6H_5(H4)-ind.), 7.42 (2H, t, J=7.8, 3-C_6H_5(H3,H5)-ind.), 7.54-7.56 (3H, m, H7, 3-C_6H_5(H2,H6)-ind.), 8.43 (1H, br. t, N_4H), 9.57 (1H, s, N_2H), 9.82 (1H, s, N_1H), 11.80 (1H, s, NH-ind.);^{13}C-NMR (HSQC) (DMSO-d_6/125MHz):δ 47.41 (NH-CH_2), 104.98 (d, J=24.0, C4-ind.), 113.67 (d, J=26.8, C6-ind.), 114.50 (d, J=9.6, C7-ind.), 119.61 (d, J=5.3, C3-ind.), 127.29 (d, J=9.6, C3a-ind.), 127.43, 127.54, 127.70 (CH_2-$\underline{C}_6H_5$(C2-6), 3-C_6H_5(C4)-ind.), 128.50 (C2-ind.), 128.83 (CH_2-$\underline{C}_6H_5$(C2,C3,C5,C6)), 129.12 (3-C_6H_5(C3,C5)-ind.), 130.52 (3-C_6H_5(C2,C6)-ind.), 132.86 (C7a-ind.), 133.82 (3-C_6H_5(C1)-ind.), 139.79 (CH_2-$\underline{C}_6H_5$(C1)), 158.37 (d, J=233.9, C5-ind.), 162.04 (C=O), 182.91 (C=S); MS (ESI-) m/z (%):417.1 ($M-H^-$, 100).Anal. Calcd for $C_{23}H_{19}FN_4OS$ (418.49): C, 66.01; H, 4.58; N, 13.39. Found: C, 66.33; H, 4.53; N, 13.24.

4.1.5.7. 1-((5-fluoro-3-phenyl-1*H*-indol-2-yl)carbonyl)-4-phenethyl-3-thiosemicarbazide (6g)

White flakes (84.3%); mp 201-202 °C; IR(KBr): υ_{max} 3343, 3296, 3265, 3159 (N-H), 1663 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 2.81 (2H, t, J= 7.8, C$\underline{H}_2$-C_6H_5), 3.63-3.67 (2H, m, NH-C$\underline{H}_2$), 7.16 (1H, td, J=9.0, 2.4, H6-ind.), 7.19-7.28 (6H, m, CH_2-$C_6\underline{H}_5$, H4-ind.), 7.35 (1H, t, J=7.3, 3-C_6H_5(H4)-ind.), 7.44 (2H, t, J=7.8, 3-C_6H_5(H3,H5)-ind.), 7.52 (2H, d, J=6.8, 3-C_6H_5(H2, H6)-ind.), 7.56 (1H, dd, J=8.8, 4.4, H7-ind.), 7.90 (1H, br. t, N_4H), 9.49 (1H, s, N_2H), 9.69 (1H, s, N_1H), 11.83 (1H, s, NH-ind.);^{13}C-NMR (APT) (DMSO-d_6/125MHz):δ 35.61 ($\underline{C}H_2$-C_6H_5),45.91 (NH-CH_2), 104.97 (d, J=23.9, C4-ind.), 113.69 (d, J=26.6, C6-ind.), 114.50 (d, J=10.0, C7-ind.), 119.53 (C3-ind.), 126.87 (CH_2-$\underline{C}_6H_5$(C4)), 127.31 (d, J=10.0, C3a-ind.), 127.60 (3-C_6H_5(C4)-ind.), 128.50 (C2-ind.), 129.11, 129.15, 129.36 (CH_2-$\underline{C}_6H_5$(C2,C3,C5,C6), 3-C_6H_5(C3,C5)-ind.), 130.57 (3-C_6H_5(C2,C6)-ind.), 132.90 (C7a-ind.), 133.85 (3-C_6H_5(C1)-ind.), 139.84 (CH_2-$\underline{C}_6H_5$(C1)), 158.37 (d, J=234.3, C5-ind.), 182.32 (C=S); MS (ESI-) m/z (%):431.1 ($M-H^-$, 100).Anal. Calcd for $C_{24}H_{21}FN_4OS$ (432.51): C, 66.65; H, 4.89; N, 12.95. Found: C, 66.77; H, 4.95; N, 12.74.

4.1.6. General procedure for the synthesis of 5-fluoro-N'-(4-oxo-3-substituted-1,3-thiazolidinon-2-ylidene)-3-phenyl-1*H*-indol-2-carbohydrazides (7a-7g)

A mixture of **6** (0.0025 mol), ethyl bromoacetate (0.0025 mol) and fused sodium acetate (0.01 mol) in absolute ethanol (15 ml) was heated under reflux for 3 h. The solid thus obtained was filtered, dried and purified by recrystallization from ethanol/chloroform.

4.1.6.1. 5-Fluoro-N'-(3-methyl-4-oxo-1,3-thiazolidinon-2-ylidene)-3-phenyl-1*H*-indol-2-carbohydrazide (7a) Beige crystals (97.9%); mp 270-273 °C; IR(KBr): υ_{max} 3318, 3232 (N-H), 1723, 1674 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 3.07 (3H, s, N-CH_3), 3.99 (2H, s, S-CH_2), 7.11 (1H, td, J=9.0, 2.4, H6-ind.), 7.15 (1H, br. d, H4-ind.), 7.37 (1H, br. s, 3-C_6H_5(H4)-ind.), 7.47-7.50 (5H, m, H7, 3-C_6H_5(H2, H6, H3, H5)-ind.), 9.68 (1H, s, NH), 11.90 (1H, s, NH-ind.);^{13}C-NMR (APT) (DMSO-d_6/125MHz): δ 29.89 (N-CH_3), 33.58 (S-CH_2), 104.73 (d, J=23.5, C4-ind.), 113.25 (d, J=26.8, C6-ind.), 114.42 (d, J=9.6, C7-ind.), 118.20 (C3-ind.), 127.52 (br. d, C3a-ind.), 127.82 (3-C_6H_5(C4)-ind.), 129.47 (C2-ind.), 129.58 (3-C_6H_5(C3,C5)-ind.), 130.49 (3-C_6H_5(C2,C6)-ind.), 132.98 (C7a-ind.), 134.03 (3-C_6H_5(C1)-ind.), 158.31 (d, J=233.8, C5-ind.), 159.06, 159.37 (C=N, C=O), 172.94 (C=O-thz.);MS (ESI-) m/z (%):381.1 ($M-H^-$, 100).Anal. Calcd for $C_{19}H_{15}FN_4O_2S$(382.41): C, 59.67; H, 3.95; N, 14.65. Found: C, 59.61; H, 4.37; N, 14.62.

4.1.6.2. N'-(3-ethyl-4-oxo-1,3-thiazolidinon-2-ylidene)-5-fluoro-3-phenyl-1*H*-indol-2-carbohydrazide (7b) White crystals (69.5%); mp 267-270 °C; IR(KBr): υ_{max} 3310, 3237 (N-H), 1710, 1654 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 1.13 (3H, br. s, N-CH_2-C$\underline{H}_3$), 3.67 (2H, br. s, N-C$\underline{H}_2$-CH_3), 4.00 (2H, s, S-CH_2), 7.12 (1H, td, J=9.3, 2.4, H6-ind.), 7.15 (1H, br. d, J=9.3, H4-ind.), 7.37 (1H, br. t, 3-C_6H_5(H4)-ind.), 7.48-7.51 (5H, m, H7, 3-C_6H_5(H2, H6, H3, H5)-ind.), 9.78 (1H, s, NH), 11.89 (1H, s, NH-ind.); ^{13}C-NMR (APT) (DMSO-d_6/125MHz): δ 12.80 (N-CH_2-$\underline{C}H_3$), 33.49 (S-CH_2), 38.23 (N-$\underline{C}H_2$-CH_3), 104.72 (d, J=23.5, C4-ind.), 113.22 (d, J=27.3, C6-ind.), 114.41 (d, J=9.6, C7-ind.), 118.20 (C3-ind.), 127.51 (C3a-ind.), 127.76 (3-C_6H_5(C4)-ind.), 129.52 (3-C_6H_5(C3,C5)-ind.), 130.50 (3-C_6H_5(C2,C6)-ind.), 132.94 (C7a-ind.), 134.04 (3-C_6H_5(C1)-ind.), 158.31 (d, J=233.9, C5-ind.), 159.16 (C=N, C=O), 171.76 (C=O-thz.);MS (ESI-) m/z (%): 395.2 ($M-H^-$, 100).Anal. Calcd for $C_{20}H_{17}FN_4O_2S$(396.44): C, 60.59; H, 4.32; N, 14.13. Found: C, 60.24; H, 4.87; N, 14.07.

4.1.6.3. 5-Fluoro-N'-(4-oxo-3-propyl-1,3-thiazolidinon-2-ylidene)-3-phenyl-1*H*-indol-2-carbohydrazide (7c) White crystals (91.4%); mp 254-256 °C; IR(KBr): υ_{max} 3308, 3253 (N-H), 1712, 1658 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 0.83 (3H, br. s, NH-CH_2-CH_2-C<u>H</u>$_3$), 1.60 (2H, br. s, NH-CH_2-C<u>H</u>$_2$-CH_3), 3.61 (2H, br. s, NH-C<u>H</u>$_2$-CH_2-CH_3), 4.02 (2H, s, S-CH_2), 7.11 (1H, td, J=9.2, 2.4, H6-ind.), 7.16 (1H, br. d, J=9.3, H4-ind.), 7.37 (1H, br. t, 3-C_6H_5(H4)-ind.), 7.48-7.51 (5H, m, H7, 3-C_6H_5(H2,H6,H3,H5)-ind.), 9.80 (1H, s, NH), 11.87 (1H, s, NH-ind.);^{13}C-NMR (APT) (DMSO-d_6/125MHz): δ 11.75 (NH-CH_2-CH_2-<u>C</u>H_3), 20.47 (NH-CH_2-<u>C</u>H_2-CH_3), 33.39 (S-CH_2), 44.67 (NH-<u>C</u>H_2-CH_2-CH_3), 104.72 (d, J=23.5, C4-ind.), 113.21 (d, J=26.4, C6-ind.), 114.40 (d, J=10.1, C7-ind.), 118.21 (d, J=4.8, C3-ind.), 127.50 (C3a-ind.), 127.73 (3-C_6H_5(C4)-ind.), 129.49 (3-C_6H_5(C3,C5)-ind.), 130.50 (3-C_6H_5(C2,C6)-ind.), 132.94 (C7a-ind.), 134.07 (3-C_6H_5(C1)-ind.), 158.31 (d, J=233.4, C5-ind.), 159.15, 159.81 (C=N, C=O), 172.11 (C=O-thz.);MS (ESI-) m/z (%):409.1 ($M-H^-$, 100).Anal. Calcd for $C_{21}H_{19}FN_4O_2S$(410.46): C, 61.45; H, 4.67; N, 13.65. Found: C, 61.29; H, 5.24; N, 13.63.

4.1.6.4. N'-[(2Z)-3-allyl-4-oxo-1,3-thiazolidinon-2-ylidene]-5-fluoro-3-phenyl-1*H*-indol-2-carbohydrazide (7d)[26] White crystals (67.3%); mp 262-265 °C; IR(KBr): υ_{max} 3309, 3247 (N-H), 1716, 1654 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 4.05 (2H, s, S-CH_2), 4.24 (2H, br. s, NH-C<u>H</u>$_2$-CH=CH_2), 5.12 (2H, m, NH-CH_2-CH=C<u>H</u>$_2$), 5.81 (1H, br. s, NH-CH_2-C<u>H</u>=CH_2), 7.11 (1H, td, J=9.1, 2.4, H6-ind.), 7.15 (1H, br. d, J=9.3, H4-ind.), 7.36 (1H, br. t, 3-C_6H_5(H4)-ind.), 7.46-7.49 (5H, m, H7, 3-C_6H_5(H2,H6,H3,H5)-ind.), 9.78 (1H, s, NH), 11.87 (1H, s, NH-ind.);^{13}C-NMR (HSQC) (DMSO-d_6/125MHz): δ 33.42 (S-CH_2), 45.02 (NH-<u>C</u>H_2-CH=CH_2), 104.71 (d, J=23.5, C4-ind.), 113.21 (d, J=25.9, C6-ind.), 114.39 (d, J=10.1, C7-ind.), 117.99 (NH-CH_2-CH=<u>C</u>H_2), 118.29 (br. d, C3-ind.), 127.51 (br. d, C3a-ind.), 127.75 (3-C_6H_5(C4)-ind.), 129.49 (3-C_6H_5(C3,C5)-ind.), 130.49 (3-C_6H_5(C2,C6)-ind.), 131.72 (NH-CH_2-<u>C</u>H=CH_2), 132.96 (C7a-ind.), 134.05 (3-C_6H_5(C1)-ind.), 158.31 (d, J=233.4, C5-ind.), 159.01, 159.18 (C=N, C=O), 171.61 (C=O-thz.);MS (ESI-) m/z (%):407.1 ($M-H^-$, 100).Anal. Calcd for $C_{21}H_{17}FN_4O_2S$(408.45): C, 61.75; H, 4.20; N, 13.72. Found: C, 61.84; H, 4.87; N, 13.69.

4.1.6.5. N'-(3-butyl-4-oxo-1,3-thiazolidinon-2-ylidene)-5-fluoro-3-phenyl-1*H*-indol-2-carbohydrazide (7e) White crystals (74.5%); mp 243-245 °C; IR(KBr): υ_{max} 3292, 3265 (N-H), 1728, 1667 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 0.87 (3H, br. s, CH_2-C<u>H</u>$_3$), 1.26 (2H, br. d, J=5.9, C<u>H</u>$_2$-CH_3), 1.56 (2H, br. s, NH-CH_2-C<u>H</u>$_2$), 3.64 (2H, br. s, NH-C<u>H</u>$_2$-CH_2), 4.01 (2H, s, S-CH_2), 7.10-7.17 (2H, m, H4, H6-ind.), 7.37 (1H, br. t, 3-C_6H_5(H4)-ind.), 7.49-7.52 (5H, m, H7, 3-

C_6H_5(H2,H6,H3,H5)-ind.), 9.79 (1H, s, NH), 11.88 (1H, s, NH-ind.); ^{13}C-NMR (APT) (DMSO-d_6/125MHz): δ 14.27 (CH_2-$\underline{C}H_3$), 20.09 ($\underline{C}H_2$-CH_3), 29.21(NH-CH_2-$\underline{C}H_2$), 33.39 (S-CH_2), 42.85 (NH-$\underline{C}H_2$-CH_2), 104.72 (d, J=23.9, C4-ind.), 113.21 (d, J=27.2, C6-ind.), 114.40 (d, J=8.7, C7-ind.), 118.19 (d, J=4.8, C3-ind.), 127.51 (C3a-ind.), 127.73 (3-C_6H_5(C4)-ind.), 129.49 (3-C_6H_5(C3,C5)-ind.), 130.51 (3-C_6H_5(C2,C6)-ind.), 132.95 (C7a-ind.), 134.07 (3-C_6H_5(C1)-ind.), 158.31 (d, J=233.9, C5-ind.), 159.14, 159.79 (C=N, C=O), 172.07 (C=O-thz.);MS (ESI-) m/z (%):423.2 ($M-H^-$, 100).Anal. Calcd for $C_{22}H_{21}FN_4O_2S$(424.49): C, 62.25; H, 4.99; N, 13.20. Found: C, 62.27; H, 4.87; N, 13.15.

4.1.6.6. N'-(3-benzyl-4-oxo-1,3-thiazolidinon-2-ylidene)-5-fluoro-3-phenyl-1*H*-indol-2-carbohydrazide (7f) White crystals (80.0%); mp 268-271 °C; IR(KBr): υ_{max} 3285, 3105 (N-H), 1726, 1655 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 4.01 (2H, s, S-CH_2), 4.84 (2H, s, N-CH_2), 7.12 (1H, td, J=9.3, 2.4, H6-ind.), 7.16 (1H, br. d, J=8.8, H4-ind.), 7.26-7.37 (6H, m, CH_2-$C_6\underline{H}_5$, 3-C_6H_5(H4)-ind.), 7.47-7.51 (5H, m, H7, 3-C_6H_5(H2, H6, H3, H5)-ind.), 9.86 (1H, s, NH), 11.91 (1H, s, NH-ind.);^{13}C-NMR (HSQC) (DMSO-d_6/125MHz): δ 33.48 (S-CH_2), 46.21 (N-CH_2), 104.74 (d, J=23.5, C4-ind.), 113.23 (d, J=26.4, C6-ind.), 114.39 (d, J=9.2, C7-ind.), 118.24 (d, J=4.8, C3-ind.), 127.52 (d, J=8.7, C3a-ind.), 127.72 (3-C_6H_5(C4)-ind.), 128.23, 128.63, 128.95, 129.06 (CH_2-$\underline{C}_6H_5$(C2-6), C2-ind.), 129.49 (3-C_6H_5(C3,C5)-ind.), 130.50 (3-C_6H_5(C2,C6)-ind.), 132.97 (C7a-ind.), 134.06 (3-C_6H_5(C1)-ind.), 136.52 (CH_2-$\underline{C}_6H_5$(C1)), 158.32 (d, J=233.4, C5-ind.), 159.07, 159.17 (C=N, C=O), 172.06 (C=O-thz.);MS (ESI-) m/z (%):457.1 ($M-H^-$, 100).Anal. Calcd for $C_{25}H_{19}FN_4O_2S$(458.51): C, 65.49; H, 4.18; N, 12.22. Found: C, 64.99; H, 4.19; N, 11.83.

4.1.6.7. 5-Fluoro-N'-(4-oxo-3-phenethyl-1,3-thiazolidinon-2-ylidene)-3-phenyl-1*H*-indol-2-carbohydrazide (7g) White flakes (78.0%); mp 245-247 °C; IR(KBr): υ_{max} 3312, 3238 (N-H), 1719, 1672 (C=O); ^{1}H-NMR (DMSO-d_6/500MHz):δ 2.92 (2H, br. s, N-CH_2-$C\underline{H}_2$), 3.85 (2H, br. s, N-$C\underline{H}_2$-CH_2), 3.95 (2H, s, S-CH_2), 7.12 (1H, br. t, J=8.9, H6-ind.), 7.17-7.28 (6H, m, CH_2-$C_6\underline{H}_5$, H4-ind.), 7.38 (1H, br. s, 3-C_6H_5(H4)-ind.), 7.50-7.54 (5H, m, H7, 3-C_6H_5(H2, H6, H3, H5)-ind.), 9.84 (1H, s, NH), 11.95 (1H, s, NH-ind.); ^{13}C-NMR (HMBC) (DMSO-d_6/125MHz): δ 32.73 (N-CH_2-$\underline{C}H_2$), 33.46 (S-CH_2), 44.29 (N-$\underline{C}H_2$-CH_2), 104.75 (d, J=23.5, C4-ind.), 113.25 (d, J=26.8, C6-ind.), 114.41 (d, J=9.1, C7-ind.), 118.22 (C3-ind.), 127.16 (CH_2-$\underline{C}_6H_5$(C4)), 127.52 (br. d, C3a-ind.), 127.77 (3-C_6H_5(C4)-ind.), 129.13, 129.37 (CH_2-$\underline{C}_6H_5$(C2,C3,C5,C6), C2-ind.), 129.53

(3-C_6H_5(C3,C5)-ind.), 130.52 (3-C_6H_5(C2,C6)-ind.), 132.99 (C7a-ind.), 134.07 (3-C_6H_5(C1)-ind.), 138.78 (CH_2-C_6H_5(C1)), 158.33 (d, J=233.4, C5-ind.), 159.19 (C=N, C=O), 171.62 (C=O-thz.);MS (ESI-) m/z (%):471.2 ($M-H^-$, 100).Anal. Calcd for $C_{26}H_{21}FN_4O_2S$(472.53): C, 66.09; H, 4.48; N, 11.86. Found: C, 66.32; H, 4.17; N, 11.53.

4.2. Antiviral activity assays

The compounds were evaluated for antiviral activity in cell culture by using cytopathic effect (CPE) reduction assays with a broad and diverse panel of DNA- and RNA-viruses.[27,28] Human cervix carcinoma HeLa cells were used to study vesicular stomatitis virus, Coxsackie B4 virus, and respiratory syncytial virus. African green monkey kidney Vero cells were used to evaluate para-influenza virus type 3, reovirus type 1, Sindbis virus, Coxsackie B4 virus, and Punta Toro virus. Viruses evaluated on human embryonic lung fibroblast cells were herpes simplex virus types 1 and 2, vaccinia virus, and vesicular stomatitis virus. Madin-Darby canine kidney (MDCK) cells were used for antiviral evaluation against influenza A and B viruses. Feline herpes virus and feline canine virus were grown in Crandell-Rees feline kidney cells. Finally, HIV-1 and HIV-2 were monitored in human T-lymphoblast MT4 cells.[29]

To perform the antiviral assays, the viruses were added to subconfluent cultures of the cells in 96-well plates, and at the same time, the test compounds were added in serial dilutions. Appropriate reference compounds were included, i.e. the viral entry inhibitor dextran sulfate (MW 5000); the broad antiviral agent ribavirin; the antiherpetic agents ganciclovir and brivudin and the HIV inhibitor azidothymidine. After 3 to 6 days incubation at 37 °C (or 35 °C in the case of influenza virus), the compounds' inhibitory effect on virus-induced cytopathic effect as well as their cytotoxicity were monitored by light microscopy or by performing the MTS cell viability assay (CellTiter 96®AQ_{ueous} One Solution Cell Proliferation Assay from Promega). Antiviral activity was expressed as the 50% effective concentration (EC_{50}) whereas cytotoxicity was defined as the minimal cytotoxic concentration (based on microscopy) or CC_{50} (50% cytotoxic concentration, assessed by the MTS assay).

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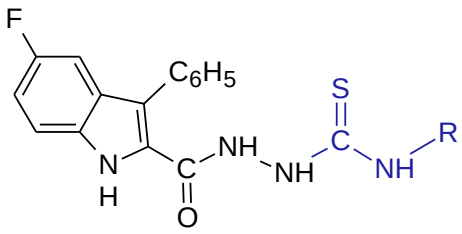
Graphical abstract



6a-g

Compounds with anti-Coxsackie B4 virus activity: **6a** (R:CH_3), **6b** (R:C_2H_5), **6c** (R:C_3H_7), **6d** (R:C_3H_5)

EC_{50}: 0.4 -2.1 µg/ml

SI: 9-56