VOL. 55 NO. 8, AUG. 2002 THE JOURNAL OF ANTIBIOTICS pp. 768 — 773

Synthesis, and Cytotoxic Activity of N^{ind}-Alkoxy Derivatives of Antibiotic Arcyriarubin and Dechloro-rebeccamycin Aglycon

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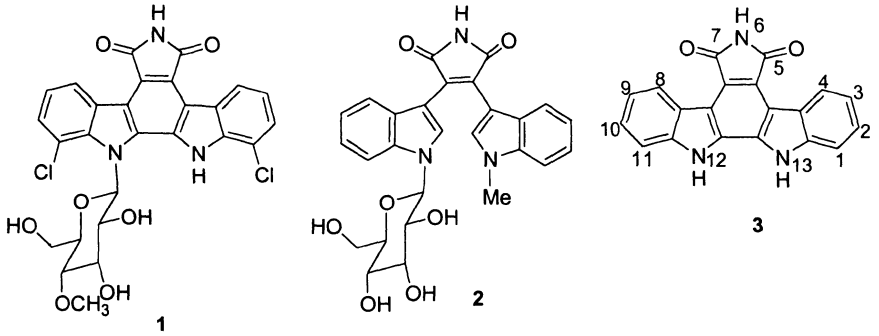
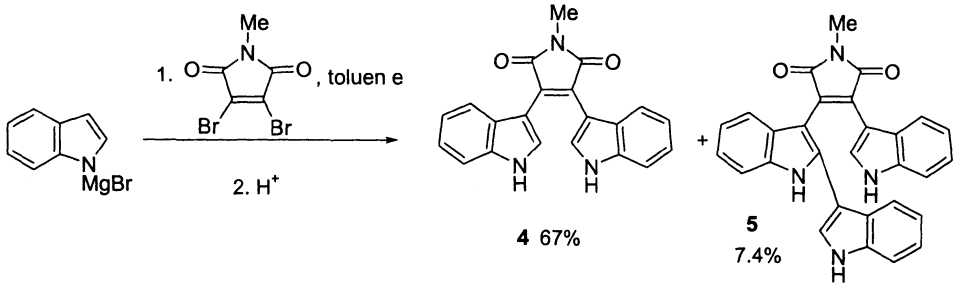
(Received for publication April 4, 2002)

Interest in rebeccamycin and arcyriarubin derivatives comes from their interesting biological properties. Rebeccamycin[1] (**1**, Fig. 1) and some of its analogs have shown remarkable activity as DNA topoisomerase I inhibitors and antitumor compounds.[2] Some of *N*-glycosyl derivatives of arcyriarubin A (*e.g.* **2**) also have demonstrated potent antiproliferative activities[3] and N^{ind}-alkyl derivatives of arcyriaflavin A (**3**) were shown to effect a potent and selective inhibition of human cytomegalovirus (HCMV) replication.[4] Although various derivatives of indolo[2,3-a]carbazole and bisindolylmaleimide have been synthesized,[2] the derivatives bearing *N*-alkoxy substituents have not been yet described. Our objective was to develop methods of synthesis of unsymmetrical *N*-alkoxy derivatives of dechloro-rebeccamycin aglycon and arcyriarubin and to study the impact of this type of substituent on cytotoxic and antiviral activities. The starting bisindolylmaleimide **4** (*N*-methyl arcyriarubin) was prepared from indole Grignard reagent and *N*-methyl-2,3-dibromomaleimide as previously described.[5] From the reaction mixture after column chromathography additionally to compound **4** (67%),

Fig. 1.



Scheme 1. Synthesis of *N*-methylarcyriarubin A (**4**) and derivative **5**.



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compound **5** was isolated in 7.4% yield (Scheme 1). It could be formed from the indolenine intermediate[5] and an excess of indolylmagnesium bromide, with subsequent oxidative aromatization (**5**, Rf 0.19 [($CHCl_3$ - EtOAc, 6 : 1), a dark red solid, H^1-NMR (DMSO-d_6, δ ppm, J Hz) 2.98 (3H, s), 6.58 (1H, t, J=6.5), 6.82 (2H, t, J=7.41), 6.94 (1H, t, J=7.17), 7.02 (2H, t, J=7.39), 7.1 (1H, d, J=7.63), 7.14 (1H, d, J=7.14), 7.28 (1H, d, J=4.8), 7.29 (1H, d, J=2.9), 7.41 (1H, d, J=7.24), 7.43 (1H, d, J=7.9), 7.65 (1H, d, J=8.05), 7.68 (1H, d, J=2.9), 11.29 (1H, d, J=2.2), 11.53 (1H, s), 11.59 (1H, d, J=2.62); ^{13}C-NMR (DMSO-d_6) 23.9 (N-CH_3), aromatic CH 111.1, 111.6, 111.7, 119.1, 119.2, 119.3, 119.5, 120.6, 120.9, 121.4, 121.5, 125.0, 129.5, aromatic C 102.0, 106.1, 108.1, 125.2, 125.3, 126.8, 128.1, 131.5, 132.2, 134.9, 136.0, 136.1, carbonyl 171.1, 171.7. HR-MS calcd for $C_{29}H_{20}N_4O_2$ M^+ 456.1586, found 456.1557.]

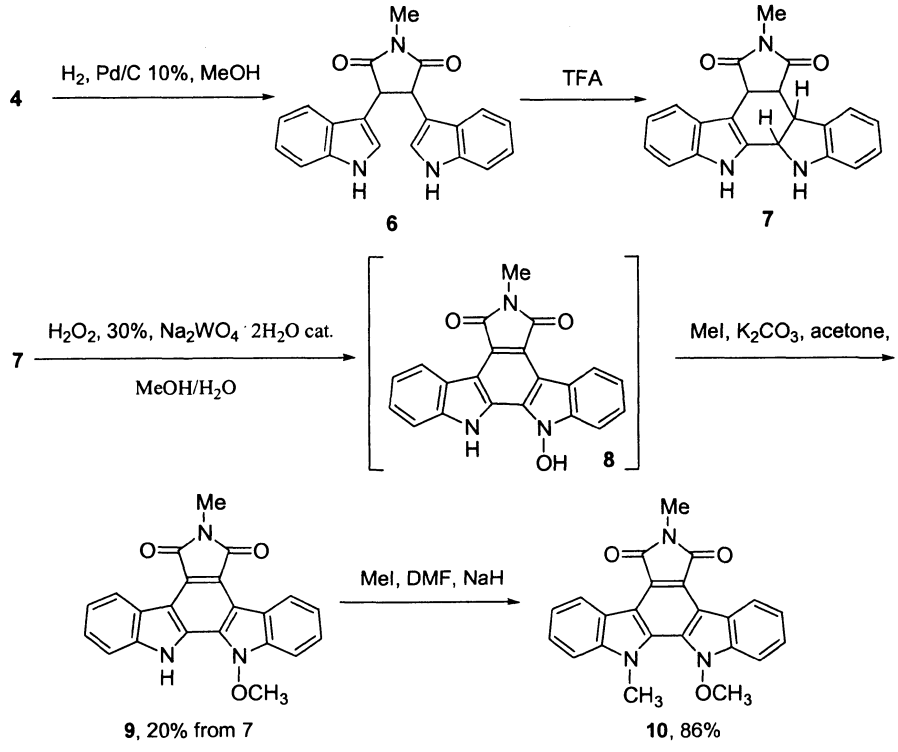
The key precursor in the synthesis of the N-methoxy-rebeccamycin aglycon analog, indolylindoline **7** was prepared from bisindolylmaleimide **4** *via* bisindolyl-succinimide **6** by treatment with TFA.[6] Compound **7** was converted into the corresponding N-hydroxy derivative **8** by the oxidation with H_2O_2 in the presence of catalytic amounts of $Na_2WO_4 \times 2H_2O$ as it was first described for the synthesis of 1-hydroxyindole.[7] Subsequent methylation led

Table 1. Cytotoxicity values for compounds **5**, **9**, **10**, and **13**~**20**.

#	Cytotoxicity, IC_{50}[a], μM		
	L1210	MOLT4/C8	CEM
5	2.3±0.9	2.7±1.2	3.4±2.5
9	100±30	13±3	65±40
10	≥500	>500	>500
13a	3.5±0.1	1.7±0.04	1.9±0.01
13b	11 ± 2	3.2 ± 0.03	3.7 ± 0.08
13c	0.51±0.01	0.25	0.38 ± 0.03
14a	32 ± 2	26 ± 2	17 ± 1
14b	22 ±1	31 ± 8	26 ± 3
15	8.2±0.8	9.3±0.1	7.7±0.4
16	24 ± 8	10 ± 0,5	21 ± 5
17	7.9 ± 3.4	8.1 ± 0,7	11 ± 1
18	1.7 ±1.2	0.57 ± 0.16	0.86 ± 0.7
19	33 ± 5	37 ±7	29 ±6
20	224 ± 29	200 ± 30	240 ± 21

[a] Compound concentration required to inhibit cell proliferation by 50%.

Scheme 2. Synthesis of N-methoxy-indolo[2,3-a]carbazoles.



to the corresponding *N*-methoxy derivative **9** as a yellow solid (Rf 0.50 ($CHCl_3$), m.p. 234~236°C (*n*-heptane - EtOAc). HR-MS calcd for $C_{22}H_{15}N_3O_3$ M^+ 369.1113, found 369.1077). Under more drastic conditions (MeI, NaH, DMF) *N,N,O*-trimethyl derivative **10** was obtained (Scheme 2). Formation of **10** was also observed by TLC when the reaction done under milder conditions (MeI, acetone, K_2CO_3) was allowed to stir overnight [**10**, a brown foam, Rf 0.22 (*n*-heptane - EtOAc, 10 : 1). HR-MS calcd for $C_{23}H_{17}N_3O_3$ 383.1270, found 383.1286]. ^{1}H- and ^{13}C -NMR data for the compounds **9**, **10** are presenteed in the Tables 2 and 3 respectively.

N^{ind}-Alkoxy-arcyriarubin derivatives (*N*-alkoxy-bis-indolylmaleimides **13a~c**) were synthesized by the condensation of (indol-3-yl)acetamide with methyl 3-(1-alkoxyindol-3-yl)glyoxylates.[8] (Scheme 3). 1-Alkoxyindoles **11a~c** were prepared from indoline by the oxidation with aqueous hydrogen peroxide in the presence of catalytic

Table 2. ^{1}H-chemical shifts (δ, ppm) and coupling constants (*J* Hz) for compounds **9**, **10**, **13a~c**, and **14a, b** (DMSO-d_6).

#	H2, H2'	H4, H4'	H5, H5'	H6, H6'	H7, H7'	$N^{indole}H$	Other
9	-	9.4; 9.0; 1H, d	7.46; 7.38; 1H, t	7.66; 7.58; 1H, t	7.82; 7.84; 1H, d;	12.42; 1H, s	-OCH$_3$ 4.27, 3H, s; N-CH$_3$ 3.16, 3H, s;
10	-	9.14; 1H, d, J=8.02; 9.08; 1H, d, J=7.96	7.48; 1H, t, J=7.50 7.43, 1H, t, J=7.49	7.66-7.72, 2H, complex	7.81, 1H, d, J=8.38; 7.78, 1H, d, J=8.05	-	-OCH$_3$ 4.28, 3H, s; N^{indole}-CH$_3$ 3.15, 3H, s; N^{mal}-CH$_3$ 3.57; 3H, s
13a	7.9; 1H, s; 7.84; 1H, d, J=2.74	7.46; 1H, d, J=7.86; 7.41; 1H, d, J=8.06	7.12; 1H, t, J=7.22 7.0, 1H, t, J=7.5	6.77, 1H, t, J=8.01; 6.65, 1H, t, J=7.1	6.96, 1H, d, J=8.14; 6.72, 1H, d, J=7.55	11.75; 1H, s	OCH$_3$ 4.08, 3H, s; N-H 11.0, 1H, s;
13b	7.83-7.58; 2H, m	7.44, 1H, d, J=7.80, 7.39, 1H, d, J=8,1	7.12; 6.99; 1H, t,	6.73; 6.63; 1H, t	6.97; 6.78; 1H, d	11.75; 1H, s	N^{mal}-H, 10.96, 1H, s; -OCH$_2$- 4.2, 2H, t; CH$_2$CH$_2$CH$_3$ 1.7, 2H, m; -CH$_2$CH$_3$ 1.0, 3H, t.
13c	7.86, 1H, s 7.84, 1H, d, J=2.78	7.44, 1H, d, J=8.24 7.38, 1H, d, J=8.09	7.10, 1H, t, J=7.63 6.99, 1H, t, J=7.62	6.74, 1H, t, J=7.52, 6.62, 1H, t, J=7.45	6.94, 1H, d, J=8.05 6.69, 1H, d, J=7.83	11.75, 1H, d, J=2.56	-OCH$_2$- 4.30, 2H, q; CH$_2$CH$_3$ 1.28, 3H, t. N^{mal}-H, 10.98, 1H, s;
14a	7.91; 7.81, 1H, s	7.47; 1H, d, J=7.5, 7.40; 1H, d, J=8.2	7.14; 1H, t, J=7.35; 7.0; 1H, t, J=7,3	6.78; 1H, t, J=8.0; 6.68; 1H, t, J=8.1	6.97; 1H, d; J=8.05; 6.71, 1H, d; J=8.15	11.9; 1H, s;	OCH$_3$ 4.08, 3H, s; N-CH$_3$ 3.06, 3H, s;
14b	7.95; 1H, d, J=2.8; 7.85; 1H, s	7.45; 7.4; 1H, d	7.13; 6.95; 1H, t	6.77; 6.63, 1H, t	6.9; 6.69; 1H, d	11.78; 1H, s;	N^{mal}-CH$_3$, 3.06, 3H, s; -OCH$_2$CH$_2$- 4.2, 2H, t; -OCH$_2$CH$_2$CH$_3$ 1.7, 2H, m -OCH$_2$CH$_2$CH$_3$ 1.0, 3H, t.

Table 3. ^{13}C-chemical shifts for compounds **9**, **10**, and **12**~**14**. ($CDCl_3$ for **10** and **12a**~**c**, DMSO-d_6 for the rest)

Comp.	Alkyl	Carbonyl	Aromatic CH	Aromatic C
9	N-$\underline{C}H_3$ 23.6, O$\underline{C}H_3$ 65.7	169.3, 169.5	108.8, 111.7, 112.2, 112.7, 116.8, 118.3, 119.8, 120.3, 120.7, 121.9, 124.2, 124.7, 125.9, 127.0, 127.2, 127.4, 137.0, 141.4	
10	N-$\underline{C}H_3$ 23.6, N^{ind}-$\underline{C}H_3$ 33.7, N-O-$\underline{C}H_3$ 65.7	169.3, 169.5	109.3, 110.7, 121.0, 122.9, 125.6, 125.9, 127.6, 127.7,	118.1, 118.7, 118.8, 121.5, 121.6, 121.7, 127.4, 131.9, 140.5, 143.2,
12a	O-$\underline{C}H_3$ 52.7, N-O-$\underline{C}H_3$ 66.9	162.8, 176.5	108.8, 122.8, 124.0, 124.6, 133.1	108.7, 123.3, 132.0
12b	-$\underline{C}H_3$ 10.1, -$\underline{C}H_2$- 21.5, O-$\underline{C}H_2$- 81.2, O-$\underline{C}H_3$ 52.6	162.9, 176.5	108.8, 122.7, 123.9, 124.5, 133.8	108.5, 123.4, 132.6
12c	$CH_2\underline{C}H_3$ 13.6, O-$\underline{C}H_2$- 75.3, O-$\underline{C}H_3$ 52.6	162.8, 176.5	108.8, 122.6, 123.9, 124.5, 133.9	108.5, 123.3, 132.7
13a	O-$\underline{C}H_3$ 52.7, N-O-$\underline{C}H_3$ 66.9	172.6, 172.7	108.3, 111.8, 119.5, 120.2, 120.9, 121.5, 121.7, 122.7, 126.1, 129.7	101.8, 105.2, 122.0, 124.9, 125.9, 129.4, 131.1, 136.1
13b	-$\underline{C}H_3$ 10.0, -$\underline{C}H_2$- 20.9, O-$\underline{C}H_2$- 80.0	172.5, 172.7	108.3, 111.8, 119.3, 120.1, 120.7, 121.4, 121.6, 122.5, 126.5, 129.6	101.7, 105.2, 121.9, 124.9, 125.9, 129.4, 131.7, 136.0
13c	$CH_2\underline{C}H_3$ 13.4, O-$\underline{C}H_2$- 74.2,	172.7, 172.5	108.4, 111.8, 119.3, 120.0, 120.7, 121.3, 121.6, 122.5, 126.7, 129.6	101.6, 105.1, 121.8, 124.9, 125.9, 129.3, 131.8, 136.0
14a	N-O-$\underline{C}H_3$ 66.5, N-$\underline{C}H_3$ 24.0	171.4, 171.5	108.3, 111.9, 119.5, 120.2, 120.9, 121.5, 121.7, 122.7, 126.1, 129.7	101.8, 105.2, 121.9, 124.8, 125.2, 128.9, 131.1, 136.1
14b	-$\underline{C}H_3$ 10.0, -$\underline{C}H_2$- 21.0, O-$\underline{C}H_2$- 80.1, N-$\underline{C}H_3$ 24.0	171.4, 174.5	108.5, 111.9, 119.4, 120.2, 120.8, 121.4, 121.7, 122.7, 126.6, 129.7	101.7, 105.2, 121.9, 124.8, 125.3, 128.9, 131.7, 136.1

Scheme 3. Synthesis of *N*-alkoxyderivatives of arcyriarubin A.

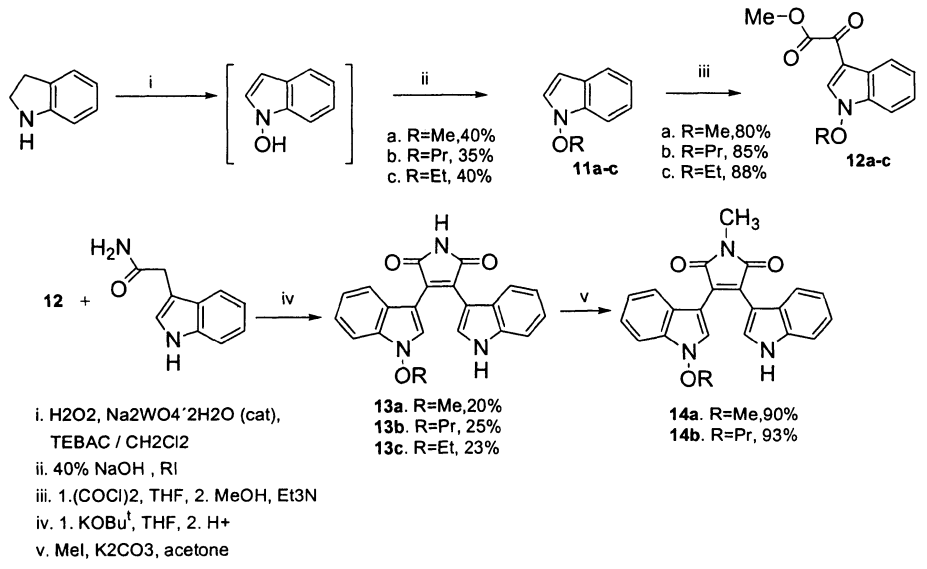
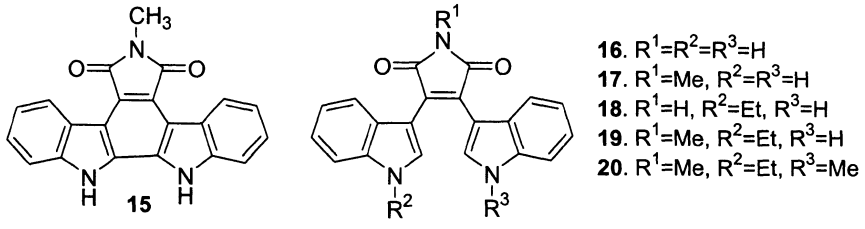


Fig. 2.



amounts of $Na_2WO_4 \times 2H_2O$ under PTC conditions (triethylbenzylammonium chloride was used as a phase transfer agent) with subsequent alkylation. The modification of this method of indoline *N*-oxidation proved to be more convenient than the oxidation in aqueous methanol followed by alkylation, as it was first described by SOMEI,[7] since oxidation and alkylation were performed as a one-pot reaction. 1-Methoxyindole (**11a**), 1-propoxyindole (**11b**), and 1-ethoxyindole (**11c**) were obtained as colorless oils in 40% yields.

The corresponding methyl 3-(1-alkoxyindol-3-yl)-glyoxylates **12a**~**c** were prepared by the reaction of **11a**~**c** with oxalyl chloride and subsequent treatment with methanolic triethylamine solution. The mixture of a methyl 3-(1-alkoxyindolyl)-glyoxylate (**12a, b** or **c**) with indolyl-3-acetamide in THF was then treated with KOBu^t solution in THF to achieve the final cyclization to give after column chromatography the corresponding bis-indolylmaleimides in 23~25% yields: 3-(indol-3-yl)-4-(1-methoxy-indol-3-yl)-pyrrole-2,5-dione (**13a**) [Rf 0.24 (*n*-heptane - EtOAc, 3 : 2), HR-MS calc. for $C_{21}H_{15}N_3O_3$ 357.1113 found 357.1091], 3-(indol-3-yl)-4-(1-propoxy-indol-3-yl)pyrrole-2,5-dione (**13b**) [Rf 0.29 (*n*-heptane - EtOAc, 3 : 2), HR-MS calc. for $C_{23}H_{19}N_3O_3$ 385.1426 found 385.1401] or 3-(indol-3-yl)-4-(1-ethoxy-indol-3-yl)-pyrrole-2,5-dione (**13c**) [Rf 0.27 (*n*-heptane - EtOAc, 3 : 2), HR-MS calc. for $C_{19}H_{17}N_3O_3$ 371.1270, found 371.1259]. Bis-indolyl-maleimides **13a, b** were then converted into N^{mal}-methyl derivatives **14a, b** in 90% yields by the treatment with MeI and K_2CO_3 in acetone (Scheme 3): [3-(Indol-3-yl)-4-(1-methoxy-indol-3-yl)-1-methyl-pyrrole-2,5-dione (**14a**), Rf 0.36 (*n*-heptane - EtOAc, 3 : 2), HR-MS calc. for

$C_{22}H_{17}N_3O_3$ 371.1269 found 371.1272], and 3-(indol-3-yl)-4-(1-propoxy-indol-3-yl)-1-methyl-pyrrole-2,5-dione (**14b**) [Rf 0.45 (*n*-heptane-EtOAc, 3:2), HR-MS calcd for $C_{24}H_{21}N_3O_3$ 399,1582 found 399,1575]. ^{1}H- and ^{13}C-NMR data for the compounds **13a, b, c** and **14a, b** are presenteed in the Tables 2 and 3 respectively.

To compare the role of an alkoxy and alkyl substituent at the indole nitrogen atom for the cytotoxic activity, 6-methyl-indolo[2,3-a]pyrrolo[3,4-c]carbazol-5,7-dione **15**, and bisindolylmaleimides **16**~**20** were synthesized as previously described (Fig. 2).[4,8]

Cytotoxicity of the compounds obtained was evaluated in three cell cultures (Table 1). The comparison of compounds **9**, **10** and **15** shows that the introduction of N^{ind}-MeO group into **15** leads to some decrease of cytotoxicity and further *N*-alkylation of the second indole nucleus (**10**) results in a dramatic decrease of cytotoxic properties. This can be explained by the role of hydrogen bonds in the biological activity of these compounds. Comparison of the cytotoxic activities of bis-indolylmaleimides (**13**~**20**) demonstrates that the unsymmetrical N^{ind}-alkoxy derivatives (**13a**~**c**) and N^{ind}-alkyl derivative (**18**) are more cytotoxic than the unsubstituted bisindolylmaleimide **16**, the most cytotoxic in this series are N^{ind}-ethoxy (**13c**) and N^{ind}-ethyl (**18**) compounds. The substitution of the nitrogen in the maleimide ring (**14a, b**, **19**) leads to a decrease of cytotoxicity; and the IC_{50} values for compounds **14a, b**, and **19** are of the same order. The substitution of the second indole nitrogen (**20**) again leads to dramatic decrease in activity. However comparison of compounds **16** and **17** shows that mono N-methylation of the maleimide ring does not influence negatively cytotoxic properties perhaps because **17** still has two N–H bonds. It is interesting to note that the maleimide derivative containing three indole nuclei (**5**) was found to be rather cytotoxic. All the compounds investigated demonstrated no antiviral activity [against HIV-1, HIV-2, HCMV (compounds **2**, **10**, **13a** and **15**) and varicella-zoster virus (compounds **9**, **10**, **13a** and **15**) and herpes simplex virus type 1 (KOS), and type 2 (G), vaccinia virus, vesicular stomatitis virus, Coxsackie, Sindbis, Punta Toro virus, reovirus-1, parainfluenza-3 and respiratory syncytial virus **13b**, **14a, b**, **16**~**20**] at subtoxic concentrations, that is at concentrations barely lower than the overtly cytotoxic concentrations.

Acknowledgements

This work was supported by the Russian Fund for Fundamental Research, grant number 01-03-33028 and the "Fonds voor Wetenschappelijk Onderzoek-Vlaanderen", grant number G.0104.98.

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