SYNTHESIS AND ANTIVIRAL ACTIVITY OF NEW THIAZOLE, 1,2,4-TRIAZOL AND OXINDOLE DERIVATIVES

[1]Oleg Radul, [1]Natalia Sucman, [1]Serghei Pogrebnoi, [1]Alic Barba,
[2]Athina Geronikaki, [1]Fliur Macaev*

[1]Institute of Chemistry of the Academy of Sciences of Moldova,
Academy str. 3, MD-2028, Chisinau, Moldova
Tel +373-22-739-754, Fax +373-22-739-954, E-mail: flmacaev@cc.acad.md
[2]Aristotelian University of Thessaloniki, Thessaloniki 54124, Greece
Tel.:+302310997616, Fax: +302310997612
E-mail: geronik@pharm.auth.gr

Dedicated to academician Pavel F. Vlad on the occasion of his 75th birthday

Abstract: The synthesis and antiviral activity evaluation of new derivatives of 2-aminothiazole, 1,2,4-triazole, as well as oxindoles has been realized. The synthesized compounds exhibited different cytotoxicity, in particular, oxindols **4** , **5** , **7** , **8** , **9**, **10**, **11**, **12**, **13**, **58** as well as thiazole/triazole **73** and **75** turned out to be the most cytotoxic for MT-4 cell lines. The compounds **11**, **12**, **73**, and **75** are more toxic than reference compound Efavirenz. As far as the antiviral activity is concerned, none of the title compounds turned out active against Reo-1, Sb-1, VSV, RSV, YFV and VV viruses. The results obtained against Bovine Viral Diarrhoea Virus (BVDV) showed that nine compounds (six from oxindol's seria **6**, **12**, **13**, **52**, **56**, **58** and three 73, **75, 77** of triazole homologues) resulted moderate active. Among all of them, the most potent compound was **52**, with EC_{50} of 6.6 μM. Studies of effect of synthesized compounds against Coxsakie Virus (CVB-2) revealed that only two compounds, **13** and **73** exhibit moderate activity (EC_{50} >40 and >18 μM, respectively). It should be noticed that eleven compounds, **4**, **5**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **58,** and **75** showed moderate activity against HIV-1 (EC_{50} >16 – m >59μM).

Keywords: 2-aminothiazole, 1,2,4-triazol, oxindoles, cytotoxicity, antiviral activity.

1. Introduction

The twentieth century has been characterized both by a drastic reduction in the mortality caused by infectious diseases and by a rise in the control of neoplastic pathologies. The treatment of infectious diseases still remains an important and challenging problem. The therapeutic problem has achieved increasing importance in hospitalised patients, in immuno suppressed patients with AIDS or undergoing anticancer therapy and organ transplants. Oxindoles has posses a different kind of physiological activity [1-21]. 4-Aryl-5-(1*H*-1,2,4-triazol-1-yl)-1,3-thiazol-2-amines are found to be associated with various biological activities such as antifungal, anti-inflammatory, plant-growth regulatory, adenosine receptor antagonists [22-24]. Prompted by these reports and in continuation of search for bioactive substituted thiazole from computer prediction [25] to synthesis and biological evaluation it was contemplated to synthesize 2-aminothiazole containing 1*H*-1,2,4-triazole's moiety starting from 1-benzoylmethyl-1*H*-1,2,4-triazoles.

2. Results and discussion

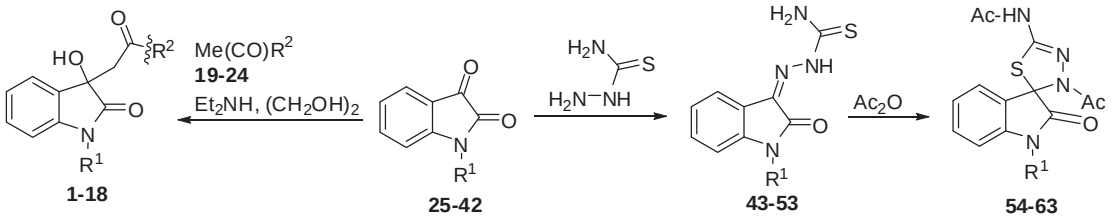
2.1 Chemistry

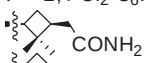
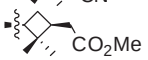
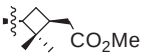
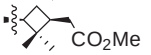
Recently, we described the synthesis of substituted oxindoles [11, 26-32]. In the present paper, the synthesis of 3-hydroxyoxindoles **1-18** from easily accessible [32, 33] indol-2,3-diones **25-35** are suggested (Scheme 1).

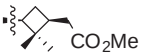
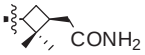
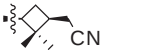
Our initial studies [27,28] were performed using as catalyst Et_2NH in aq. MeOH, aq. EtOH, aq. *i*-PrOH. The presence of water in reaction media at room temperature showed a positive effect on the reaction of isatine **25** with acetone **19**. However, the use of Et_2NH as a catalyst in 25% aq. ethylene glycol gives the best result (98 %) compared to using of Et_2NH in aq. MeOH (77 %), aq. EtOH (81 %) or aq. *i*-PrOH (88 %). When the temperature was decreased to $5\text{-}10^0$ C, the 3-hydroxyoxindole **1** was obtained only with 60 %. Finally, all the interactions of isatines **26-35** with methylketones **19-24** carried out in 25% aq. ethylene glycol as a solvent using 1 eq. of Et_2NH as catalyst at room temperature gave the novel oxindoles **3-8** and known derivatives **1,2,9-18** with moderate to good yields (see Table 1 and experimental part).

The known transformation [34] of 1-methylindoline-2,3-dione **26** into thiosemicarbazone **44** afforded an antiviral drug that works by inhibiting mRNA and protein synthesis, especially in pox viruses. On the other side, the synthetic approach to Δ^2-1,3,4-thiadiazoline **56** from thiosemicarbazone **46** we published early [35]. This prompted us to find new compounds having a thiadiazoline moiety attached to oxindoles. These compounds can be considered as cyclic thiosemicarbazide derivatives as well.

The synthesis of compounds **43, 45, 47-53** from easily accessible isatines **25, 29, 36-42** was performed according to the method previously reported [35]. Here we report the reaction of 2-oxoindolin-3-ylidene-hydrazinecarbothioamides **43, 45, 47-53** with boiling acetic anhydride (Scheme 1).



1 R^1= H, R^2=Me
2 R^1= R^2=Me
3 R^1= Et, R^2=Me
4 R^1= Bn, R^2=Me
5 R^1= CH_2CH_2Cl, R^2=Me
6 R^1=$CH_2N(CH_2CH_2)_2O$, R^2=Me
7 R^1=$CH_2N(CH_2CH_2)_2NPh$, R^2=Me
8 R^1= Pr, R^2 = C_6H_5
9 R^1= Me, R^2 = 2,4-Cl_2-C_6H_3
10 R^1= Et, R^2 = 2,4-Cl_2-C_6H_3
11 R^1= Bu, R^2 = 2,4-Cl_2-C_6H_3
12 R^1= hexyl, R^2 = 2,4-Cl_2-C_6H_3
13 R^1= decyl, R^2 = 2,4-Cl_2-C_6H_3
14 R^1= H, R^2= 
15 R^1= H, R^2= 
16 R^1=H, R^2= 
17 R^1= Et, R^2= 
18 R^1= Bn, R^2= 

19 R^2 = Me
20 R^2 = Ph
21 R^2 = 2,4-Cl_2-C_6H_3
22 R^2 = 
23 R^2 = 
24 R^2 = 

25, **43** R^1= H
26, 44 R^1= Me
27 R^1= Et
28 R^1= Pr
29 45, 55 R^1= Bu
30 R^1= hexyl
31 R^1= decyl
32, 46, 56 R^1= Bn
33 R^1= CH_2CH_2Cl
34 R^1=$CH_2N(CH_2CH_2)_2O$
35 R^1=$CH_2N(CH_2CH_2)_2NPh$
36, 47, 57 R^1= heptyl
37, 48, 58 R^1= nonyl
38, 49, 59 R^1= COMe
39, 50, 60 R^1= $CH_2C(O)CMe_3$
40, 51, 61 R^1= $CH_2CH=C(Me)Cl$
41, 52, 62 R^1= $(CH_2)_2COMe$
42, 53, 63 R^1= $CH_2CH=CH_2$
54 R^1= Ac

Scheme 1

The reaction of thiosemicarbazone **43** with Ac_2O at high temperature afforded isatine-derived Δ^2-1,3,4-thiadiazoline **54** as colorless needles in 71 % yield. Similarly**,** by the reaction of thiosemicarbazone **45, 47-52** and Ac_2O, solid spirooxindoles **54, 55, 57-62** were obtained in good to excellent yields (see Table 1).

Table 1

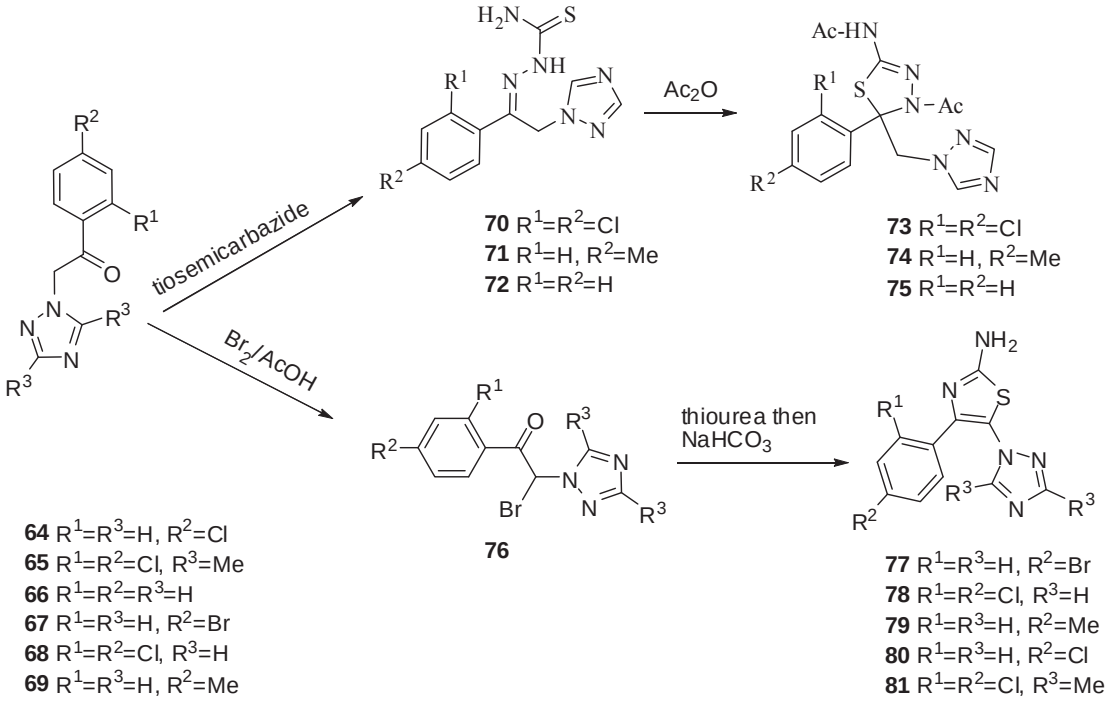
An analytical data of the synthesized compounds

Compounds	Aggregation M.p. (°C) from EtOH	Yield (%)	Mol. Formula	Elemental analyses (%)		
				C	H	N
				Calcd. / Found	Calcd. /Found	Calcd. / Found
3	155-156	76	$C_{13}H_{15}NO_3$	66.94 / 67.23	6.48 / 6.67	6.00 / 6.32
4	155-157	80	$C_{18}H_{17}NO_3$	73.20 / 73.44	5.80 / 6.21	4.74 / 4.72
5	150-152	78	$C_{13}H_{14}ClN_2O$	58.32 / 58.22	5.27 / 5.68	5.23 / 5.48
6	107-108	76	$C_{18}H_{17}NO_3$	73.20 / 73.44	5.80 / 6.21	4.74 / 4.72
7	165-166	73	$C_{16}H_{20}N_2O_4$	63.14 / 63.22	6.62 / 6.26	9.20 / 9.33
8	149-150	76	$C_{22}H_{25}N_2O_3$	69.64 / 69.77	6.64 / 6.99	11.07 / 10.92
43	252	92	$C_9H_8N_4OS$	49.08/ 49.08	3.66/ 3.49	25.44/ 25.27
45	155	91	$C_{13}H_{16}N_4OS$	56.50/56.34	5.84/6.02	20.27/20.45
46	250	96	$C_{16}H_{14}N_4OS$	61.92/61.77	4.55/4.68	18.05/18.34
47	166-168	90	$C_{15}H_{20}N_4OS$	59.18/59.00	6.62/6.49	18.40/18.11
48	131-132	88	$C_{18}H_{26}N_4OS$	62.39/62.22	7.56/7.33	16.17/16.01
49	251-254	97	$C_{12}H_{12}N_4O_2S$	52.16/51.89	4.38/4.55	20.28/20.11
50	236-237	82	$C_{15}H_{18}N_4O_2S$	56.58/56.38	5.70/5.56	17.60/17.46
51	228	79	$C_{13}H_{13}ClN_4OS$	50.57/50.69	4.24/4.47	18.14/18.11

52	203-205	77	$C_{13}H_{14}N_4OS$	53.78/54.09	4.86/5.02	19.30/19.10
53	176-178	81	$C_{12}H_{12}N_4OS$	55.37/55.21	4.65/4.69	21.52/21.63
54	246-248	71	$C_{13}H_{12}N_4O_3S$	51.31/51.59	3.97/4.25	18.41/18.66
55	211-213	65	$C_{17}H_{20}N_4O_3S$	56.65/56.66	5.59/5.77	15.54/15.34
56	248-249	88	$C_{20}H_{18}N_4O_3S$	60.90/60.88	4.60/4.44	14.20/14.32
57	170-171	71	$C_{19}H_{24}N_4O_3S$	58.74/58.77	6.23/6.50	14.42/14.65
58	202-203	70	$C_{22}H_{30}N_4O_3S$	61.37/61.55	7.02/7.32	13.01/12.96
59	247	59	$C_{16}H_{16}N_4O_4S$	53.32/53.61	4.47/4.35	14.20/14.20
60	250(decom)	82	$C_{19}H_{22}N_4O_4S$	56.70/56.55	5.51/5.69	13.92/14.12
61	176-178	60	$C_{17}H_{17}ClN_4O_3S$	51.97/51.84	4.36/4.54	14.26/14.02
62	189-192	57	$C_{17}H_{18}N_4O_4S$	54.53/54.59	4.85/4.68	14.96/14.89
63	173-175	40	$C_{16}H_{16}N_4O_3S$	55.80/55.98	4.68/4.41	16.27/16.45
70	162-163	75	$C_{11}H_{12}N_6S$	50.75/ 50.97	4.65/ 4.83	32.28/ 32.55
71	194-199	66	$C_{12}H_{14}N_6S$	52.54/52.66	5.14/5.33	30.63/30.76
72	187-190	79	$C_{11}H_{10}Cl_2N_6S$	40.13/40.34	3.06/3.06	25.53/25.67
73	226-236	50	$C_{15}H_{16}N_6O_2S$	52.31/52.33	4.68/4.77	24.40/24.23
74	199	56	$C_{16}H_{18}N_6O_2S$	53.65/53.44	5.07/4.77	24.40/23.37
75	250 (decom)	63	$C_{15}H_{14}Cl_2N_6O_2S$	43.59/43.52	3.41/3.49	20.33/20.31
77	230-232	89	$C_{11}H_8BrN_3S$	C 41.01/40.98	2.50/2.71	21.74/21.72
78	245-246	88	$C_{11}H_8ClN_5S$	47.57/47.83	2.90/2.59	25.22/25.03
79	250(decom)	93	$C_{12}H_{11}ClN_5S$	56.01/56.21	4.31/4.00	27.22/27.47
80	243-244	77	$C_{11}H_7Cl_2N_5S$	42.32/41.98	2.26/2.08	22.43/22.65
81	285-286	77	$C_{13}H_{11}Cl_2N_5S$	45.89/46.32	3.26/3.11	20.58/20.68

N-Allylisatine derivative **53** readily underwent cyclization with Ac_2O to produce the corresponding 1,3,4-thiadiazoline **63**. This compound also appeared to be quite stable in the reaction mixture at this temperature. However, acetamide **63** was obtained in a low yield (only a 40 %). At this condition polymerization to a dark solid material was observed.

The successfull use of thiosemicarbazones in heterocyclization encouraged us to apply available 1-aryl-2-(1*H*-1,2,4-triazol-1-yl)-1-ethanones **66, 68, 69** [36] for the synthesis of compounds **70-72** (schema 2).



Scheme 2

The thiosemicarbazones **70-72** when reacted with boiling acetic anhydride gave variable yields of 2-acetamido-4-acetyl-5-(aryl)-5-(1*H*-1,2,4-triazol-1-yl-methyl)-Δ^2-1,3,4-thiadiazoline **73-75**.

The second strategy has include transformations of ethanones **64, 65,** and **67-69** into the 1,3-thiazol-2-amines derivative 1,2,4-triazoles **77-81** *via* corresponding α-bromoketones **76**. In the beginning our study the compound **76** (R^1=R^3=H, R^2=Br) was obtained by the halogenation of ketone **67** with Br_2 in mixture HBr/AcOH at 10^0C according to the procedure described in the literature [37]. However, this compound was apparently not very stable. It is worth noting that allylic bromination of ketone **67** using Br_2 followed by condensation of product with thiourea gave the 2-aminothiazole **77** in 89 % yield. The optimized two steps protocol was then expanded to ketones **64, 65, 68,** and **69**. The targets 2-aminothiazole-1*H*-1,2,4-triazoles **78-81** were conveniently isolated as precipitates with good yields (see Table 1). All synthesized compounds were characterized by ellemental analysis (Table 1) as well as spectroscopically (H-NMR, Table 2, MS experimental part).

Table 2

^{1}H NMR spectral data of synthesized compounds

Comp.	δ (ppm)
3	DMSO-d_6: 1.20 (t, 3H, J= 9.17, C$\underline{H}_3$CH$_2$); 2.10 (s, 3H, Me); 2.97 (dd, 2H, CH_2, AB-system, *J*= 11.20 and 14.49), 4.80 (q, 2H, J=6.48 Hz, Me$\underline{CH}_2$); 4.39 (s, 1H, OH), 6.81-7.44 (m, 4H, ArH)
4	DMSO-d_6:2.11 (s, 3H, Me); 2.89, 2.99 (dd, 2H, CH_2, AB-system, *J*=11.55 and 24.32); 4.48 (s, 1H, OH); 4.88 (s, 2H, N-$\underline{CH}_2$-Ph); 6.24-7.41 (м, 9H, arom).
5	$CDCl_3$:2.14 (s, 3H, Me); 3.11-3.66 (m, 3CH_2); 3.97 (s, 1H, OH); 6.91-7.23 (м, 4H, arom).
6	DMSO-d_6:2.09 (s, 3H, Me); 2.50-3.65 (m, 3CH_2); 4.41 (s, 2H, NCH_2), 6.08 (s, 1H, OH); 6.77-7.11 (м, 4H, arom).
7	$CDCl_3$:2.17 (s, 3H, Me); 2.48-3.44 (m, 3CH_2); 4.58 (s, 1H, OH); 4.45 (s, 2H, NCH_2), 6.43-7.27 (м, 9H, arom).
8	$CDCl_3$: 0.90 (t, 3H, J=8.7, C$\underline{H}_3$CH$_2$); 1.62-3.38 (m, 4H, 2CH_2), 2.06 (s, 3H, Me); 2.76 (dd, 2H, CH_2, AB-system, *J* =11.01 and 14.49), 5.99 (s, 1H, OH), 6.83-7.98 (m, 4H, ArH)
43	DMSO-d_6: 7.23-7.89 (m, 6H, NH_2, ArH); 9.10 (s, 1H, NH-CO); 11.13 (s, 1H, NH-CS)
45	DMSO-d_6: 1.00 (t, 3H, J= 8.76, C$\underline{H}_3$CH$_2$); 1.31-3.97 (m, 6H, 3CH_2); 7.21-7.98 (m, 6H, NH_2, ArH), 10.92 (s, 1H, NH-CS)
46	DMSO-d_6: 4.94 (s, 2H, CH_2); 6.93-7.75 (m, 9H, ArH); 8.74-9.10 (m, 2H, NH_2); 12.43 (s, 1H, NH-CS)
47	$CDCl_3$: 0.78 (t, 3H, CH_2-C$\underline{H}_3$, *J*=7.28); 1.02-1.79 (m, 6H, $(CH_2)_3$); 3.64 (q, 2H, CH_2-N, *J*=7.14)
48	$CDCl_3$: 0.78 (t, 3H, CH_2-C$\underline{H}_3$, *J*=5.76); 1.0-1.83 (m, 14H, $(C\underline{H}_2)_7$); 3.64 (t, 2H, CH_2-N, *J*=7.28); 6.65-7.54 (m, 5H, ArH, NH_2); 12.26 (s, 1H, N$\underline{H}$-CS)
49	DMSO-d_6: 2.11 (s, 3H, $COCH_3$); 2.85 (t, 2H, C$\underline{H}_2$-CO-CH_3, *J*=7.28); 3.89 (t, 2H, N-C$\underline{H}_2$-CH_2, *J*=7.2); 7.00-7.70 (m, 4H, ArH); 8.65-9.05 (m, 2H, NH_2); 12.36 (s, 1H, NH-CS)
50	DMSO-d_6:1.20(s,9H,3CH_3); 4.80 (s,2H,CH_2); 6.69-7.76 (m,4H, ArH); 8.71, 8.08(s,s,2H,NH_2);12.29 (s,1H,NH)
51	DMSO-d_6: 2.11 (s, 3H, CH_3); 4.42 (d, 2H, CH_2, *J*=6.06); 5.72 (t, 1H, CH=, , *J*=6.29), 6.93-7.72 (m, 4H, ArH); 8.67, 8.9.06 (s, s , 2H, NH_2); 12.34 (s, 1H, NH)
52	$CDCl_3$:2.13 (s, 3H, Me); 2.70-3.24 (m, 2CH_2); 7.21-7.98 (m, 6H, NH_2, ArH), 10.92 (s, 1H, NH-CS)
53	DMSO-d_6: 4.32 (s, 2H, CH_2); 4.89-5.39 (m, 2H, =CH_2), 5.49-6.2 (m, 1H, CH=), 6.97-7.73 (m, 4H, ArH); 8.59, 8.9.03 (s, s , 2H, NH_2); 12.45 (s, 1H, NH)
54	DMSO-d_6: 2.1, 2.15 (s, s, 6H, 2$COCH_3$); 2.56 (s, 3H, $CONCOCH_3$); 6.65-7.5 (m, 4H, ArH); 12.05 (s, 1H, NH)
55	$CDCl_3$: 0.812 (t, 3H, CH_2-C$\underline{H}_3$, *J*=6.28); 1.13-1.64 (m, 4H, C$\underline{H}_2$-C$\underline{H}_2$-CH_3); 1.91, 2.13 (s, s, 6H, 2$COCH_3$); 3.69 (t, 2H, N- CH_2, *J*=6.51); 6.74-7.39 (m, 4H, ArH); 10.40 (s, 1H, NH)
56	DMSO-d_6: 2.08 (s, s, 6H, 2$COCH_3$); 2.21 (c, 3H, CO-C$\underline{H}_3$), 4.94 (q, 2H, J-16.11, N-C$\underline{H}_2$-C_6H_5); 6.73-7.45 (м, 9H, аром); 12.00 (c, 1H, N$\underline{H}$- CO-CH_3)
57	$CDCl_3$: 0.80 (t, 3H, CH_2-C$\underline{H}_3$, *J*=5.49); 1.15-1.65 (m, 8H, $(\underline{CH}_2)_4$Me); 1.92 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$); 3.67 (t, 2H, N- CH_2, *J*=6.91); 6.73-7.38 (m, 4H, ArH); 10.40 (s, 1H, NH)
58	$CDCl_3$: 0.80 (t, 3H, CH_2-C$\underline{H}_3$, *J*=6.64); 1.21-1.84 (m, 14H, $(C\underline{H}_2)_7$Me); 1.93 (s, 3H, $COCH_3$); 2.13 (s, 3H, $COCH_3$); 3.67 (t, 2H, N-CH_2, *J*=6.69); 6.74-7.39 (m, 4H, ArH); 10.35 (s, 1H, NH)
59	$CDCl_3$:2.1, 2.16, 2.22 (s, s, s, 9H, 3$COCH_3$); 4.67 (s, 2H, CH_2); 6.68-7.36 (m, 4H, ArH); 11.72 (s, 1H, NH)
60	DMSO-d_6: 1.19 (s, 9H, CMe_3); 2.08, 2.14 (s, s 6H, 2$COCH_3$); 4.83 (s, 2H, CH_2); 6.79-7.43 (m, 4H, ArH)
61	$CDCl_3$: 1.08 (s, 3H, =C-CH_3); 1.96, 2.12 (s, s, 6H, 2$COCH_3$); 4.45 (d, 2H, CH_2, *J*=7.21); 5.45 (t, 1H, CH=C, *J*=7.07); 6.74-7.38 (m, 4H, ArH); 10.17 (s, 1H, NH)
62	$CDCl_3$: 1.97, 2.02 (s, s, 6H, 2$COCH_3$); 2.05 (s, 3H, $COCH_3$); 2.73 (t, 2H, C$\underline{H}_2$CO, *J*=7.4); 3.82 (t, 2H, N-CH_2, *J*=7.6); 6.69-7.28 (m, 4H, ArH); 11.06 (s, 1H, NH)
63	DMSO-d_6: 2.09, 2.2.16 (s, s, 6H, 2$COCH_3$); 4.20-4.39 (m, 2H, CH_2); 4.89-5.48 (m, 2H, =CH_2), 5.63-5.89 (m, 1H, CH=), 6.88-7.59 (m, 4H, ArH); 12.03 (s, 1H, NH)
70	DMSO-d_6: 5.71 (s, 2H, CH_2); 7.36 (m, 5H, ArH); 7.93 (s, 1H, triazole $C_{(3)}$H); 8.12-8.52 (m, 2H, NH_2); 8.68 (s, 1H, triazole $C_{(5)}$H); 10.97 (s, 1H, NH)

71 DMSO-d_6: 2.26 (s, 3H, CH_3); 5.69 (s, 2H, CH_2); 7.09-7.68 (m, 4H, ArH); 7.94 (s, 1H, triazole $C_{(3)}H$); 8.09-8.44 (m, 2H, NH_2); 8.66 (s, 1H, triazole $C_{(5)}H$); 10.94 (s, 1H, NH)

72 DMSO-d_6: 5.29 (s, 2H, CH_2); 6.98-8.48 (m, 5H, ArH, NH_2); 7.92 (s, 1H, triazole $C_{(3)}H$); 8.48 (s, 1H, triazole $C_{(5)}H$); 10.21 (s, 1H, NH)

73 DMSO-d_6: 1.93, 2.17 (s,s, 6H, $2COCH_3$); 5.30, 5.36 (d,d, 2H, CH_2, AB system, J = 14.43 and 23); 7.41 (m, 5H, ArH); 8.01 (s, 1H, triazole $C_{(3)}H$); 8.55 (s, 1H, triazoile $C_{(5)}H$); 11.55 (s, 1H, NH)

74 DMSO-d_6:1.94, 2.16 (s,s, 6H, $2COCH_3$); 5.24, 5.31 (d,d, 2H, CH_2, AB system, J = 18.67 and 28.92); 7.20, 7.34 (d,d, 4H, ArH, J = 8.65); 8.00 (s, 1H, triazole $C_{(3)}H$); 8.54 (s, 1H, triazole $C_{(5)}H$); 11.50 (s, 1H, NH)

75 DMSO-d_6: 1.97, 2.07 (s,s, 6H, $2COCH_3$); 5.25, 5.32 (d,d, 2H, CH_2, AB system, J = 18.67 and 25.79); 7.54-7,66 (m, 3H, ArH); 8.01 (s, 1H, triazole $C_{(3)}H$); 8.55 (s, 1H, triazole $C_{(5)}H$); 11,55 (s, 1H, NH)

77 DMSO-d_6: 7.31-7.67 m (6H, NH_2, arom), 8.11 s, 8.45 s (2H, 2 Tr-H).

78 DMSO-d_6: 7.18-7.40 m (6H, NH_2, arom), 8.18 s, 8.56 s (2H, 2 Tr-H).

79 DMSO-d_6: 2.26 s (3H, Me), 7.05 broad s (4H, arom), 7.3 s (2H, NH_2), 8.15 s, 8.47 s (2H, 2 Tr-H)

80 DMSO-d_6: 7.23-7.51 m (5H, NH_2, arom), 7.97 s, 8.51 s (2H, 2 Tr-H)

81 DMSO-d_6: 2.00 s, 2.18 s (6H, 2 Me), 7.20-7.50 m (5H, NH_2, arom)

2.2. Antiviral activity

The synthesized thiazole/triazol/oxindols/thiosemicarbazones were evaluated *in vitro* in parallel cell-based assays for cytotoxicity and antiviral activity (Tables **4**) against viruses representative of two of the three genera of the Flaviviridae family, i.e. Flaviviruses (YFV) and Pestiviruses (BVDV), as Hepaciviruses can hardly be used in routine cell-based assays. Title compounds were also tested against representatives of other virus families. Among $ssRNA^+$ were HIV-1 (Retroviridae), CVB-2 and Polio-1 (Picornaviridae); among $ssRNA^-$ were RSV (Paramyxoviridae) and VSV (Rhabdoviridae); among double-stranded RNA (dsRNA) viruses were Reo-1 (Reoviridae). Two representatives of DNA virus families were also included: HSV-1 (Herpesviridae) and VV (Poxviridae).

Compounds exhibited different cytotoxicity, in particular, oxindoles **4** , **5** , **7** , **8** , **9**, **10**, **11**, **12**, **13**, **58** as well as thiazole/triazole **73** and **75** turned out to be the most cytotoxic for MT-4 cell lines (table 3).

Table 3

Bioactivity synthesized compounds against MT-4, MDBK, BHK-21, Vero-76, HIV-1, BVDV, YFV, CVB-2, Sb-1, VSV, VV, HSV-1 (in vitro)

Comps	MT-4	MDBK	BHK-21	Vero-76	HIV-1	BVDV	YFV	CVB-2	Sb-1	VSV	VV	HSV-1
	CC50 [μM]				EC50 [μM]							
1	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
2	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4	47	>100	>100	>100	>47	>100	>100	>100	>100	>100	>100	>100
5	55	>100	>100	>100	>55	>100	>100	>100	>100	>100	>100	>100
6	>100	93	>100	>100	>100	>93	>100	>100	>100	>100	>100	>100
7	59	>100	>100	>100	>59	>100	>100	>100	>100	>100	>100	>100
8	49	>100	>100	>100	>49	>100	>100	>100	>100	>100	>100	>100
9	33	>100	>100	>100	>33	>100	>100	>100	>100	>100	>100	>100
10	29	>100	>100	>100	>29	>100	>100	>100	>100	>100	>100	>100
11	17	>100	>100	>100	>17	>100	>100	>100	>100	>100	>100	>100
12	19	>100	>100	>80	>19	85	>100	>80	>80	>80	>80	>100
13	45	>18	>19	>40	>45	>18	>19	>40	>40	>40	>40	>19
14	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
15	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
16	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
17	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
18	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
52	>100	>100	>100	>100	>100	6.6	>100	>100	>90	>90	>100	>100
54	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
56	>100	>100	>100	>100	>100	>94	>100	>100	>100	>100	>100	>100
55	>100	>100	>100	>80	>100	>100	>100	>100	>100	>100	>100	>100
57	>100	>100	>100	>100	>100	>100	>100	>80	>80	>80	>80	>100
58	51	>100	>100	>100	>51	22	>100	>100	>100	>100	>100	>100

59	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
61	>100	>100	>100	>90	>100	>100	>100	>90	>90	>90	>90	>100
62	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
63	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
70	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
71	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
72	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
73	≤3.7	16	>100	18	>100	>16	>100	>18	>18	>18	>18	>100
74	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
75	16	55	>100	90	>16	>55	>100	>90	>90	>90	>90	>100
77	>100	>100	>100	>100	>100	>87	>100	>100	>100	>100	>100	>100
78	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
79	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
80	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
81	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

It is worth noting that compounds **11**, **12**, **73**, and **75** are more toxic than reference compound Efavirenz.

As far as the antiviral activity is concerned, none of the title compounds exhibited any activity against Reo-1, Sb-1, VSV, RSV, YFV and VV viruses.

The results obtained against Bovine Viral Diarrhoea Virus (BVDV) showed that nine compounds (six from oxindol's seria **6**, **12**, **13**, **52**, **56**, **58** and three 73, **75, 77** of triazole homologues) resulted moderate active. Among all of them, the most potent compound was **52**, with EC_{50} of 6.6 μM.

Studies of effect of synthesized compounds against Coxsakie Virus (CVB-2) revealed that only two compounds, **13** and **73** exhibit moderate activity (EC_{50} >40 and >18 μM, respectively).

It should be noticed that eleven compounds, **4**, **5**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **58,** and **75** showed moderate activity against HIV-1 (EC_{50} >16 – m >59μM).

3. Conclusions

In the light of the above-mentioned results, we conclude that synthesized compounds in general not so active as antiviral agent. But some of them showed, good activity against viruses containing a single-stranded positive-sense RNA genome ($ssRNA^+$). In particular, in cell-based assays the compounds **11**, **12**, **52**, **57**, **73**, and **75** results the most potent against MT4 cells, BVDV, and HIV-1, respectively.

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5. Experimental methods

Solvents and commercially available reagents (1*H*-indole-2,3-dione **25**, 1-methyl-1*H*-indole-2,3-dione **26**, 1-ethyl-1*H*-indole-2,3-dione **27**, 1-benzyl-1*H*-indole-2,3-dione **46**) were purchased from Aldrich and used without additional purification. 1-Alkylindoline-2,3-diones **28-31**, **33-42** were obtained by a known method [32,33]. Melting points were determined on a Boëtius melting point apparatus (PHMK, VEB Wägetechnik Rapido, Radebeul, Germany) and are uncorrected. ^{1}H - NMR spectra were acquired on a Bruker Avance III 400 spectrometer operating at 400.13 MHz for ^{1}H. Chemical shifts δ are given in ppm referring to the signal center using the solvent peaks for reference: DMSO-d_6 2.50 ppm. IR spectra were acquired on apparatus "Perkin-Elmer Spectrum 100 FTIR". Electron ionisation mass-spectra were recorded on a VG-250 spectrometer (VG Labs., Tritech England) with ionisation energy maintained at 70 eV.

General procedure for synthesis of oxindoles 1-18

A mixture of indoline-2,3-dione (10 mmol), ketone (10 mmol), and Et_2NH (0.73 g, 10 mmol) in 25% aq. ethylene glycol (20 ml) was stirred for 6-8 hours at room temperature (TLC control). The precipitate was filtered off, and washed with 25% aq. ethylene glycol (3x10 ml). The crystalline product was used for further synthesis without purification. For analytical purposes, a sample was recrystallized from a suitable solvent.

3-Hydroxy-3-(2-oxopropyl)indolin-2-one 1. M.p. 168-169°C, Ref. [38] 160-165 °C.

3-Hydroxy-1-methyl-3-(2-oxopropyl)indolin-2-one 2. M.p. 147-148°C, Ref. [38] 140-145 °C.

3-(2-(2,4-Dichlorophenyl)-2-oxoethyl)-3-hydroxy-1-methylindolin-2-one 9. Yield 87%, M.p. 162-164°C. Ref. [27] M.p. 161-164 °C.

3-(2-(2,4-Dichlorophenyl)-2-oxoethyl)-1-ethyl-3-hydroxyindolin-2-one 10. Yield 78 %, M.p. 137-138°C. Ref. [27] M.p. 135-138°C.
1-Butyl-3-(2-(2,4-dichlorophenyl)-2-oxoethyl)-3-hydroxyindolin-2-one 11. Yield 95 %, oil. Cal. C 61.24; H 4.88; N, 3.57. $C_{20}H_{19}Cl_2NO_3$. Find. C 61.29; H 4.60; N, 3.58. Ref. [27] Oil.
3-(2-(2,4-Dichlorophenyl)-2-oxoethyl)-1-hexyl-3-hydroxyindolin-2-one 12. Yield 98 %, oil. Cal. C 62.86; H 5.52; N 3.33. $C_{22}H_{23}Cl_2NO_3$. Find. C 62.77; H 5.52; N 3.26. Ref. [27] Oil.
1-Decyl-3-(2-(2,4-dichlorophenyl)-2-oxoethyl)-3-hydroxyindolin-2-one 13. Yield 99 %, oil. Cal. C 65.54; H 6.56; N 2.94. $C_{26}H_{31}Cl_2NO_3$. Find. C 65.39; H 6.56; N 2.88. Ref. [27] Oil.
2-((1S,3S)-3-(2-(3-Hydroxy-2-oxoindolin-3-yl)acetyl)-2,2-dimethylcyclobutyl)-acetamide 14. Yield 68 %, M.p. 206-207°C. Ref. [28] M.p. 205-207°C.
2-((1S,3S)-3-(2-(3-Hydroxy-2-oxoindolin-3-yl)acetyl)-2,2-dimethylcyclobutyl)-acetonitrile 15. Yield 81 %, M.p. 163-164°C. Ref. [28] M.p. 163-164°C.
Methyl 2-((1S,3S)-3-(2-(3-hydroxy-2-oxoindolin-3-yl)acetyl)-2,2-dimethyl-cyclobutyl)acetate 16. Yield 88 %, M.p. 76-77°C. Ref. [28] M.p. 76°C.
Methyl 2-((1S,3S)-3-(2-(1-ethyl-3-hydroxy-2-oxoindolin-3-yl)acetyl)-2,2-dimethylcyclobutyl)acetate 17. Yield 55 %, M.p. 162-163°C. Ref. [28] M.p. 162-164°C.
Methyl 2-((1S,3S)-3-(2-(1-benzyl-3-hydroxy-2-oxoindolin-3-yl)acetyl)-2,2-dimethylcyclobutyl)acetate 18. Yield 79 %, M.p. 152-153°C. Ref. [28] M.p. 152-153°C.

General procedure for synthesis of thiosemicarbazones 43-53, 70-72

The ketones (1 mmol) in EtOH (10 ml) and thiosemicarbazide (1 mmol) in EtOH (10 ml) were mixed at 50°C. Three drops of 30% HCl were added to the mixed solution following by reflux for 1-6 hours (TLC control). The mixture was cooled and precipitate was filtered and recrystallized using an appropriate solvent.

General procedure for heterocyclization of thiosemicarbazones into 1,3,4-thiadiazolines 73-75

After refluxing during 3-6 hours of thiosemicarbazones **70-72** (10 mmol) in Ac_2O (25 ml) the solution was cooled and after addition of H_2O (5 ml) kept overnight at room temperature. The solid was filtered off, washed with cold H_2O, dried over P_2O_5 and recrystallized from appropriate solvent.
N-{5-[(1*H*-1,2,4-Triazol-1-yl)methyl)-4-acetyl-5-(2,4-dichlorophenyl]-4,5-dihydro-1,3,4-thiadiazol-2-yl} acetamide 73. MS, *m*/*z* (relative intensity %): $[M]^+$ 277 (100), 208 (23), 188 (61), 181 (15), 163 (11), 155 (10), 140 (23), 139 916), 138 (56), 137 (38), 132 (8), 123 (7), 114 (8), 113 (9), 111 (17), 102 (12), 90 (8), 86 (10), 81 (8), 76 (8), 75 (13), 70 (10), 60 (89), 32 (25).
N-{5-[(1*H*-1,2,4-Triazol-1-yl)methyl]-4-acetyl-5-p-tolyl-4,5-dihydro-1,3,4-thiadiazol-2-yl}acetamide 74. MS, *m*/*z* (relative intensity %): $[M]^+$ 269 (16), 259(27), 258 (92), 203 (15), 189 (14), 188 (27), 162 (8), 161 (22), 147 (12), 145 (10), 143 (10), 135 (9), 119 (21), 118 (100), 117 (34), 116 (18), 103 (10), 92 (8), 91 (37), 90 (16), 89 (17), 86 (13), 77 (10), 76 (9), 71 (7), 69 (10), 65 (19), 64 (11), 63 (21), 62 (14), 61 (17), 60 (51), 59 (12), 54 (16), 53 (11), 51 (22), 50 (14), 45 (10), 42 (10), 41 (13).
N-{5-[(1*H*-1,2,4-Triazol-1-yl)methyl]-4-acetyl-5-phenyl-4,5-dihydro-1,3,4-thiadiazol-2-yl}acetamide 75. MS, *m*/*z* (relative intensity %): $[M]^+$315 (9), 314 (19), 313 (47), 312 (28), 311 (66), 279 (8), 278 (54), 277 (12), 276 (100), 275 (12), 224 (15), 222(21), 197 (11), 195 (22), 194 (8), 174 (9), 173 (11), 172 (20), 171 (19), 136 (9), 60 (32), 59 (12).

General procedure for the preparation of 4-aryl-5-(1*H*-1,2,4-triazol-1-yl)-1,3-thiazol-2-amines 77-81

The bromination of ketones **64, 65,** and **67-69** were realized with Br_2 in mixture HBr/AcOH at 10°C. The crude product **76** was used without purification in the next step. Mixture of bromide (0.01 mol) **76** and thiourea (0.01 mol) in acetone (20 ml) was stirred at room temperature for 1 hour and after removal of the solvent, the resulting solid was refluxed in ethanol (50 ml) for additional 2 hours. After evaporation of the EtOH, 5% water solution of $NaHCO_3$ (50 ml) was added and mixture was refluxed 8 hours. The solid was collected by filtration, dried and recrystallized from ethanol.

Biological Assays

Compounds were dissolved in DMSO at 100 mM and then diluted in culture medium.

Cells and Viruses.

Cell lines were purchased from American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Cell lines supporting the multiplication of RNA viruses were the following: $CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4); Madin Darby Bovine Kidney (MDBK); Baby Hamster Kidney (BHK-21) and Monkey Kidney (Vero 76) cells.

Cytotoxicity Assays

For cytotoxicity tests, run in parallel with antiviral assays, MDBK and BHK cells were re-suspended in 96 multi-well plates at an initial density of $6x10^5$ and $1x10^6$ cells/mL, respectively, in maintenance medium, without or with serial dilutions of test compounds. Cell viability was determined after 48-96 hrs at 37 °C in a humidified CO_2 (5%) atmosphere by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [39].

Vero 76 cells were re-suspended in 24 multi-well plates at an initial density of $4x10^5$ cells/mL. The cell number of Vero 76 monolayers was determined by staining with the crystal violet dye.

For cytotoxicity evaluations, exponentially growing cells derived from human haematological tumors [$CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4)] were seeded at an initial density of $1x10^5$ cells/mL in 96 well plates in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 units/mL penicillin G and 100 μg/mL streptomycin. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 hrs at 37 °C by the MTT method.

Antiviral assay

Activity of compounds against Human Immunodeficiency Virus type-1 (HIV-1) was based on inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected with a multiplicity of infection (m.o.i.) of 0.01. Briefly, 50 μL of RPMI containing $1x10^4$ MT-4 were added to each well of flat-bottom microtitre trays containing 50 μL of RPMI, without or with serial dilutions of test compounds. Then, 20 μL of an HIV-1 suspension containing 100 $CCID_{50}$ were added. After a 4-day incubation, cell viability was determined by the MTT method.

Activity of compounds against Yellow Fever Virus (YFV) and Reo Virus type-1 (Reo-1) was based on inhibition of virus-induced cytopathogenicity in acutely infected BHK-21 cells. Activities against Bovine Viral Diarrhoea Virus (BVDV), in infected MDBK cells, were also based on inhibition of virus-induced cytopathogenicity.

BHK and MDBK cells were seeded in 96-well plates at a density of $5x10^4$ and $3x10^4$ cells/well, respectively, and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in a humidified CO_2 (5%) atmosphere. Cell monolayers were then infected with 50 μL of a proper virus dilution (in serum-free medium) to give an m.o.i = 0.01. 1 hr later, 50 μL of MEM Earle's medium, supplemented with inactivated foetal calf serum (FCS), 1% final concentration, without or with serial dilutions of test compounds, were added. After 3-4 days incubation at 37 °C, cell viability was determined by the MTT method.

Activity of compounds against Coxsackie Virus, B-2 strain (CVB-2), Polio Virus type-1 (Polio-1), Sabin strain, and Vesicular Stomatitis Virus (VSV), Vaccinia Virus (VV) and Herpes Virus 1 (HSV-1) and against Respiratory Syncytial Virus (RSV), A-2 strain, in infected Vero 76 cells, was determined by plaque reduction assays in Vero 76 cell monolayers. To this end, Vero 76 cells were seeded in 24-well plates at a density of $2x10^5$ cells/well and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in a humidified CO_2 (5%) atmosphere. Then, monolayers were infected with 250 μL of proper virus dilutions to give 50-100 PFU/well. Following removal of unadsorbed virus, 500 μL of Dulbecco's modified Eagle's medium supplemented with 1% inactivated FCS and 0.75% methyl cellulose, without or with serial dilutions of test compounds, were added. Cultures were incubated at 37 °C for 2 (Sb-1 and VSV), 3 (CVB-2, VV and HSV-1) or 5 days (RSV) and then fixed with PBS containing 50% ethanol and 0.8% crystal violet, washed and air-dried. Plaques were then counted. 50% effective concentrations (EC_{50}) were calculated by linear regression technique.

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