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SYNTHESIS, ANTI-HEPATITIS B AND C VIRUS ACTIVITY AND ANTITUMOR SCREENING OF NOVEL THIAZOLO[4,5-*D*]-PYRIMIDINE DERIVATIVES

LILIANNA BECAN[1*] and ANNA WÓJCICKA[1]

[1]Department of Drugs Technology, Wroclaw Medical University, Borowska 211A, 50-556 Wrocław, Poland

Abstract: The paper describes the synthesis, antivirus and antitumor evaluation of novel thiazolo[4,5-d]pyrimidine derivatives. The target compounds **3a**-**h** were synthesized by cyclocondensation of 4-amino-N'-(phenylmethylidene)-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carbohydrazides **2a**-**d** with aromatic aldehydes. The structures of new compounds were determined by IR, ^{1}H-NMR and elemental analysis. Thiazolopyrimidines **3a** and **3d**-**h** were screened by the National Institute of Allergy and Infectious Diseases against various viruses. Four compounds **3e**-**h** showed *in vitro* anti-HCV activity. One (**3e**) demonstrated significant activity against HBV and was submitted to an anti-HBV *in vivo* assay but had a low bioavailability. As a result of antitumor study, compound **3h** was found to be most potent against leukemia SR.

Keywords: thiazolo[4,5-d]pyrimidines synthesis, anticancer screening, anti-HCV agents, anti-HBV agents

In the present work, novel thiazolo[4,5-*d*]pyrimidine derivatives were synthesized and evaluated for their antivirus and antitumor activity. Thiazolo[4,5-*d*]pyrimidines, 7-thia analogues of purine bases of nucleotides, have acquired a growing importance in medicinal chemistry. Purine antagonists may inhibit normal production of DNA and stop cell replication. Modified nucleotides and nucleosides represent an important class of antiviral drugs such as acyclovir, lamivudine, abacavir, zidovudine, tenofovir. Anti-hepatitis B and C virus activity of synthetic pyrimidine and purine analogues has been reported (1-5). Although thiazolo[4,5-*d*]pyrimidines are known to possess a variety of biological activities including antiviral (6-8), however there are no reports about their anti HBV and HCV bioactivity. Herein, the synthesis of new derivatives of thiazolo[4,5-*d*]pyrimidines as well as their evaluation for anti-virus and anticancer activities are reported. The newly synthesized compounds **3a** and **3d**-**h** were screened *in vitro* for the activity against various viruses: the BK Virus, SARS, Influenza A and B, Measles, Yellow Fever, Dengue, Herpesviruses (EBV, CMV, HHV-6, HHV-8), Hepatitis B and Hepatitis C viruses. The experimental results showed activity of the new thiazolo[4,5-*d*]pyrimidines **3a** and **3d**-**h** only against hepatitis B (HBV) and hepatitis C virus (HCV) replication. They were found to be inactive towards all the other screened viruses. Both hepatitis viruses are one of the common agents of chronic liver diseases (9, 10). The long term consequences of these persistent infections can caused chronic active hepatitis. All current agents have some serious liabilities, thus intense efforts in drug discovery are still needed. Especially, the development of drug-resistance has been observed in the treatment of HBV and HCV infections (11, 12). Furthermore, two of the synthesized compounds **3d** and **3h** were selected by the National Cancer Institute (Bethesda, MD, USA) for a primary *in vitro* antitumor assay and showed high inhibitory effects against leukemia cell lines.

EXPERIMENTAL

Materials and methods

Melting points were measured with a Boethius heating-table microscope apparatus and are uncorrected. Elemental analyses for the synthesized compounds were performed on a Perkin Elmer 2400 (Waltham, MA, USA) analyzer and results are within ±0.4% of the theoretical values. ^{1}H-NMR, spectra were acquired in dimethyl sulfoxide-d_6 on a Bruker ARX 300 MHz (Bruker Analytic,

* Corresponding author: e-mail: lilianna.becan@umed.wroc.pl

Karlsruhe, Germany; Bruker AG, Fallanden, Switzerland) instrument. Tetramethylsilane was used as the internal standard and all chemical shift values were recorded as δ (ppm) values. IR spectra were recorded on a Specord M80 spectrometer using KBr pellets. The course of the reactions and the purity of the obtained compounds were monitored by thin-layer chromatography on Merck silica gel plates (Merck F_{254}, Darmstadt, Germany) using the solvent system dichloromethane: 1-propanol : n-hexane (10 : 1 : 3, v/v/v) for elution. Iodine was used as a visualization agent. The chemicals and reagents for syntheses were obtained from Alfa Aesar (Karlsruhe, Germany), Chempur (Piekary Sl. Poland), and Lancaster (Frankfurt am Main, Germany).

General method for the synthesis of 2-cyano-N'-[(phenyl/substituted phenyl)-methylidene]-acetohydrazides 1a-d

An equimolar (0.1 mol) mixture of cyanoacetic acid hydrazide and the appropriate aromatic aldehyde in 60 mL absolute ethanol was heated under reflux for 6 h. The precipitate formed after cooling was filtered, washed thoroughly with ethanol, dried, and recrystallized (Table 1).

2-Cyano-N'-phenylmethylideneacetohydrazide (1a)

IR (KBr, cm^{-1}): 3400 (NH), 2250 (C≡N) 1690 (C=O), 1570 (NH), 1530 (C=N), 760 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 4.18 (s, 1H, CH_2), 7.27-7.43 (m, 5H, arom.), 8.11 (s, 1H, CH=N), 10.87 (s, 1H, NH). Analysis: calcd. for $C_{10}H_9N_3O$: C, 64.16, H, 4.85, N, 22.45%; found: C, 64.22, H, 4.72, N, 22.68%.

N'-(2-chlorophenyl)methylidene-2-cyanoacetohydrazide (1b)

IR (KBr, cm^{-1}): 3390 (NH), 2260 (C≡N) 1680 (C=O), 1570 (NH), 1530 (C=N), 770 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 4.22 (s, 1H, CH_2), 7.21-7.32 (m, 4H, arom.), 8.08 (s, 1H, CH=N), 11.15 (s, 1H, NH). Analysis: calcd. for $C_{10}H_8ClN_3O$: C, 54.19, H, 3.64, N, 18.96%: found; C, 54.27, H, 3.68, N, 19.10%.

Table 1. Characterization data of compounds **2a-d** and **3a-h**.

Comp.	R^1	R^2	M.p. (°C)	Yield %	Molecular formula	Molecular weight	Solvent
1a	H	-	164-5	92.4	$C_{10}H_9N_3O$	187.20	methanol
1b	2-Cl	-	188-9	87.6	$C_{10}H_8ClN_3O$	221.65	1-propanol
1c	4-Cl	-	210-1	90.0	$C_{10}H_8ClN_3O$	221.65	1-propanol
1d	4-F	-	192-3	89.8	$C_{10}H_8FN_3O$	205.06	ethanol
2a	H	-	230-1	87.6	$C_{17}H_{14}N_4OS_2$	354.45	1-propanol
2b	2-Cl	-	252-3	79.1	$C_{17}H_{13}ClN_4OS_2$	388.89	1-propanol
2c	4-Cl	-	255-6	88.8	$C_{17}H_{13}ClN_4OS_2$	388.89	1-propanol
2d	4-F	-	260-1	76.5	$C_{17}H_{113}FN_4OS_2$	372.44	1-propanol
3a	H	H	351-2	59.3	$C_{24}H_{16}N_4OS_2$	440.54	1,4-dioxane
3b	H	2-Cl	376-7	53.3	$C_{24}H_{15}ClN_4OS_2$	475.01	DMF-1-propanol 3 : 1 (v/v)
3c	4-Cl	H	334-5	47.3	$C_{24}H_{15}ClN_4OS_2$	474.99	DMF-1-propanol 3 : 1 (v/v)
3d	4-F	H	388-90	57.6	$C_{24}H_{15}FN_4OS_2$	458.56	DMF-1-propanol 3 : 1 (v/v)
3e	2-Cl	2-Cl	366-7	54.8	$C_{24}H_{14}Cl_2N_4OS_2$	509.45	DMF-H_2O 9 : 1 (v/v)
3f	4-F	4-F	347-8	59.5	$C_{24}H_{14}F_2N_4OS_2$	476.55	DMF-H_2O 9 : 1 (v/v)
3g	4-F	2-Cl	342-3	52.9	$C_{24}H_{14}ClFN_4OS_2$	493.00	DMF-H_2O 9 : 1 (v/v)
3h	2-Cl	4-F	359-60	58.3	$C_{24}H_{14}ClFN_4OS_2$	493.00	DMF-H_2O 9 : 1 (v/v)

N'-(4-chlorophenyl)methylidene-2-cyanoacetohydrazide (1c)

IR (KBr, cm^{-1}): 3420 (NH), 2220 (C≡N) 1680 (C=O), 1570 (NH), 1540 (C=N), 760 (phenyl). ^{1}H-NMR (DMSO-d_6, δ, ppm): 4.26 (s, 1H, CH_2), 7.11-7.52 (m, 4H, arom.), 8.00 (s, 1H, CH=N), 11.09 (s, 1H, NH). Analysis: calcd. for $C_{10}H_8ClN_3O$: C, 54.19, H, 3.64, N, 18.96%; found: C, 54.36, H, 3.70, N, 18.88%.

2-Cyano-N'-(4-fluorophenyl)methyleneacetohydrazide (1d)

IR (KBr, cm^{-1}): 3400 (NH), 2260 (C≡N) 1680 (C=O), 1570 (NH), 1530 (C=N), 770 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 4.28 (s, 1H, CH_2), 6.97-7.46 (m, 4H, arom.), 8.09 (s, 1H, CH=N), 10.89 (s, 1H, NH). Analysis: calcd. for $C_{10}H_8FN_3O$: C, 58.53, H, 3.93, N, 20.48%; found: C, 58.71, H, 3.98, N, 20.60%.

General method for the synthesis of compounds 2a-d

To a stirred suspension of **1a-d** (0.1 mol), finely divided sulfur (3.2 g, 0.1 mol) and triethylamine (12.5 mL) in ethanol (50 mL), phenyl isothiocyanate (1.35 g, 0.1 mol) was dropped. The mixture was refluxed for 4 h. The solid product formed after cooling was filtered, washed thoroughly with cold ethanol, dried, and recrystallized from proper solvent (Table 1).

4-Amino-3-phenyl-N'-phenylmethylidene-2-thioxo-2,3-dihydrothiazole-5-carbohydrazide (2a) (14)

4-Amino-N'-(2-chlorophenyl)methylidene-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carbohydrazide (2b)

IR (KBr, cm^{-1}): 3450 (NH), 1680 (C=O), 1570 (NH), 1530 (C=N), 1240 (C-S-C), 760 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 7.32 (s, 2H, NH_2), 7.37-8.07 (m, 9H, arom.), 8.44 (s, 1H, CH=N), 11.72 (s, 1H, NH). Analysis: calcd. for $C_{17}H_{13}ClN_4OS_2$: C, 52.50, H, 3.37, N, 14.41%; found: C, 52.44, H, 3.52, N, 14.27%.

4-Amino-N'-(4-chlorophenyl)methylidene-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carbohydrazide (2c) (14)

4-Amino-N'-(4-fluorophenyl)methylidene-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carbohydrazide (2d)

IR (KBr, cm^{-1}): 3450 (NH), 1680 (C=O), 1570 (NH), 1530 (C=N), 1240 (C-S-C), 780 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 7.25 (s, 2H, NH_2), 7.36-7.75 (m, 9H, arom.), 8.12 (s, 1H, CH=N), 11.43 (s, 1H, NH). Analysis: calcd. for $C_{17}H_{13}FN_4OS_2$: C, 54.82, H, 3.52, N, 15.04%; found: C, 54.34, H, 3.50, N, 14.92%.

General method for the synthesis of compounds 3a-h

A mixture of the proper **2a-d** (0.1 mol) and the appropriate aromatic aldehyde (0.2 mol) was heated and stirred under reflux for 5-6 h in the presence of lithium hydroxide monohydrate (4.2 g, 0.1 mol). The resulting solid precipitated by cooling, was filtered, washed thoroughly twice with hot methanol (2 × 50 mL), dried, and purified by recrystallization from proper solvent (Table 1).

6-(Benzylideneamino)-3,5-diphenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one (3a)

IR (KBr, cm^{-1}): 1690 (C=O), 1640, 1490 (C=N), 1260 (N-C=S), 1040 (C-S-C), 760 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 7.25-7.88 (m, 15H, arom.), 10.79 (s, 1H, CH=N). Analysis: calcd. for $C_{24}H_{16}N_4OS_2$: C, 65.43, H, 3.66, N, 12.72%; found: C, 65.34, H, 3.62, N, 12.78%.

6-(Benzylideneamino)-5-(2-chlorophenyl)-3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one (3b)

IR (KBr, cm^{-1}): 1680 (C=O), 1640, 1480 (C=N), 1240 (N-C=S), 1030 (C-S-C), 780 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 7.30-8.18 (m, 14H, arom.), 9.24 (s, 1H, CH=N). Analysis: calcd. for $C_{24}H_{15}ClN_4OS_2$: C, 60.69, H, 3.18, N, 11.80%; found: C, 60.77, H, 3.36, N, 11.58%.

6-(Benzylideneamino)-5-(4-chlorophenyl)-3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one (3c)

IR (KBr, cm^{-1}): 1670 (C=O), 1630, 1480 (C=N), 1260 (N-C=S), 1050 (C-S-C), 760 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 7.32-7.89 (m, 14H, arom.), 9.52 (s, 1H, CH=N). Analysis: calcd. for $C_{24}H_{15}ClN_4OS_2$: C, 60.69, H, 3.18, N, 11.80%; found: C, 60.80, H, 3.32, N, 11.63%.

6-[(4-Fluorophenyl)methyleneamino]-3,5-diphenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one (3d)

IR (KBr, cm^{-1}): 1680 (C=O), 1640, 1490 (C=N), 1230 (N-C=S), 1040 (C-S-C), 770 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 7.20-7.89 (m, 14H, arom.), 9.37 (s, 1H, CH=N). Analysis: calcd. for $C_{24}H_{15}FN_4OS_2$: C, 62.87, H, 3.30, N, 12.22%; found: C, 62.66, H, 3.22, N, 11.99%.

5-(2-Chlorophenyl)-6-[(2-chlorophenyl)methyleneamino]-3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one (3e)

IR (KBr, cm^{-1}): 1680 (C=O), 1650, 1470 (C=N), 1250 (N-C=S), 1020 (C-S-C), 760 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 7.32-7.89 (m, 13H, arom.), 9.52 (s, 1H, CH=N). Analysis: calcd. for $C_{24}H_{14}Cl_2N_4OS_2$: C, 56.58, H, 3.14, N, 11.00%; found: C, 56.82, H, 3.00, N, 11.29%.

5-(4-Fluorophenyl)-6-[(4-fluorophenyl)methyleneamino]-3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one (3f)

IR (KBr, cm^{-1}): 1690 (C=O), 1640, 1490 (C=N), 1260 (N-C=S), 1040 (C-S-C), 780 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 7.34-7.82 (m, 13H, arom.), 9.54 (s, 1H, CH=N). Analysis: calcd. for $C_{24}H_{14}F_2N_4OS_2$: C, 60.49, H, 2.96, N, 11.76%; found: C, 60.66, H, 3.04, N, 11.54%.

5-(2-Chlorophenyl)-6-[(4-fluorophenyl)methyleneamino]-3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one (3g)

IR (KBr, cm^{-1}): 1680 (C=O), 1660, 1490 (C=N), 1240 (N-C=S), 1050 (C-S-C), 760 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 7.32-8.14 (m, 13H, arom.), 9.26 (s, 1H, CH=N). Analysis: calcd. for $C_{24}H_{14}ClFN_4OS_2$: C, 58.47, H, 2.86, N, 11.37%; found: C, 58.82, H, 2.99, N, 11.29%.

6-[(2-Chlorophenyl)methyleneamino]-5-(4-fluorophenyl)-3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one (3h)

IR (KBr, cm^{-1}): 1690 (C=O), 1650, 1480 (C=N), 1250 (N-C=S), 1040 (C-S-C), 770 (phenyl). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 7.23-7.88 (m, 13H, arom.), 9.56 (s, 1H, CH=N). Analysis: calcd. for $C_{24}H_{14}ClFN_4OS_2$: C, 58.47, H, 2.86, N, 11.37%; found: C, 58.76, H, 3.06, N, 11.51%.

Antiviral activity assays

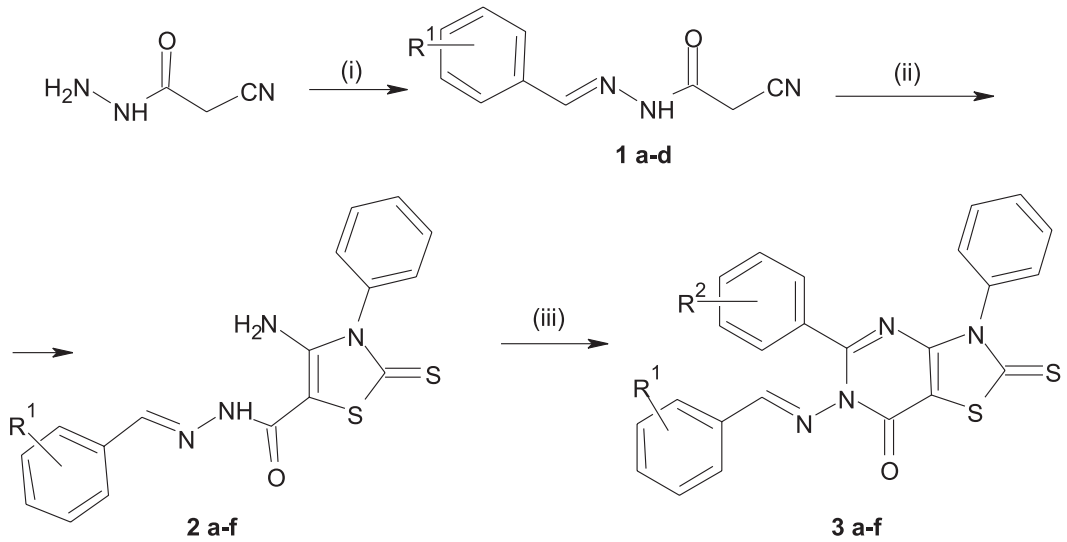
Anti-HBV *in vitro* testing

Compounds **3a** and **3d**-**h** were tested in the primary antiviral evaluations in cultured HepG2 cell line 2.2.15 for the inhibition of replication of hepatitis B virus (HBV) (17). HepG2 2.2.15 is a stable cell line containing HBV ayw strain genome. It was measured whether the compounds will reduce the production of secreted HBV from cells using real time quantitative PCR assay. Toxicity studies were conducted to determine cellular sensitivities CC_{50}. The cytotoxic activity of a test compounds is a significant aspect of understanding the compound's antiviral activity, because virus replication depends on the cell. EC_{50} and EC_{90} values are compound concentrations which give 50% and 90% inhibition of viral replication, respectively. The activity of compounds is confirmed in secondary antiviral evaluations using a different HepG2 stable cell containing an HBV adr1 strain genome. In addition, intracellular HBV DNA replication was measured by quantitative Southern blot hybridization analysis (17). EC_{50}, EC_{90} and SI values were calculated for both extracellular virion DNA and intracellular HBV DNA replication intermediates. Compounds with desirable therapeutic index SI = CC_{50} /EC_{90} are candidates for further *in vivo* studies in animal models (Table 2). Compound **3e** exhibited a good toxicity/activity profile against HBV by inhibition of the synthesis of extracellular virion release EC_{50} = 1.4 μM, EC_{90} = 3.6 μM, CC_{50} = 148 μM, SI = 41 and intracellular HBV replication intermediates EC_{50} =

Table 2. Anti-HBV activity and cytotoxicity of compounds **3a**-**h**.

Comp.	Assay/level	Antiviral activity (μM)		Toxicity (μM) CC_{50}	Selectivity index SI
		EC_{50}	EC_{90}		
3a	Virion/primary	> 10	> 10	> 100	
3d	Virion/primary	> 10	> 10	> 100	
3e	Virion/primary Virion/secondary RI/secondary	1 1.4 3.5	3.6 3.6 15	> 100 148 148	> 28 41 10
3f	Virion/primary	> 10	> 10	> 100	
3g	Virion/primary	> 10	> 10	> 100	
3h	Virion/primary	> 10	> 10	> 100	

Virion - data are based on extracellular HBV virion DNA. RI - data are based on intracellular HBV DNA replication intermediates. SI - are calculated as CC_{50}/EC_{90} ratio.



Scheme 1. Reagent and reaction conditions: (i) aromatic aldehydes, EtOH, reflux 6 h; (ii) H_5C_6NCS, S, TEA, EtOH, reflux 4 h; (iii) aromatic aldehydes, $LiOH{\cdot}H_2O$, reflux 6 h

3.5 μM, EC_{90} = 15 μM, CC_{50} = 148 μM, SI = 10. SI (selectivity index) was calculated as CC_{50} (toxicity) and EC_{90} (activity) ratio.

Anti-HBV *in vivo* testing

As a result of *in vitro* screens, compound **3e** was tested *in vivo* in the chimeric mouse model suffering from a transgene-induced liver disease (18). The compound was diluted in DMSO, then 10% cremaphor ELP and saline at ratio 1 : 8 : 8, respectively. The compound was prepared at concentrations of 10, 3.2, 1.0, and 0.32 mg/mL and dosages of 100, 32, 10, 3.2 mg/kg/day. The vehicle group received the DMSO, cremaphor, and saline solution (placebo) in the same ratio as the treatment groups. Homozygous transgenic HBV mice were administered *i.p.* the test agents or placebo twice daily until day 14. At least 3 h after the last treatment on day 14 mice were necropsied to obtain whole blood, serum and liver samples for complete blood cell count, serum chemistry and liver HBV DNA. Compound **3e** did not produce any signs of toxicity as determined as weight change, blood chemistry, or blood cells. Liver HBV DNA appeared to be lowered in the group receiving the highest dose of compound **3e**, but not to the level of Advantan (ADV) (Fig. 1). Based on histological report, it appears that most of the investigated compound precipitated out of solution when injected *i.p.* and that it probably did not have good tissue distribution. The phenomenon is probably unique to the compound's insolubility properties *in vivo*.

Anti-HCV *in vitro* testing

Anti-HCV activity and toxicity were assessed by the primary HCV RNA replicon assay (19) The assay was performed using Huh 7 ET cell line which contains the HCV RNA replicon with stable luciferase (LUC) reporter. HCV RNA-derived LUC activity is used as a measure of HCV RNA levels. Human interferon a-2b was included as positive control compound. Four compounds **3e-h** showed antiviral activity; they inhibited HCV replication in 48-68%. Compound cytotoxicity was evaluated as the percent viable cells comparative to cell control. In further testing, **3e** and **3h** exhibited 50% effective concentration EC_{50} = 0.41 μM but their activity has not been confirmed in HCV RNA replicon confirmatory assay (Table 3).

Anticancer activity assay

All synthesized compounds were submitted for testing at the National Cancer Institute (Bethesda, MD, USA), to evaluate the cytotoxic effect. Only two compounds **3d** and **3h** were selected for *in vitro* antitumor assay against 60 different human tumor cell lines representing leukemia, melanoma, and cancers of the lung, colon, brain, breast, ovary, prostate, and kidney. The tumor cells were cultured in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. The tumor cells were plated in standard 96-well microtiter plates in 100 μL of medium. Density of the inoculum depended on the type of tumor cells and their growth charac-

Table 3. Anti-HCV activity and cytotoxicity for compounds **3a-h**, single dose 20 μM.

Comp.	Activity (% inhibition virus control)	Cytotoxity (%cell control)	SI	Confirmatory dose-response assay						RNA-based confirmatory assay		
				IC_{50}	IC_{90}	EC_{50}	EC_{90}	SI_{50}	SI_{90}	IC_{50}	EC_{50}	SI_{50}
3a	19.5	98.8	< 1									
3d	34.6	118.1	< 1									
3e	63.1	34	< 1	11.25	> 20	0.41	> 20	27.43	1	8.25	> 20	0.41
3f	48.7	42.9	< 1	> 20	> 20	4.53	> 20	> 4.42	1			
3g	68.1	101.8	> 1	> 20	> 20	> 20	> 20	1	1			
3h	58.7	24.6	< 1	> 20	> 20	0.41	> 20	> 48.8	1	11.05	> 20	0.55
rINFa-2b	97.3	108.2	> 1	> 2	> 2	0.09	0.48	> 22.2	> 4.17	> 2	0.11	> 18.2

n/s = compound not screened; IC_{50} = the compound's 50% inhibitory concentration against virus RNA replication; IC_{90} = the compound's 90% inhibitory concentration against virus RNA replication; EC_{50} = effective concentration which reduces viral replication by 50%; EC_{90} = effective concentration which reduces viral replication by 90%; SI_{50} = selectivity index calculated as IC_{50}/EC_{50}; SI_{90} = selectivity index calculated as IC_{90}/EC_{90}.

teristics (5,000 to 40,000 cells/well). The cells cultures were incubated at 37°C on the microtiter plate for 24 h before adding the compounds at 10^{-5} molar (M) concentration. After 48 h incubation under the same conditions with the tested agent, the sulforhodamine B (SRB) protein assay was used to estimate cell viability and growth The details of used technique has been previously described (20). The one dose data were reported as a mean graph of the percent growth of the treated cells. Both compounds **3d** and **3h** exhibited a noticeable antiproliferative effect against leukemia K-562 cell line, resulting in 68.58% and 70.14% growth inhibition, respectively. Compound **3h** proved to be very sensitive towards leukemia SR, caused 88.19% growth inhibition of tumor cell line.

RESULTS AND DISCUSSION

Chemistry

Novel thiazolo[4,5-*d*]pyrimidine derivatives **3a**-**d** were synthesised as presented in Scheme 1. At first the starting 2-cyano-*N*'-[(phenyl/substituted phenyl)methylidene]acetohydrazides **1a**-**d** were formed, with excellent yield, through the reaction of 2-cyanoacetic acid hydrazide with various aromatic aldehydes, followed by the elimination of water, based on the reported procedures (13, 14). The 2-cyano-acetohydrazides **1a**-**d** were subsequently cyclized with sulfur and phenyl isothiocyanate in the presence of basic catalyst to the 4-amino-*N'*-[(phenyl/substituted phenyl)methylidene]-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carbohydrazides **2a**-**d**, by using the method of Gewald (15) and Rida et al. (14). The required 5-substituted thiazolo[4,5-*d*]pyrimidines **3a**-**h** were achieved by heating the former in the twofold excess of appropriate substituted aromatic aldehydes under base-catalyzed conditions, according to the known procedure (16). These compounds were formed by a pyrimidine ring closure reaction between the 6-substituted hydrazide moiety and the primary 5-amino group. The yields of the newly synthesized thiazolo[4,5-*d*]pyrimidines **3a**-**h** ranged from 47 to 59% (Table 1). Their structures were established on the basis of spectroscopic data and elemental analysis. Absorption peaks in IR spectrum were found at 1690 (C=O), 1640, 1490 (C=N), 1260 (N-C=S), 1040 (C-S-C), 760 (phenyl) cm^{-1} for **3a** and similarly for others, respectively. In the ^{1}HNMR spectra of **3a**-**h** derivatives, besides aromatic protons multiplet signal ranged between 7.20-8.18 ppm, characteristic one proton singlet of 6 CH=N resonated between 9.26 and 10.79 ppm.

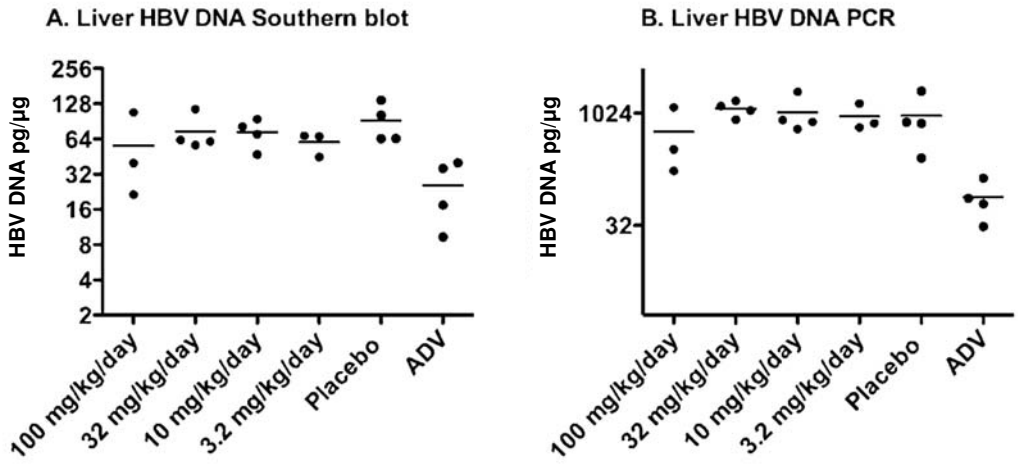


Figure 1. Effect of compound **3e** administered *i.p.* mg/kg/day for 14 days on liver HBV DNA in HBV transgenic mice (obtained from the Institute for Antiviral Research, Utah State University (Logan, UT, USA)

Biology

Compounds **3a** and **3d**-**h** were screened *in vitro* against various viruses (the BK Virus, SARS, Influenza A and B, Measles, Yellow Fever, Dengue, Herpesviruses, Hepatitis B and Hepatitis C) through contractual arrangements with the National Institute of Allergy and Infectious Diseases (Bethesda, MD, USA). Compound **3e** exhibited promising toxicity/activity profile against HBV and as a result of *in vitro* screens was tested *in vivo* in the transgenic HBV mice. Unfortunately, most of examined compound **3e** appeared to precipitate in the peritoneal cavity as suggested by the pathology report and showed no activity *in vivo*. The compounds **3e**-**h** were found to be active *in vitro* against HCV, they inhibited HCV replication in 48-68%. In further testing, **3e** and **3h** exhibited 50% effective concentration EC_{50} = 0.41 µM but their activity has not been confirmed in HCV RNA replicon confirmatory assay EC_{50} > 20 µM. Summing up, only compound **3e** exhibited *in vitro* activity against both hepatitis viruses HBV and HCV. The biological screening results for anti-HBV and anti-HCV activities of compounds **3a** and **3d**-**h** are presented in Tables 2, 3 and Figure 1. Two compounds **3d** and **3h** were selected by the National Cancer Institute (Bethesda, MD, USA) for *in vitro* antitumor assay against 60 different human tumor cell lines. Compound **3d** exhibited significant antiproliferative effect against leukemia K-562 cell line, resulting in 68.58% growth inhibition. Compound **3h** proved to be very sensitive towards leukemia SR and K-562 cell lines, caused 88.19% and 70.14% growth inhibition, respectively. The other tumor cell lines were slightly inhibited by these compounds, values below 20%.

CONCLUSION

The novel 3-aryl-6-(arylideneamino)-3-phenyl-2-thioxo-2,3-dihydrothiazolo[4,5-*d*]pyrimidin-7(6H)-ones **3a**-**h** were synthesized. Compounds **3a** and **3d**-**h** were evaluated for their *in vitro* antivirus activity, **3d** and **3h** were also screened for *in vitro* antitumor NCI assay. Four of six tested compounds, **3e**-**h** exhibited *in vitro* activity against HCV replication. Compound **3e** demonstrated significant *in vitro* activity against HBV and was submitted to an anti-HBV *in vivo* assay but had a low bioavailability. These results indicate that further structural modification of screened compounds may be of value in search for new antivirus agents. In antitumor screening compound **3h** was found to be the most potent against leukemia SR.

In conclusion, the presence of electron-withdrawing groups (F or Cl) in the phenyl rings increased biological activity. Devoid of these elements derivative **3a** was inactive.

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screening program, in cooperation with the Institute for Antiviral Research, Utah State University (Logan, UT, USA), wherein compound **3e** was tested in the HBV transgenic mouse model. We also thank the staff of the National Institutes of Cancer of the NIH (Bethesda, MD, USA), for *in vitro* evaluation of anticancer activity.

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