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Short communication

New 1-indanone thiosemicarbazone derivatives active against BVDV

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Abstract

Identification of new therapeutic agents for the treatment of viral diseases represents an area of active investigation. In an effort to develop new antiviral compounds, a series of 1-indanone thiosemicarbazone derivatives were synthesized. These derivatives were structurally characterized using several spectroscopic techniques and evaluated against bovine viral diarrhoea virus as a surrogate model for hepatitis C virus. Thiosemicarbazone **2m** showed potent anti-bovine viral diarrhoea virus activity with a higher selectivity index (SI = 80.29) than that of ribavirin (SI = 11.64). This result determines the potentiality of these thiosemicarbazones as antiviral agents for the treatment of infections caused by other highly related members of Flaviviridae family, as hepatitis C virus.

Keywords: Thiosemicarbazones derived from 1-indanones; Antiviral activity

1. Introduction

Hepatitis C virus (HCV) infection remains a main public health problem with 175 million people infected in the world. It is a major cause of cirrhosis and primary hepatocellular carcinoma and the main reason for liver transplant among adults in western countries [1].

Currently, the best treatment available consists in the combination of pegylated alpha interferon and the nucleoside analogue ribavirin, but its effectiveness is only of about 50–60% in patients suffering from chronic HCV infection and is associated with important side effects [2]. Consequently, there is an urgent need for highly effective and selective inhibitors of HCV replication to improve the current antiviral strategies against chronic HCV infection.

HCV is the sole member of the *Hepacivirus* genus, which along with the flaviviruses and pestiviruses belong to the family Flaviviridae. The member of *Pestivirus* genus bovine viral diarrhoea virus (BVDV), a single-stranded RNA virus of positive polarity, is one of the best characterized and has one of the largest RNA genomes (12.5 Kb) in this family [3]. The genome organization of the pestiviruses closely resemble those of hepatitis C virus. Because of this close relation, BVDV may provide a surrogate model for HCV [4], both for the molecular study of viral proteins [5] and for the evaluation of antiviral compounds [6].

Biological properties of thiosemicarbazone derivatives have been studied since 1946 [7], when their activity against Mycobacterium tuberculosis was reported. Since then, this and other biological properties of thiosemicarbazone derivatives such as antibacterial [8], antitumoral [9], antiprotozoal [10] and cytotoxic activity [11] have been described. In 1950, Hamre et al. [12] found that thiosemicarbazone derivatives from several benzaldehydes were active against neurovaccinial infection in mice when given orally. It was the first study on the antiviral activity of thiosemicarbazone derivatives that prompted further investigation of their properties in this area.

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Several studies made with thiosemicarbazone derivatives of *N*-methylisatine and *N*-allylisatine showed that these compounds were capable of inhibiting structural protein synthesis in human immunodeficiency virus (HIV) [13]. The thiosemicarbazone of isatin was also found strongly active as anti-poxvirus [14].

Previously we have reported the inhibitory activity of several thiosemicarbazone derivatives synthesized from aromatic ketones and terpenones against Junin virus (JUNV), a RNA virus member of the Arenaviridae family and causative agent of argentine hemorrhagic fever (AHF) [15]. The results obtained showed that from 25 compounds tested, six of them having the thiosemicarbazone group had a selective antiviral effect, with SI values over 10. Among the thiosemicarbazone derivatives the highest activity was found in those derived from 1-indanone, particularly 5-methoxy-1-indanone (**2k**). This initiated our interest to evaluate the antiviral activity of a series of 1-indanone thiosemicarbazone derivatives (TSCs), with different patterns of substitution in the aromatic ring. In this way, some new TSCs were synthesized and evaluated against BVDV, a surrogate model for evaluation of anti-HCV activity.

2. Results and discussion

2.1. Synthesis

The TSCs **2a**–**p** (Table 1) were obtained from the corresponding 1-indanones (**1a**–**p**) by treatment with thiosemicarbazide (Scheme 1).

Ketones (1-indanones) **1a**–**c** and **1j**–**p** are of commercial source; **1d**, **e**, **h**, **i** were synthesized following the described procedures [16,17], and **1h** and **1i** were obtained as a mixture, as they cannot be separated they were transformed into the corresponding TSCs in this way.

Finally, compounds **1f** and **1g** are new compounds and were obtained following the protocol presented in Scheme 2.

All the compounds were identified by spectral data (^{1}H, ^{13}C NMR and IR). The synthesized TSCs and their spectroscopic characteristics are shown in Table 1.

The products obtained were isolated by conventional work-up and purified by crystallization in ethanol. Melting point determination was carried out to check the purity of compounds. Both analytical and spectral data (IR and NMR) of all the compounds are in full agreement with the proposed structures. The IR spectra show bands in the region 3419–3435 cm^{-1} and 3198–3263 cm^{-1} due to stretching frequencies for N–H. The absence of S–H stretch at 2500–2600 cm^{-1} and the presence of N–H stretch at 3118–3153 cm^{-1} in the spectra, suggest that all compounds are in the thione form and it is confirmed by the presence of a band in the region 1083–1096 cm^{-1} for C=S. Finally a band for C=N appears in the region 1582–1595 cm^{-1} in all the TSCs. In all the ^{1}H NMR were observed three broad singlets for the three hydrogen atoms bonded to the nitrogen atoms (δ between 7.00 and 10.30 ppm). In the ^{13}C NMR spectra were observed the corresponding signals to *C*=N (δ between 155.3 and 160.5 ppm) and to *C*=S (δ between 176.7 and 179.0 ppm).

2.2. Evaluation of antiviral activity

The synthesized compounds were screened for antiviral activity on the replication of BVDV type-1 NADL strain in the Madin–Darby bovine kidney (MDBK) cell line by reduction of cytopathic effect, as it was previously described (Table 2) [18]. The cytotoxicity of all compounds evaluated was determined by means of the MTS/PMS method (Promega).

Among the TSCs assayed, six of them exhibited a selectivity index (SI = 12.24–80.29) higher than that of the reference ribavirin value (SI = 11.64). Compound **2m** showed the highest antiviral activity with an EC_{50} value of 1.75 μM. Moreover, compound **2m** presented a SI of 80.29, this value is around seven times higher than that of ribavirin value.

The EC_{50} value of compound **2m** lies in the micromolar range and hence it is in accordance with that required for new leads suitable for pharmaceutical development. The activity of this compound appears to be pestivirus specific and was essentially inactive against others positive sense single-stranded RNA viruses, such as polio virus type-1 Sabin strain ($EC_{50} = 64$ μM) and human immunodeficiency virus type-1 ($EC_{50} > 38$ μM). Moreover, **2m** had no activity against the negative stranded RNA vesicular stomatitis virus type-1 Indiana strain ($EC_{50} > 75.5$ μM) and the double stranded DNA herpes simplex virus type-1 F strain ($EC_{50} > 64$ μM).

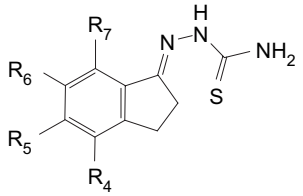
Despite the interesting antiviral properties of some of these molecules, it is difficult to highlight in detail their structure–activity relationship. Nevertheless, some general aspects merit to be commented. Compound **2m**, with two methoxy groups in the aromatic ring, was identified as highly selective and potent in vitro inhibitor of the replication of BVDV. The absence of substituents (**2a**) or the presence of only one methoxy group in the aromatic ring (**2j**, **2k** and **2l**), reduced markedly the anti-BVDV activity. The incorporation of a halogen atom in the aromatic ring (**2o**–**p**), a lipophilic but electron-withdrawing group, leads to very toxic compounds but with an interesting activity. Further studies, in order to determine the mechanism of action of these compounds, could explain the influence of the type and position of the substituents in the observed antiviral action. However, it is important to note that the presence of the thiosemicarbazone group is essential for the antiviral activity; 1-indanones, thiosemicarbazide and semicarbazone derivatives of 1-indanones showed to be inactive against BVDV.

The thiosemicarbazones possess the ability to form metallic complexes and hence they can remove metals from biological systems and in this way they are capable of inhibiting the activity of metal-requiring proteins [19]. Further studies in the action mechanism of this kind of compounds represent a challenge of our investigation.

3. Conclusion

In the present study, the most potent evaluated TSC (**2m**) showed a level of in vitro activity against BVDV better than that of ribavirin (Table 2) and consequently, it is a promising lead compound to pursue further analysis of other chemical

Table 1
Synthesized TSCs and their physical, analytical and spectral data



2a-p

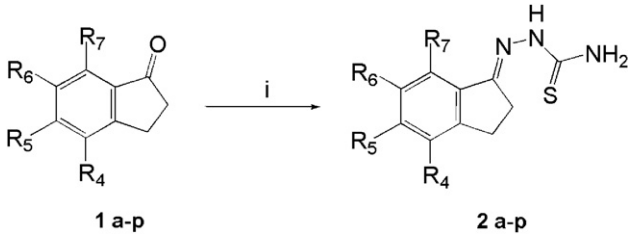
2 (TSC)	R_4	R_5	R_6	R_7	MW[a]	Experimental elemental analysis[b]	M.p.[c] (°C)	Yield (%)	^{1}H NMR (δ, ppm; JHz)	^{13}C NMR (δ, ppm)	IR (cm^{-1})
a (Ref. [15])	H	H	H	H	205.2852		175–177	61	2.88 (2H, m); 3.07 (2H, m); 7.34–7.87 (4H, m); 7.90 (1H, s); 8.17 (1H, s); 10.21 (s, 1H)	27.3; 28.4; 157.1; 178.7; among others	3395.9; 3253.4; 3171.7; 3408.0; 1602.6; 1485.4; among others
b	H	CH_3	H	H	219.3122	H: 5.96%; C: 60.31%; N: 19.13%; S: 14.64%	201–203	59	2.33 (3H, s); 2.85 (2H, m); 3.01 (2H, m); 7.10 (1H, d, $J = 7.8$); 7.18 (1H, s); 7.74 (1H, d, $J = 7.8$); 7.87 (1H, s); 8.12 (1H, s); 10.08 (1H, s)	22.0; 28.0; 28.8; 122.4; 126.8, 128.8; 135.6; 141.6; 149.9; 158.4; 178.8	3224.3; 3199.2; 3118.2; 1583.4; 1512.7; 1303.1; 1090.2; 858.8
c	CH_3	H	H	H	219.3122	H: 5.98%; C: 60.33%; N: 19.12%; S: 14.60%	223–224	60	2.21 (3H, s); 2.83 (2H, m); 2.93 (2H, m); 7.18 (2H, d, $J = 4.3$); 7.63 (1H, t, $J = 4.3$); 7.86 (1H, s); 8.03 (1H, s); 10.08 (1H, s)	18.3; 27.4; 119.6; 127.6, 131.5; 135.0; 137.6; 148.2; 158.4; 178.5	3387.2; 3173.1; 3128.92, 1583.7; 1640.0; 1583.7; 1515.5; 1296.6; 1096.2; 779.8; 711.6
d	H	CH_3	H	CH_3	233.3392	H: 6.46%; C: 61.82%; N: 18.00%; S: 13.73%	258–259	83	2.30 (3H, s); 2.80 (2H, m); 2.98 (2H, m); 6.92 (1H, s); 7.02 (1H, s); 7.20 (1H, s); 8.26 (1H, s); 10.14 (1H, s)[e]	20.7; 21.0; 27.6; 27.7; 123.4; 130.0; 132.1; 135.1; 139.8; 150.0; 160.3; 178.3	3434.6; 3198.5; 3127.4; 2999.8; 1582.9; 1512.9; 1504.1; 1298.8; 1083.4; 855.8
e	Br	CH_3	H	CH_3	312.2354	H: 4.52%; C: 46.13%; N: 13.44%; S: 10.26%; Br: 25.62%	d[d]	82	2.34 (3H, s); 2.48 (3H, s); 2.94 (4H, s); 7.11 (1H, s); 7.23 (1H, s); 8.30 (1H, s); 10.30 (1H, s)	21.9; 27.9; 28.6; 122.2; 126.6; 128.6; 135.5; 141.4; 149.8; 158.3; 178.6	3417.7; 3263.2; 3198.6; 3153.5; 1594.8; 1505.1; 1298.9; 1090.9; 859.8
f	H	CH_3	Cl	CH_3	267.7841	H: 5.28%; C: 53.87%; N: 15.66%; S: 11.96%; Cl: 13.26%	267–269	66	2.36 (3H, s); 2.67 (3H, s); 2.92–2.97 (4H, m); 7.23 (1H, s); 7.28 (1H, s); 8.30 (1H, s); 10.17 (1H, s)	17.5; 21.7; 27.8; 28.7; 123.0; 125.8; 133.7; 134.4; 138.4; 148.8; 160.5; 179.0	3419.2; 3247.8; 3200.2; 3141.1; 1595.0; 1514.1; 1299.8; 1092.8; 860.8
g	CH_3	Cl	CH_3	H	267.7841	H: 5.27%; C: 53.89%; N: 15.67%; S: 11.95%; Cl: 13.25%	260–262	65	2.28 (3H, s); 2.35 (3H, s); 2.88 (2H, m); 2.98 (2H, m); 7.74 (1H, s); 7.96 (1H, s); 8.21 (1H, s); 10.28 (1H, s)	16.1; 20.6; 27.2; 27.6; 121.1; 132.2; 134.5; 135.3; 135.9; 146.7; 156.0; 178.4	3394.7; 3253.6; 3209.0; 3143.1; 3003.9; 1584.1; 1516.2; 1294.6; 1094.9; 856.3
h	H	CH_3	CH_3	H	233.3392	H: 6.50%; C: 61.84%; N: 17.99%; S: 13.72%		48	2.15 (3H, s, **2i**); 2.23 (3H, s, **2h**); 2.24 (3H, s, **2h**); 2.26 (3H, s, **2i**); 2.95–2.98 (m, **2h**); 2.82–2.87 (m, **2h** + **2i**); 7.10 (1H, d, $J = 7.8$, **2i**); 7.14 (1H, s, **2h**); 7.58 (1H, d, $J = 7.8$, **2i**); 7.64 (1H, s, **2h**); 7.86 (s, **2h** + **2i**); 8.11 (s, **2h** + **2i**); 10.12 (s, **2h** + **2i**)	15.4; 20.0; 20.1; 20.5; 28.0; 28.3; 119.5; 122.8; 126.9; 129.6; 133.6; 135.9; 139.6; 140.4; 147.3; 158.5; 178.6	3426.6; 3235.3; 3167.6; 3131.9; 1586.8; 1511.9; 1297.8; 1093.2; 859.7
i	CH_3	CH_3	H	H							

(*continued on next page*)

Table 1 (*continued*)

2 (TSC)	R_4	R_5	R_6	R_7	MW[a]	Experimental elemental analysis[b]	M.p.[c] (°C)	Yield (%)	^{1}H NMR (δ, ppm; JHz)	^{13}C NMR (δ, ppm)	IR (cm^{-1})
j	OCH_3	H	H	H	235.3117	H: 5.58%; C: 56.21%; N: 17.84%; S: 13.62%	227–228	80	2.83 (2H, s); 2.91 (2H, s); 6.95 (1H, d, $J = 7.7$); 7.26 (1H, t, $J = 7.7$); 7.41 (1H, d, $J = 7.7$); 8.05 (1H, s); 10.11 (1H, s)[e]	25.8; 27.7; 55.8; 112.4; 114.5; 129.4; 137.4; 139.7; 156.6; 158.1; 178.8	3431.6; 3247.7; 3179.1; 3140.4; 1637.7; 1587.4; 1515.1; 1264.5; 1094.3; 773.5; 699.3
k (Ref. [15])	H	OCH_3	H	H	235.3117		179–180	76	2.82 (2H, m); 3.04 (2H, m); 3.80 (3H, s); 6.85–7.80 (3H, s); 7.82 (1H, s); 8.07 (1H, s); 10.08 (1H, s)	27.6; 28.4; 156.9; 178.5; among others	3400.8; 3275.2; 3181.5; 3041.0; 1602.8; 1526.3; among others
l (Ref. [15])	H	H	OCH_3	H	235.3117		217–219	35	2.87–2.89 (4H, m); 3.79 (3H, s); 6.92–7.48 (3H, s); 8.03 (1H, s); 8.18 (1H, s); 10.18 (1H, s)	27.5; 27.9; 156.9; 178.5; among others	3398.3; 3254.7; 3158.9; 3050.2; 1603.3; 1481.4; among others
m	H	OCH_3	OCH_3	H	265.33.81	H: 5.71%; C: 54.41%; N: 15.85%; S: 12.06%	246–247	75	2.83 (2H, m); 2.95 (2H, m); 6.93 (1H, s); 7.41 (1H, s); 7.97 (1H, s); 8.11 (1H, s); 10.08 (1H, s)[e]	28.1; 28.5; 56.1; 56.2; 104.3; 108.3; 130.0; 142.8; 149.3; 152.4; 158.5; 178.3	3373.3; 3259.4; 3151.9; 1616.6; 1594.9; 1264,3; 1092.9; 853.0
n	OCH_3	OCH_3	H	H	265.3581	H: 5.70%; C: 54.31%; N: 15.83%; S: 12.09%	212–213	75	2.82 (2H, m); 2.98 (2H, m); 7.01 (1H, d, $J = 8.5$); 7.53 (1H, d, $J = 8.5$); 7.81 (1H, s); 7.98 (1H, s); 10.02 (1H, s)[e]	23.9; 26.2; 54.9; 58.4; 111.6; 116.4; 130.0; 140.2; 143.5; 156.0; 176.7	3390.2; 3255.5; 3153.0; 1609.8; 1515.2; 1274.6; 1092.9; 802.7
o	H	Cl	H	H	239.7301	H: 4.20%; C: 50.13%; N: 17.51%; S: 13.39%; Cl: 14.81%	214–215	88	2.89 (2H, m); 3.06 (2H, m); 7.34 (1H, dd, $J = 8.2$, $J = 1.2$); 7.40 (1H, d, $J = 1.2$); 7.91 (1H, d, $J = 8.2$); 8.01 (1H, s); 8.21 (1H, s); 10.30 (1H, s)	27.8; 28.6; 123.8; 126.2; 127.8; 135.7; 137.2; 151.3; 156.5; 178.9	3395.3; 3246.9; 3136.5; 1582.8; 1525.1; 1300.9; 1093.7; 865.9; 854.9; 706.5
p	H	Br	H	H	284.1814	H: 3.55%; C: 42.31%; N: 14.77%; S: 11.27 Br: 28.15%	213–215	89	2.88 (2H, m); 3.07 (2H, m); 7.48 (1H, d, $J = 8.3$); 7.60 (1H, s); 7.84 (1H, d, $J = 8.3$); 8.01 (1H, s); 8.21 (1H, s); 10.30 (1H, s)	27.2; 28.1; 123.6; 123.7; 128.5; 129.9; 137.3; 150.8; 155.3; 178.5	3430.0; 3253.6; 3145.9; 1594.7; 1501.0; 1463.6; 1288.2; 1096.4; 847.4; 798.4

[a] Molecular weight.
[b] Elemental analysis found was within ±0.4% of the theoretical values.
[c] Melting point.
[d] Decompose before melting.
[e] The signals corresponding to methyl and methoxy groups are overlapped by DMSO-d_6/water.



Scheme 1. Reagents and conditions: (i) $NH_2NHCSNH_2$, EtOH, reflux.

modified derivatives to establish their therapeutic potential against BVDV and HCV.

4. Experimental protocols

4.1. Chemistry

Melting points (uncorrected) were determined on a Thomas Hoover apparatus. Thin layer chromatography (TLC) was used to monitor reactions. Flash chromatography was performed with silica gel (Merck silica gel 60, 230–400 mesh). IR spectra were recorded as KBr pellets using a Perkin–Elmer Spectrum One FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 500 MHz spectrometer. Mass spectra were recorded on a Shimadzu QP 5000 spectrometer. Micro-analyses (C, H, N) were carried out on a Carlo Erba elemental analyser (Model 1106) and were within 0.4% of the theoretical values.

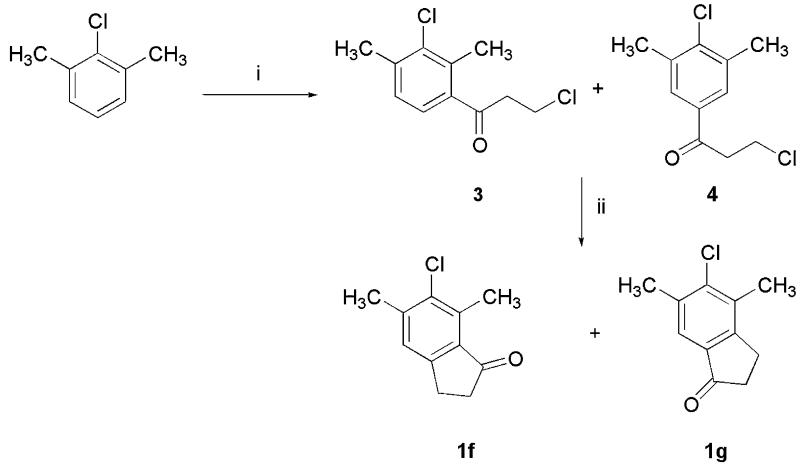
4.1.1. 3-Chloro-1-(3-chloro-2,4-dimethylphenyl)propan-1-one (**3**) and 3-chloro-1-(4-chloro-3,5-dimethylphenyl)-propan-1-one (**4**).

$AlCl_3$ (3.07 g, 23.0 mmol) was added to a magnetically stirred solution of 2-chloro-1,3-dimethylbenzene (1.20 g, 8.5 mmol) and β-chloro-propionyl chloride (1.08 mL, 11.3 mmol) in CS_2 (10 mL) at 0 °C over a period of 30 min. The reaction mixture was heated under reflux for a further 30 min. The resulting dark-brown solution was cooled at room temperature and carefully poured onto ice and was extracted with CH_2Cl_2 (20 mL), washed with water (2 × 10 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo to yield the crude product (1.87 g, 94%). ^{1}H NMR ($CDCl_3$): δ 2.42 (s, CH_3Ar, compd. **3**); 2.43 (s, 2 × CH_3Ar, compd. **4**); 2.48 (s, CH_3Ar, compd. **3**); 3.32 (t, $J = 6.6$ Hz, CH_2CO, compd. **3**); 3.41 (t, $J = 7.0$ Hz, CH_2CO, compd. **4**); 3.89 (m, CH_2Cl, compds. **3** and **4**); 7.15 (d, $J = 7.9$ Hz, HAr, compd. **3**); 7.34 (d, $J = 7.9$ Hz, HAr, compd. **3**); 7.66 (s, HAr, compd. **4**). Anal. Calc. for $C_{11}H_{12}Cl_2O$ (231.12) C: 57.16; H: 5.23; Cl: 30.68. Found C: 57.21; H: 5.21; Cl: 30.58.

4.1.2. 6-Chloro-5,7-dimethylindan-1-one (**1f**) and 5–chloro-4,6-dimethylindan-1-one (**1g**)

The mixture of compounds **3** and **4** (1.25 g, 5.4 mmol) was added in small portions with swirling to concentrated H_2SO_4 (15 mL). The resulting solution was heated on a silicon bath at 90 °C. After 1 h, the reaction mixture was cautiously poured onto ice and then extracted with $CHCl_3$ (40 mL). The organic layer was washed with a solution of 10% NaOH (2 × 15 mL), water (15 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue containing the mixture of indanones **1f** and **1g** was separated by flash chromatography using hexane–ethyl acetate (9:1) as eluent.

Compound **1f** (0.47 g, 28%): m.p. 94–96 °C. ^{1}H NMR ($CDCl_3$): δ 2.44 (3H, s, CH_3Ar); 2.68 (2H, m, CH_2CO); 2.71 (3H, s, CH_3Ar); 2.99 (2H, m, CH_2Ar); 7.19 (1H, s, HAr). ^{13}C NMR ($CDCl_3$): δ 15.8; 21.0; 25.0; 36.3; 122.4; 134.0; 134.7; 136.0; 141.4; 152.7; 206.4. MS *m/z*: 194 $[M]^+$ (100), 196 $[M+2]^+$ (28), 159 $[M-Cl]^+$ (49), 131 $[M-(Cl+CO)]^+$ (91). Anal. Calc. for $C_{11}H_{11}ClO$ (194.66) C: 67.87; H: 5.70; Cl: 18.21. Found C: 67.84; H: 5.69; Cl 18.17. Compound **1g** (0.60 g, 37%): m.p. 92–94 °C. ^{1}H NMR ($CDCl_3$): δ 2.39 (3H, s, CH_3Ar); 2.42 (3H, s, CH_3Ar); 2.70 (2H, t, $J = 9.4$ Hz, CH_2CO); 3.02 (2H, t, $J = 9.4$ Hz, CH_2Ar); 7.49 (1H, s, HAr). ^{13}C NMR ($CDCl_3$): δ 14.4; 21.9; 24.5; 37.3; 125.8; 133.5; 134.6; 136.7; 142.8; 153.8; 206.7. MS *m/z*: 194 $[M]^+$ (100), 196 $[M+2]^+$ (37), 159 $[M-Cl]^+$ (24), 131 $[M-(Cl+CO)]^+$ (77). Anal. Calc. for



Scheme 2. Reagents and conditions: (i) β-chloro-propionyl chloride, $AlCl_3$, CS_2, 0 °C, 30 min; (ii) H_2SO_4 (c), 90 °C, 1 h.

Table 2
Antiviral activity of 1-indanone TSCs against BVDV strain NADL in MDBK cells

2	R_4	R_5	R_6	R_7	CC_{50} (μM)[a]	EC_{50} (μM)[b]	SI[c]
a	H	H	H	H	156.30	18.10	8.60
b	H	CH_3	H	H	130.00	6.48	20.06
c	CH_3	H	H	H	164.80	8.13	20.27
d	H	CH_3	H	CH_3	51.70	ND	ND
e	Br	CH_3	H	CH_3	139.10	8.94	15.50
f	H	CH_3	Cl	CH_3	80.70	7.40	10.91
g	CH_3	Cl	CH_3	H	72.50	>11.10	< 6.53
h	H	CH_3	CH_3	H	161.30[d]	6.74[d]	23.93[d]
i	CH_3	CH_3	H	H			
j	OCH_3	H	H	H	822.50	242.40	3.39
k	H	OCH_3	H	H	149.00	18.80	7.93
l	H	H	OCH_3	H	106.30	26.00	4.09
m	**H**	**OCH_3**	**OCH_3**	**H**	**140.50**	**1.75**	**80.29**
n	OCH_3	OCH_3	H	H	140.80	11.50	12.24
o	H	Cl	H	H	>27.00	8.80	3.07
p	H	Br	H	H	>15.00	1.81	8.29
Ribavirin					**54.00**	**4.62**	**11.64**

[a] **50% cytotoxic concentration**; concentration required to reduce MDBK cells viability by 50%.
[b] **50% effective concentration**; concentration required to inhibit BVDV cytopathic effect in MDBK cells by 50%.
[c] **Selectivity index** or ratio of CC_{50} to EC_{50}.
[d] Values corresponding to the mixture of compounds **2h** and **2i**.

$C_{11}H_{11}ClO$ (194.66) C: 67.87; H: 5.70; Cl: 18.21. Found C: 67.94; H: 5.68; Cl: 18.27.

4.1.3. General procedure for the synthesis of TSCs (*2a–p*)

The TSC derivatives (Table 2) were obtained from the corresponding 1-indanones (**1a–1p**) by treatment with thiosemicarbazide (Scheme 2). A suspension of 1-indanone (1.2 mmol) and thiosemicarbazide (2.7 mmol) in absolute ethanol (20 mL) was heated under reflux for 30 min, then concentrated H_2SO_4 (0,10 mL) was added and the heating was continued until the 1-indanone was consumed. The conversion of 1-indanone in the corresponding TSC was monitored by TLC on silica gel 60 F_{254} using chloroform–ethanol (1:0.1) as eluent. The solvent was removed in vacuo and the solid was suspended in water (20 mL), filtered and washed with water (2 × 5 mL), EtOH (5 mL), CH_2Cl_2 (5 mL) and finally hexane (2 × 5 mL).

The compounds were identified by spectral data (^1H, ^{13}C NMR and IR). The synthesized TSCs, their yield, physical and spectroscopic characteristics are summarized in Table 2.

4.2. Antiviral activity

4.2.1. Cells and viruses

The synthesized TSCs, dissolved in DMSO (1 mg/mL), were tested against BVDV type-1 NADL strain (ATCC VR 534) in MDBK cells culture. The activity on herpes simplex virus type-1 (HSV-1), vesicular stomatitis virus type-1 (VSV-1) and polio virus type-1 (PV-1) replication was evaluated in Vero cells culture. The activity on human immunodeficiency virus type-1 (HIV-1) replication was evaluated in Peripheral Blood Mononuclear cells (PBMC).

4.2.2. Cytotoxic assay

MDBK cells were seeded in microplates at a density of 1×10^4 cells per well of a 96-well plate in Minimal Essential Medium (Gibco) supplemented with Fetal Bovine Serum (MEM-FBS); 24 h later, serial dilutions of the test compounds were added. Cells were allowed to proliferate for 3 days at 37 °C, after which the cell number was determined by means of MTS/PMS method (Promega). The yield of formazan product is proportional to the number of living cells. After 3 h at 37 °C, the absorbance was determined at 490 nm. The percent cell viability was calculated as follows:

$$\text{Cell viability} = (\text{Abs}_t \times 100/\text{Abs}_c)$$

Abs_c = absorbance of cells untreated; Abs_t = absorbance of cells treated with a certain dilution of compound.

The 50% cytotoxic concentration (CC_{50}) was defined as the concentration that inhibited the proliferation of exponentially growing cells by 50% and was calculated using logarithmic interpolation from dose-response curves.

4.2.3. Anti-BVDV assay

MDBK cells were seeded in microplates (96 wells) at a density of 1×10^4 cells per well, in MEM-FBS. Following 24 h of incubation at 37 °C and 5% CO_2, medium was removed and a serial dilution of the test compounds was added. After which, the BVDV inoculum was added to each well. This inoculum resulted in a greater than 80% of cytophatic effect after 3 days of incubation at 37 °C. Uninfected cells and cells receiving virus without compound were included in each assay plate. After 3 days medium was removed and MEM-DHS (donor horse serum), supplemented with MTS/PMS solution, was added to each well. The absorbance of each well was read at 490 nm. The 50% effective concentration (EC_{50}) was defined as the concentration of compound that offered 50% protection of the cells against virus-induced cytopathic effect (CPE) and was calculated using logarithmic interpolation from dose-response curves.

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