Synthesis and Biological Activity Evaluation of Schiff Bases of 5-Acyl-1,2,4-Triazine

[1] Mariusz Mojzych and [2]Marek Sebela
[1] *Department of Chemistry, Siedlce University of Natural Sciences and Humanities, 3 Maja 54, 08-110 Siedlce, Poland.*
[2]*Department of Biochemistry, Faculty of Science, Polacky University, Slechtitelu 11, CZ-783 71, Olomouc, Czech Republik.*
mojzych@uph.edu.pl

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Summary: A simple and general method has been developed for the synthesis of various Schiff bases (oximes, hydrazones, semicarbazones and thiosemicarbazones) derived from 5-acyl-1,2,4-triazines. Some of the new synthesized Schiff bases were tested for biological activity but only oximes **2a-c** shown poor antiviral activity. The oxime derivatives of 5-acyl-3-methylsulfanyl-1,2,4-triazine were tested with pea-seedling diamine oxidase as the enzyme is known to be inhibited by oxime compounds. However, only weak non-competitive inhibitory effects were observed (K_i of 10^{-2} M).

Keywords: Oximes of 5-acyl-1,2,4-triazines, Hydrazones of 5-acyl-1,2,4-triazines, Thiosemicarbazones and Semicarbazones of 5-acyl-1,2,4-triazines, Antiviral activity, Schiff base.

Introduction

1,2,4-Triazine derivatives constitute an important class of heterocyclic compounds. Both naturally occurring and synthesized derivatives of this ring system occupy an important position in medicinal chemistry and agro-chemistry due to a wide range of biological activities [1]. Furthermore, 1,2,4-triazine derivatives have been shown to be intermediates for the synthesis of other nitrogen-containing heterocycles, *via* electron demand Diels-Alder reactions with rich dienophiles [2], or nucleophilic substitution [3]. It is worthy of mentioning that the synthesis of poly-substituted or fused 1,2,4-triazines has been an active research area for many years in our laboratory [4-6]. Recently we reported that the reaction of 1,2,4-triazines with alkyl nitronate anions has considerable synthetic utility and allows a highly efficient entry into oximes of alkyl (1,2,4-triazyn-5-yl)ketones [7]. The latter may serve as common intermediates for construction of 5-acyl-1,2,4-triazines [8], chiral 1,2,4-triazine alcohols [9], 2-acylpyridines [10], pyrazolo[4,3-*e*][1,2,4]triazines [11], and 3-acyl-5,6,7,8-tetrahydroisoquinolines, the valuable precursor for the synthesis of sempervirine, and its analogues [12], possessing interesting pharmacological activity. 5-Acyl-1,2,4-triazines are useful building blocks for the preparation of Schiff bases *e.g.* semicarbazides, thiosemicarbazides and hydrazones are intermediates for the construction of pyrazolo[4,3-*e*][1,2,4]triazines [13].

Numerous heterocyclic Schiff bases exhibit a broad spectrum biological activity [14] including bactericidal, fungicidal, antipyretic, antitumour, antitubercular, anticancer and sterease inhibitory activities. Furthermore, some of the Schiff bases have shown antiviral, antimicrobial and anti-inflammatory activities. In analytical chemistry, Schiff bases find applications as chelating agents and analytical reagents for transition metal analysis. As a result of these useful properties, a large number of Schiff bases with 1,2,4-triazine core have been developed. Considering these applications it was planed to evaluate Schiff bases derived from 5-acyl-1,2,4-triazines with the hope to find new biologically active compounds.

In the present work, we report synthesis and antiviral evaluation of new Schiff bases containing 1,2,4-triazine moiety. As inhibitory properties towards pea seedling amine oxidase of various oxime compounds have been reported in the literature [15], two selected oxime derivatives of 5-acyl-3-methylsulfanyl-1,2,4-triazine were analyzed toward this end.

Experimental

Melting points were determined in open capillaries and are uncorrected. ^{1}H-NM spectra were recorded on a Varian Gemini 200MHz spectrometer using tetramethylsilane as the internal standard. IR spectra were measured with a Magna IR-760 spectrometer in KBr pellets. Mass spectra were acquired using an AMD 604 spectrometer (electron impact, 70eV). Elemental analyses were obtained on a Perkin-Elmer 2400-CHN analyzer and the results

*To whom all correspondence should be addressed.

for the indicated elements were within 0.3 % of the calculated values. Compounds **2a-e**, **4a-e**, **5**, **6-36** and **37-41** was synthesized according to literature procedures [8, 9, 11, 13, 16-19].

General method for the synthesis of oxime dimers (**3ab)**.

To a solution of oxime of 5-acyl-3-methylsulfamyl-1,2,4-triazine (1.84g, 10 mmol) in anhydrous DMF (20 mL), potassium hydroxide (0.6 g, 11 mmol) and appropriate dihalogeno compound (5 mmol) were added. The reaction mixture was stirred at rt for 2 h. Then the reaction mixture was poured into ice/water and the formed precipitate was collected by filtration, washed with cold water and recrystallized from aqueous ethanol to yield the corresponding dimer.

(1Z,1'Z)-1-(3-(methylthio)-1,2,4-triazin-5-yl)ethanone O-2-(1-(3-(methylthio)-1,2,4-triazin-5-yl)ethylidene-aminooxy)ethyl oxime **(3a)**: Yield: 95%; m.p.: 167-169^{o}C; ^{1}H-NMR ($CDCl_3$) δ: 2.25 (s, 6H), 2.67 (s, 6H), 4.63 (s, 4H), 9.41 (s, 2H); ^{13}C-NMR ($CDCl_3$) δ: 9.90, 14.03, 141.68, 152.11, 153.85, 173.55; Anal. Calcd. for $C_{14}H_{18}N_8O_2S_2$: C, 42.63; H, 4.60; N, 28.41. Found: C, 42.43; H, 4.63; N, 28.27.

(1Z,1'Z)-1-(3-(methylthio)-1,2,4-triazin-5-yl)ethanone O-5-(1-(3-(methylthio)-1,2,4-triazin-5-yl)ethylidene-aminooxy)pentyl oxime **(3b)**: Yield: 90%; m.p.: 110-112^{o}C; ^{1}H-NMR (DMSO-d_6) δ: 1.51-1.56 (m, 2H), 1.78-1.85 (m, 4H), 2.19 (s, 6H), 2.66 (s, 6H), 4.34-4.38 (t, J = 6.4 Hz, 4H), 9.38 (s, 2H); ^{13}C-NMR (DMSO-d_6) δ: 9.50, 13.84, 22.23, 28.82, 75.63, 141.53, 152.16, 152.49, 173.13; Anal. Calcd. for $C_{17}H_{24}N_8O_2S_2$: C, 46.77; H, 5.54; N, 25.67. Found: C, 46.57; H, 5.57; N, 25.48.

Results and Discussion

Chemistry

The route used for the synthesis of Schiff bases assessed in this study is outlined in Scheme 1. The key intermediates for the presented synthesis are oximes **2a-e** prepared, in good yield, by nucleophilic substitution reaction of hydrogen with nitronate anions under basic condition according to published procedure [7]. The oximes **2a-e** occurred to be useful intermediates for the synthesis of ketones **4a-e** [8] and dimers **3ab**. In order to obtain the desired bis-oxime ethers **3ab**, the oxime **2a** was allowed to react with appropriate dihalogeno alkyl compounds, in a molar ratio of 2:1, in DMF in the presence of potassium carbonate at room temperature. The reaction of ketone **4a** (R^1 = SCH_3, R^2 = CH_3) with hydrazine hydrochloride in ethanol produced a derivative (**5**) [16, 20]. Condensation of 5-acetyl-1,2,4-triazines **4a-e** with phenylhydrazine derivatives in ethyl alcohol containing catalytic amount of hydrochloric acid formed appropriate arylhydrazone of 5-acyl-1,2,4-triazine **6-36** in excellent yield [13, 17, 18, 21]. In an attempt to prepare the thio- and semi-carbazide derivatives **37-41** the carbonyl compounds **4a** and **4d** were treated with thio- and semi- carbazide in ethanol under acidic conditions [18].

The structures of the evaluated compounds were established by spectroscopic methods ^{1}H-NMR, ^{13}C-NMR, MS and elemental analysis [8, 9, 11, 13, 16-19] and some of them were subjected to x-ray analysis and the results were published elsewhere [20, 21].

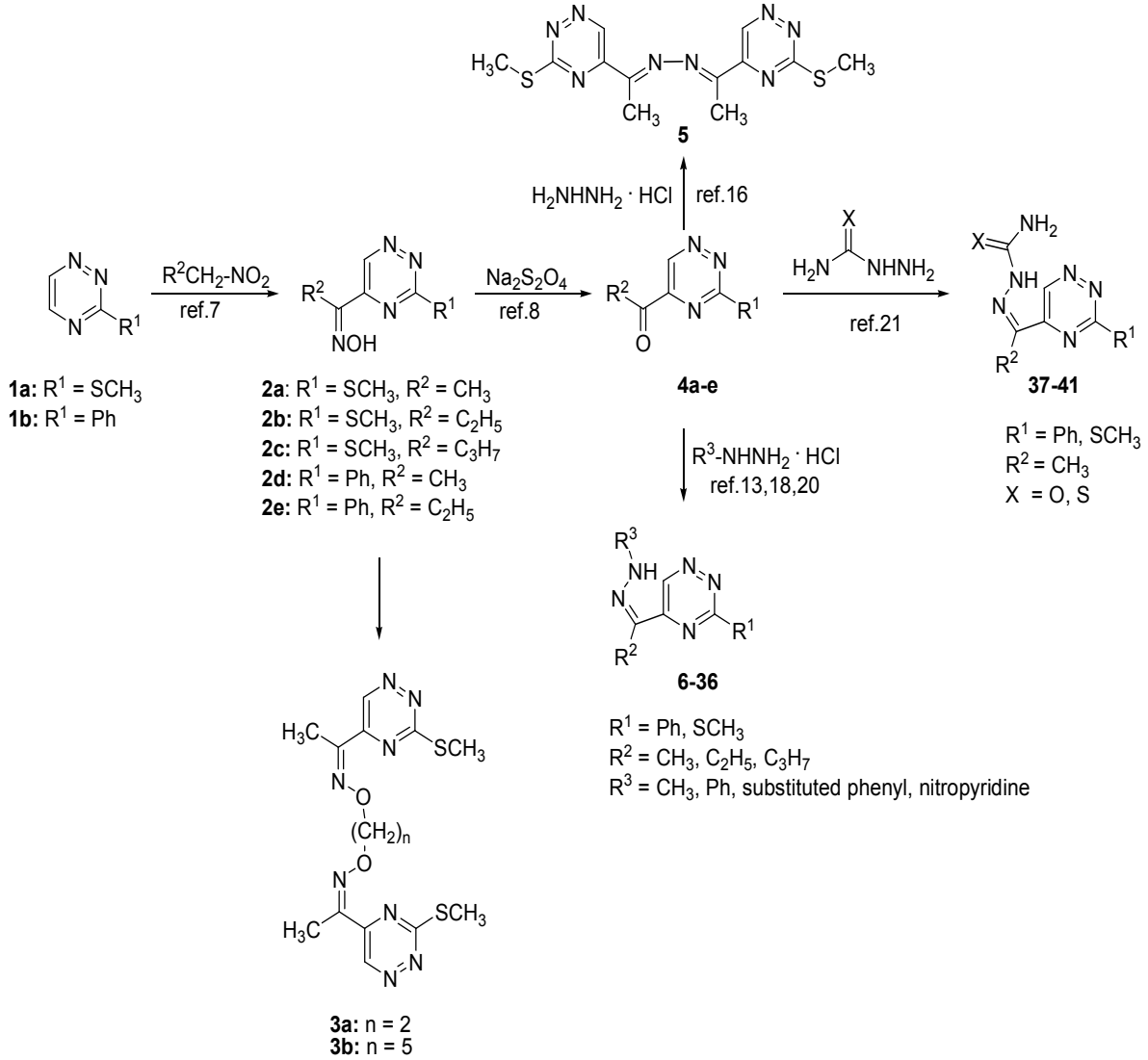
Biological Activity

Pea diamine oxidase was isolated from 7-day-old etiolated seedlings following a published procedure [22]. The final enzyme preparation showed a specific activity of 42 Umg^{-1} determined by the guaiacol spectrophotometric method with putrescine as a substrate [23]. Kinetic parameters of inhibitors were characterized by measuring Lineweaver-Burk plots, stock solutions of the measured compounds were made in methanol.

The synthesized Schiff bases were evaluated for activity against several RNA- and DNA-viruses, using the following cell-based-assays: (a) Vero cells infected with parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B4 virus, or Punta Toro virus; (b) human embryonic lung (HEL) fibroblasts infected with herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), acyclovir-resistant herpes simplex virus-1 (KOS ACV^{r} TK^{-}), vaccinia virus or vesicular stomatitis virus; (c) human epithelial (HeLa) cells infected with vesicular stomatitis virus, coxsackie B4 virus or respiratory syncytial virus and (d) Madin Darby canine kidney (MDCK) cells infected with influenza virus, subtype A/H1N1, A/H3N2 or B. Antiviral activity of tested compounds in HEL, Vero and HeLa cell cultures are presented in Table-2. Against MDCK cell culture none of the tested compounds were active. As a results of broad spectrum antiviral screening of the derivatives, which had minimal antivirally effective concentration less than one-fifth of minimal cytotoxic concentration, were considered active. Compounds **2b** and **2c** emerged as the best antiviral agents among the tested Schiff bases against Coxsackie Virus B4 (EC_{50}=2 μg/mL for **2b** and EC_{50}=9 μg/mL for **2c**, Table-2) and Punto Toro virus (EC_{50}=59 μg/mL for **2b** and

EC_{50}=12 μg/mL for **2c**, Table-2) in Vero cell culture, Vesicular Stomatitis virus (EC_{50}=50 μg/mL for **2b** and EC_{50}=9 μg/mL for **2c**, Table-2) and Coxsackie Virus B4 (EC_{50}=2 μg/mL for **2b** and EC_{50}=12 μg/mL for **2c**, Table 2) in HeLa cell culture. Against viruses in HEL cell culture, no activity was observed with any of the screened derivatives, except of oxime **2b** (Table 2) which was much less active than the reference compounds: brivudin, cidofovir and ganciclovir. Further, oxime **2a** emerged as promising antiviral agent against both Coxsackie Virus B4 in Vero cell culture and Coxsackie Virus B4 in HeLa cell culture with an EC_{50} value of 20 and 45μg/mL, respectively. It would be worthwhile to design several analogues of the evaluated oximes (**2a-c**) to optimize them antiviral potency and this will be the subject of our future research.

Various oximes have been shown to inhibit copper-containing amine oxidases due to their interaction with the enzyme-bound cupric ions [15]. For that reason, we performed also an enzymological experiment as a part of this study. Two oxime compounds, 1-(3-methylsulfanyl-[1,2,4]triazin-5-yl)-ethanone oxime (**2a**) and 1-(3-methylsulfanyl-[1,2,4]triazin-5-yl)-propan-1-one oxime (**2b**) displayed only a very weak effect on pea diamine oxidase. They were found to be non-competitive inhibitors characterized by high K_i values of 14 and 16 mM, respectively. Thus, their inhibitory properties can be considered negligible, which becomes apparent namely in comparison with the behaviour of previously studied aliphatic, alicyclic and aromatic oximes [15].



Scheme-1: Synthetic route of the screened compounds.

Table-1: The minimum cytotoxic concentration of Schiff bases.

Compounds	R^1	R^2	R^3	X	Minimum cytotoxic concentration (μg/mL)[a]		
					HEL	Vero	HeLa
2a	SCH_3	CH_3			>100	>100	>100
2b	SCH_3	C_2H_5			≥100	≥100	>100
2c	SCH_3	C_3H_7			20	100	100
3a	SCH_3	CH_3			100	100	100
3b	SCH_3	CH_3			20	100	100
5	SCH_3	CH_3			100	100	≥20
6	SCH_3	CH_3	CH_3		100	100	100
7	OCH_3	CH_3	Ph		100	100	≥20
8	SCH_3	CH_3	*p*-CH_3-Ph		≥0.8	100	100
9	SCH_3	CH_3	*p*-OCH_3-Ph		20	100	100
10	SCH_3	CH_3	*m*-Cl-Ph		100	100	100
11	SCH_3	CH_3	*o,p*-diCl-Ph		≥20	100	100
12	SCH_3	CH_3	*p*-NO_2-Ph		100	100	100
13	SCH_3	CH_3	*o,p*-diNO_2-Ph		100	100	100
14	SCH_3	C_2H_5	Ph		100	100	100
15	SCH_3	C_2H_5	*o*-CH_3-Ph		100	20	100
16	SCH_3	C_2H_5	*m*-CH_3-Ph		20	20	20
17	SCH_3	C_2H_5	*p*-CH_3-Ph		20	100	100
18	SCH_3	C_2H_5	*p*-OCH_3-Ph		100	100	100
19	SCH_3	C_2H_5	*p*-Cl-Ph		100	100	100
20	SCH_3	C_2H_5	*m*-Cl-Ph		≥20	100	≥20
21	SCH_3	C_2H_5	*o,p*-diCl-Ph		100	≥100	100
22	SCH_3	C_3H_7	*p*-CH_3-Ph		100	100	100
23	SCH_3	C_3H_7	*p*-OCH_3-Ph		100	100	≥20
24	SCH_3	C_3H_7	*p*-Cl-Ph		100	100	100
25	SCH_3	C_3H_7	*m*-Cl-Ph		100	20	≥20
26	SCH_3	C_3H_7	*p*-NO_2-Ph		100	100	100
27	Ph	CH_3	*p*-CH_3-Ph		≥100	100	≥100
28	Ph	CH_3	*m*-CH_3-Ph		100	>100	100
29	Ph	CH_3	*p*-OCH_3-Ph		100	100	100
30	Ph	CH_3	*m*-Cl-Ph		100	100	100
31	Ph	CH_3	*o,p*-diNO_2-Ph		100	100	100
32	Ph	CH_3	*o,p*-diCl-Ph		100	100	100
33	Ph	C_2H_5	*o,p*-diCl-Ph		100	100	100
34	Ph	CH_3	5-NO_2-2-pyridyl		≥20	20	≥20
35	Ph	CH_3	3-NO_2-4-pyridyl		>100	>100	>100
36	Ph	CH_3	3-NO_2-2-pyridyl		>100	>100	>100
37	SCH_3	CH_3		S	≥20	100	≥20
38	SCH_3	CH_3		O	100	100	100
39	Ph	CH_3		O	≥100	>100	≥100
40	SCH_3	CH_3		S	100	100	100
41	SCH_3	CH_3		O	>100	>100	>100
DS-5000					-	>100	>100
(S)-DHPA					-	>250	>250
Ribavirin					>250	>250	>250
Brivudin					>250	-	-
Cidofovir					>250	-	-
Ganciclovir					>100	-	-

[a]Required to cause a microscopically detectable alteration of normal cell morphology.

Table-2: Antiviral activity of Schiff bases in HEL, Vero and HeLa cell cultures.

Compounds	EC_{50}[a] (μg/mL)												
	HEL							Vero			HeLa		
	Herpes Simplex Virus-1(KOS)	Herpes Simplex virus-2 (G)	Vaccinia virus	Vesicular Stomatitis virus	Herpes Simplex virus-1 KOS ACV^r(TK)	Para-Influenza-3 virus	Reovirus- 1	Sindbis virus	Coxsackie Virus B4	Punta Toro virus	Vesicular Stomatitis virus	Coxsackie Virus B4	Respiratory Syncytial virus
2a	>100	>100	>100	>100	>100	>100	>100	>100	20	100	>100	45	>100
2b	59	59	>100	>100	100	>100	>100	>100	2	59	50	2	>100
2c	>4	>4	>4	>4	>4	>20	>20	>20	9	12	9	12	>20
3a	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20
3b	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20
5	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20
6	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20
7	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20
8	>0.8	>0.8	>0.8	>0.8	>0.8	>20	>20	>20	>20	>20	>20	>20	>20

Table-2 continue

9	**>4**	**>4**	**>4**	**>4**	**>4**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
10	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
11	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
12	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
13	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
14	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
15	**>20**	**>20**	**>20**	**>20**	**>20**	**>4**	**>4**	**>4**	**>4**	**>4**	**>20**	**>20**	**>20**
16	**>4**	**>4**	**>4**	**>4**	**>4**	**>4**	**>4**	**>4**	**>4**	**>4**	**>4**	**>4**	**>4**
17	**>4**	**>4**	**>4**	**>4**	**>4**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
18	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
19	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
20	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
21	**>20**	**>20**	**>20**	**>20**	**>20**	**>100**	**>100**	**>100**	**>100**	**>100**	**>20**	**>20**	**>20**
22	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
23	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
24	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
25	**>20**	**>20**	**>20**	**>20**	**>20**	**>4**	**>4**	**>4**	**>4**	**>4**	**>20**	**>20**	**>20**
26	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
27	**>100**	**>100**	**>100**	**>100**	**>100**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
28	**>20**	**>20**	**>20**	**>20**	**>20**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**
29	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
30	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
31	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
32	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
33	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
34	**>20**	**>20**	**>20**	**>20**	**>20**	**>4**	**>4**	**>4**	**>4**	**>4**	**>4**	**>4**	**>4**
35	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**
36	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>20**	**>20**	**>20**
37	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>0.16**	**>0.16**	**>0.16**
38	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>0.8**	**>0.8**	**>0.8**
39	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>20**	**>20**	**>20**
40	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>0.8**	**>0.8**	**>0.8**
41	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>100**	**>0.16**	**>0.16**	**>0.16**
DS-5000	**-**	**-**	**-**	**-**	**-**	**>100**	**>100**	**59**	**>100**	**9**	**12**	**100**	**0.5**
(S)-DHPA	**-**	**-**	**-**	**-**	**-**	**>250**	**>250**	**>250**	**>250**	**>250**	**250**	**>250**	**>250**
Ribavirin	**>250**	**>250**	**>250**	**126**	**>250**	**112**	**>250**	**>250**	**>250**	**112**	**22**	**146**	**29**
Brivudin	**0.04**	**126**	**4**	**>250**	**250**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Cidofovir	**1**	**4**	**3**	**>250**	**4**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Ganciclovir	**0.06**	**0.03**	**>100**	**>100**	**2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

[a] Required to reduce virus- induced cytopathogenicity by 50%

Conclusion

A series of new Schiff bases (oximes, hydrazones, semicarbazones and thiosemicarbazones) derived from 5-acyl-1,2,4-triazines were synthesized in order to evaluate their biological activities. Among the synthesized compounds only oximes: **2a**, **2b**, and **2c** showed significant antiviral activities. The results of antiviral activity of the tested compounds are shown in table 2. None of the other Schiff bases exhibited specific antiviral activity, which means that they do not inhibit replication (formation of viral cytopathogenicity) of any the viruses tested at a concentration that was ≥5-fold lower than the minimum cytotoxic concentration. In our opinion only oximes **2a-c** are suitable as a subject to further structural modifications for biological study.

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References

1. H. Neunhoeffer, In *Comprehensive Heterocyclic Chemistry*, Katritzky, A. R., Rees, C. W.; Ed. Pergamon: Oxford 1984; Vol. 3, pp 385-456.
2. D. Branowska and A. Rykowski, Application of 1-Vinylimidazole in Diels-Alder Reaction of 5,5´-bi-1,2,4-Triazines, *Synlett*, **11**, 1892 (2002**)**.
3. D. Branowska, S. Ostrowski and A. Rykowski, Tandem Vicarious Nucleophilic Substitution of Hydrogen/Intramolecular Diels-Alder Reaction of 1,2,4-Triazines into Functionalized Cycloalkenopyridines, *Chem. Pharm. Bull.*, **50**, 463 (2002).
4. M. Mojzych, Z. Karczmarzyk and A. Fruziński, Synthesis and Structure of (E)-1-(3-Methylsulfanyl-1,2,4-triazin-5-yl)-ethanone O-Acryloyl Oxime, *Analytical Sciences: X-ray Structure Analysis Online*, **23**, x205 (2007**)**.
5. J. Ławecka, E. Olender, P. Piszcz and A. Rykowski, Sequential homo-coupling Diels–Alder/retro Diels–Alder reaction of 5,5´-bi-1,2,4-triazine-containing thiamacrocycles as a new

route to thiacrown ethers incorporating a 2,2´-bipyridine subunit, *Tetrahedron Lett.,* **49**, 723 (2008).

6. Z. Karczmarzyk, M. Mojzych and A. Rykowski, Synthesis and structure of a novel mesomeric betaine 6,7-dimethyl-2H-pyrazolo[4,3-e]tetrazole[4,5-b][1,2,4]triazine, *J. Mol. Struct.,* **829**, 22 (2007).
7. A. Rykowski and M. Mąkosza, Reaction of 1,2,4-triazines with nitronate anions, direct nucleophilic acylation of 1,2,4-triazines, *Tetrahedron Lett.*, **25**, 4795 (1984).
8. A. Rykowski and T. Lipińska, A Concise Route to a Key Intermediate in the Total Synthesis of Sempervirine, *Synth. Commun.,* **26**, 4409 (1996).
9. A. Rykowski, T. Lipinska, E. Guzik, M. Adamiuk and E. Olender, Enantioselective Reduction of 5-Acyl-1,2,4-Triazines and their Oximes by Bakers-Yeast, Polish Journal of Chemistry, *Pol. J. Chem.,* **71**, 69 (1997**)**.
10. A. Rykowski, E. Olender, D. Branowska and H. C. Van der Plas, A novel synthesis of 2-acylpyridines via inverse electron demand Diels-Alder reaction of 5-acyl-1,2,4-triazines, *Org. Prep. Proced. Int.,* **33**, 501 (2001).
11. M. Mojzych and A. Rykowski, Direct synthesis of pyrazolo[4,3-e][1,2,4]triazine derivatives from oximes of 5-acyl and 5-formyl-1,2,4-triazines. *Heterocycl. Commun.,* **12**, 191 (2006).
12. T. M. Lipińska, Total synthesis of new indolo[2,3-a]quinolizine alkaloids sempervirine type, potential pharmaceuticals, *Tetrahedron*, **62**, 5736 (2006).
13. A. Rykowski, M. Mojzych and Z. Karczmarzyk, A new synthesis of pyrazolo[4,3-*e*][l,2,4]-triazines via acid promoted ring closure of the phenylhydrazones of 5-acyl-l,2,4-triazines. *Heterocycles*, **53**, 2175 (2000).
14. (a) A. P. Rajput and S. S. Rajput, Synthesis of benzaldehyde substituted phenyl carbonyl hydrazones and their formylation using Vilsmeier-Haack reaction, *International Journal of PharmTech Research,* **1**, 1605 (2009), and literature cited therein; (b) M. S. Karthikeyan, D. J. Prasad, B. Poojary, K. S. Bhat, B. S. Holla and N. S. Kumarib, Synthesis and biological activity of Schiff and Mannich bases bearing 2,4-dichloro-5-fluorophenyl moiety, *Bioorg. Med. Chem*., **14**, 7482 (2006).
15. K. Mlickova, M. Sebela, R. Cibulka, I. Frebort, P. Pec, F. Liska and K. Tanizawa, Inhibition of copper amine oxidases by pyridine-derived aldoximes and ketoximes, *Biochimie*, **83**, 995 (2001).
16. M. Mojzych and A. Rykowski, One-step synthesis and regioselective alkylation of substituted 1*H*-pyrazolo[4,3-*e*][l,2,4]triazine, *Pol. J. Chem*, **77**, 1797 (2003).
17. M. Mojzych and A. Rykowski, Thermal transformations of phenylhydrazones of 5-acyl-1,2,4-triazines towards pyrazolo[4,3-*e*][1,2,4]-triazine and 4-cyanopyrazole derivative under acidic and no acidic conditions. *J. Heterocycl. Chem.,* **44**, 1003 (2007).
18. M. Mojzych, 3-Nitropyridin-2-yl hydrazone of 5-Acetyl-3-phenyl-1,2,4-triazine, *Molbank*, M433 (2005).
19. M. Mojzych, Semicarbazone and Thiosemicarbazone of 5-acetyl-3-(methylsulfanyl)-1,2,4-triazine, *Molbank,* M434 (2005).
20. M. Mojzych, Z. Karczmarzyk, Z. Urbańczyk-Lipkowska and P. Kalicki, 1,2-Bis[1-(3-methylsulfanyl-1,2,4-triazin-5-yl)ethylidene]-diazane. *Acta Crystallogr. Sect. E: Struct. Rep. Online,* **65**, o1772 (2009).
21. Z. Karczmarzyk, M. Mojzych and A. Rykowski, Synthesis and structure of *p*-chlorophenyl-hydrazone of 3-(methylthio)-5-propanoyl-1,2,4-triazine. *J. Chem. Crystallogr.,* **30**, 423 (2000).
22. M. Sebela, D. Kopecny, Z. Lamplot, J. Havlis, H. Thomas and A. Shevchenko, Thermostable β-cyclodextrin conjugates of two similar plant amine oxidases and their properties, *Biotechnol. Appl. Biochem.,* **41**, 77 (2005).
23. T. A. Smith, Polyamine oxidase (oat seedlings*), Methods Enzymol.,* **94***,* 311 (1983).