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Testing of Silanes for Antiviral Activity

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Abstract

Forty-three organosilicon class of compounds were tested for antiviral activity using a wide scope screening program *in vitro* (in cell cultures) including eight model strains of viruses belonging to seven taxonomic groups including causative agents of infections in which applications of chemotherapy is indicated. The results obtained demonstrated a marked activity of di(hexadecanoyloxy)diphenylsilan (compound 27) only against human cytomegalovirus (SI = 30.9). A marked activity toward this virus at a low m.o.i. was recorded also by 1-o-dimethyl(octadecyl)silyl-(2,3,4,6-o-tetraacetyl-β-D-glucopyranosid) (compound 20). Small to borderline effect against this virus was found by silanes 2, 7, 15, 19 and 34, at silanes 2, 3 and 25 toward influenza virus A(H3N2) and at silane 9 versus vaccinia virus. As concerns the cytotoxicity it was established a strong variation towards different cell cultures used, the higher susceptibility of the HEp-2 cells been recorded.

Keywords: silanes, antiviral testing, *in vitro*, cytotoxicity

Резюме

Четиридесет и три съединения от класа органосиликони бяха изпитани за антивирусна активност чрез широко-спектърна скринингова програма *in vitro*, състояща се от осем моделни щамове на вируси, принадлежащи към седем таксономични групи, включващи причинители на инфекции, при които е показано приложение на химиотерапия. Получените резултати показаха отчетлива активност на ди(хексаноилокси)дифенилсилан (съединение 27) само срещу човешкия цитомегаловирус (SI = 30.9). Отчетлива активност спрямо този вирус при ниска множественост на инфекцията бе отчетена също от 1-о-диметил(октадецил)силил-(2,3,4,6-о-β-D-глюкопиранозид) (съединение 20). Слаб до граничен ефект спрямо този вирус бе намерена при силани 2, 7, 15, 19 и 34, при силани 2, 3 и 25 срещу грипен вирус A(H3N2) и при силан 9 срещу вирус вакциние. Що се касае до цитотоксичността, установено бе силно вариране спрямо различните използвани клетъчни култури, като най-висока чувствителност бе отчетена при клетки HEp-2.

Introduction

The presented organosilicon class of compounds comprises di(acyloxy)dialkyl silanes, di(acyloxy)diaryl silanes, di(acyloxy)dialkoxysilanes, tetra(acyloxy)silanes and sugar-organosilicon compounds – mono-, di- or trialkyl, alkoxysilyl sugar (saccharide(s)) as pyranosid.

By appropriate composition of the four substituents at the central silicon atom, the properties and uses of both the non-aggregated organo- and sugar-organosilicon compounds as well as the vesicles formed by these compounds (Siosomes®) can be varied on a broad basis, thus enabling adaptation to a particular problem. A large number of the silanes and sugar silanes such as alkylsilylsilanes, alkoxysilylsilanes, alkylsilylphosphonate, alkylsilylphosphites, hydroxyl and methoxy derivatives of the sugar silanes and diemethylalkylsilylethyl saccharides have been prepared and physicochemically characterised These compounds can be used: (i) in non-aggregated form, as organosilicon and sugar-organosilicon molecules; (ii) in aggregated

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form, as vesicles (Siosomes®) (Kunath *et al.,*1992; Richter *et al.,* 1992a, b, c, d, e; 1993a, b; Richter and Salama*,* 1994; Pallas and Richter, 1995; Mühl and Richter, 1997; Aisa *et al.,* 1998, 1999; Aisa and Richter, 1999; Ardah *et al.,* 2000; Uhr *et al.,* 2003).

As a general principle, organosilicon compounds (compounds containing one or several Si – C bonds), although not known in nature, can exhibit biological activity. This statement is the most important result of extensive interdisciplinary research in the field of bio-organosilicon chemistry, carried out by chemists, biologists, pharmacologists, toxicologists and physicians during the past 20 years. Several tests involving compounds which contain silicon in the molecule, used as therapeutic agents in human medicine, have been successful (Voronkov, 1979; Tacke, 1985a,b; Tacke and Wannagat, 1997). A large number of organosilicon compounds with high and specific biological activity has been synthesized and subjected to pharmacological and toxicological testing in recent years [Tacke, 1985b]. So far, we can differentiate three main groups of research in the field of biologically active organosilicon compounds, however, these cannot be separated from each other in all cases: (1) synthesis and biological investigation of silylated derivatives of well-known bioactive organic compounds; (2) synthesis and biological investigation of organosilicon compounds which either have no organic analogs or which do have organic analogs with unknown biological activity; (3) synthesis and biological investigation of organosilicon compounds which have analogous sila-substituted structures of organic compounds with well-known bioactivity (so-called sila drugs) (Voronkov, 1979; Tacke, 1985a, b; Tacke and Wannagat, 1997).

Sugar–silanes and sugar–Siosomes® can be considered as innovative drug delivery and targeting system for pharmaceuticals, biologics and cosmetics an as possible candidates for new pharmaceutical agents. A number of sugar organosilicon containing compounds have shown pharmacological efficacy (anti-cancer activities) (Salama, 2016) and others may have anti-viral, anti-bacterial, anti-fungal and immunological activities (Salama, 2016). There are many examples of pharmaceutically active silicon organic compounds in various different therapeutic fields. These include Karenitecin, and TAC-101, which are used to treat various types of cancers (Okusaki *et al.,* 2012; Bio Numerik Pharmaceuticals, 2017), Silperisone, which is used as a central nervous system relaxant, Zifrosilon, which is used in the treatment of dementia and Cisobiatan which in an Oestrogen analogue (Farkas, 2006). Thus, it is not inconceivable that Silanes exhibiting a therapeutic effect could be discovered, developed and clinically used. This development has given rise to therapeutic Siosomes®, where the therapeutic effect of the broken down drug transport system could act in synergy with the encapsulated drug e.g. anti cancer agent(s) encapsulated in Siosomes® where the Silanes have proven chemotherapeutic activity (Salama, 2016).

A special interest represents Siosomes®. They are vesicles consisting of at least one concentric, self-contained layer of organosilicon compounds of the general formula 1 given above. Like liposomes, they can contain a great variety of active substances encapsulated in their compartments. Depending on substance-specific properties such as chain length of the compounds' lateral chains, injection rate, temperature and agitation rate, Siosomes® of various size and structures (unilamellar, multilamellar and heterolamellar) can be obtained which, when compared to liposomes consisting of phospholipids, have the advantage of chemical stability and a defined composition (Kunath *et al.,* 1992; Richter *et al.,* 1992a, b, c, d, e; 1993a, b; Richter and Salama, 1994; Mühl and Richter, 1997).

Siosomes® with a heterolamellar structure are either unilamellar vesicles enclosed in the larger multilamellar vesicles, or they are multilamellar vesicles enclosed in larger unilamellar vesicles. When Siosomes® are synthesized; this may also produce a heterogeneous mixture of unilamellar and multilamellar vesicles (Richter *et al.,* 1992a, b, c, d, e; 1993a,b; Kunath *et al.,*1992; Richter and Salama, 1994). The encapsulation capacities of Siosomes® are good, with encapsulated parameters which make them preferred vehicles for different active substances (Kunath *et al.,*1992; Richter *et al.,* 1992a, b, c, d, e; 1993a, b; Richter and Salama, 1994; Mühl and Richter, 1997; Salama, 2016).

The aim of the present study is a wide scale screening of series of 43 silanes for antiviral activity against model viruses belonging to taxonomic groups including causative agents of infections in which applications of chemotherapy is strongly indicated: a) *Enterovirus* B (or C) (family *Picornaviridae*); b) bovine viral diarrhea virus (a surrogate hepatitis C virus) (family *Flaviviridae*); c) influenza virus A (family *Orthomyxoviridae*); d) respiratory syncytial virus (family *Paramyxoviridae*); e) human adenovirus type 2 (or 5) (family *Adenoviridae*); f) herpes simplex virus type 1, and g) human cytomegalovirus (family *Herpesviridae*);

h) vaccinia virus (family *Poxviridae*). The antiviral screening will be *in vitro* in cell cultures. The CPE inhibition test in monolayer cell cultures (in micro plates); photometrical (optical density) measurement of neutral red uptake will be carried out. In parallel, cytotoxicity will be determined.

Materials and Methods

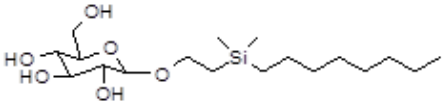
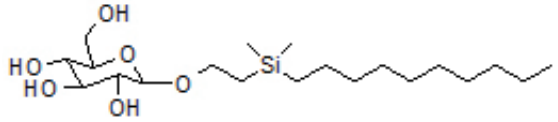
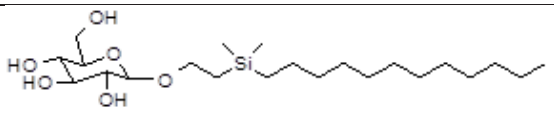
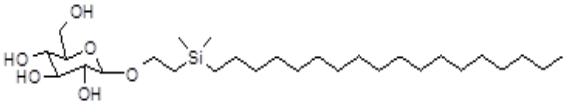
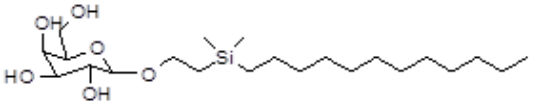
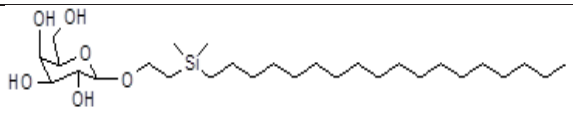
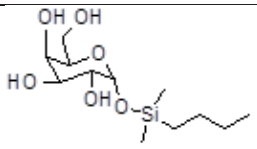
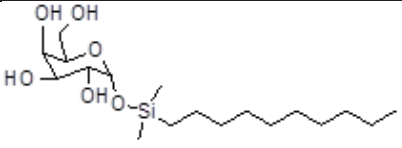
Compounds tested

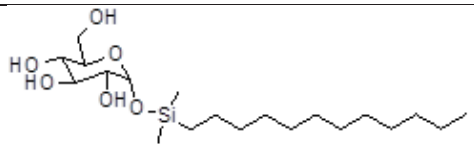
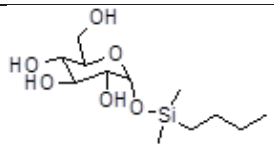
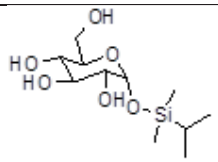
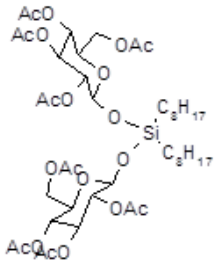
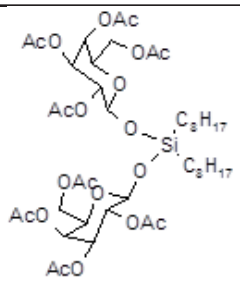
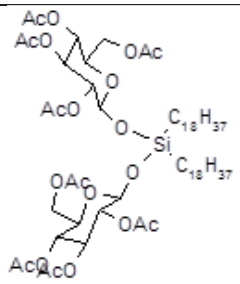
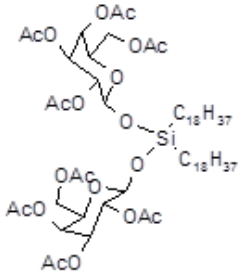
Silanes. The chemical structures of the tested 43 compounds supplied by Dr.Zoser B. Salama are presented in Figure 1. They are used as stock solution of 10 mM in ethanol of each compound.

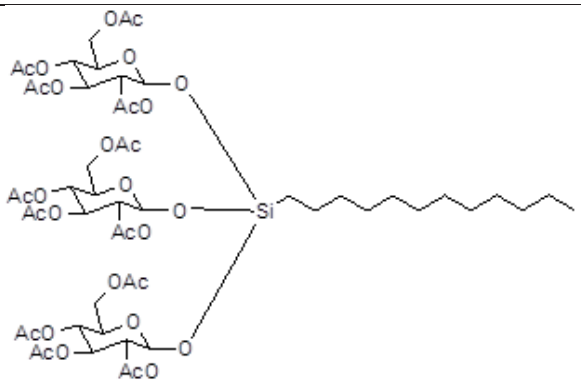
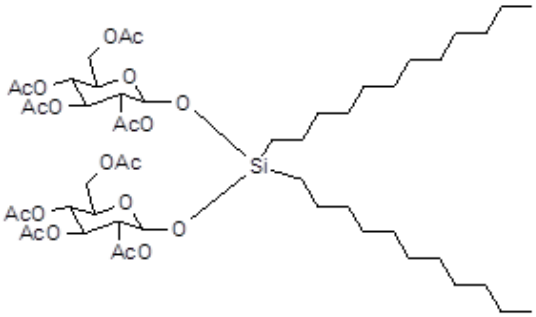
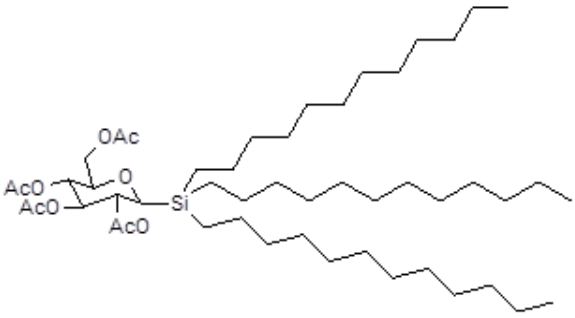
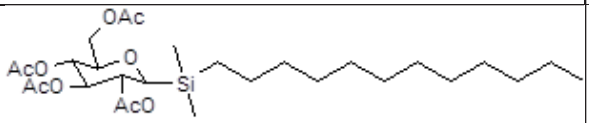
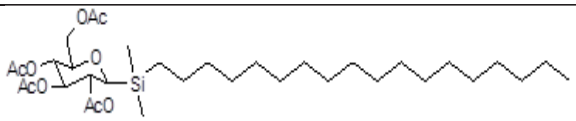
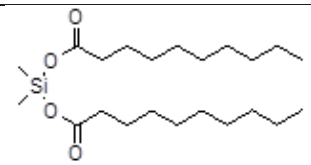
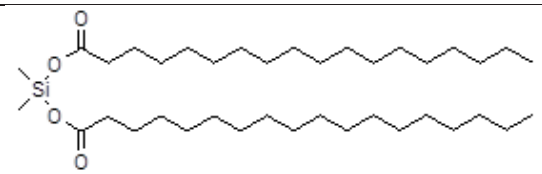
Reference antivirals: disoxaril vs. poliovirus 1; ribavirin vs.human respiratory syncytial virus A2, bovine viral diarrhea virus and human adenovirus 2; rimantadine.HCl vs. influenza virus A(H3N2); acyclovir vs. herpes simplex virus type 1; gancyclovir vs human cytomegalovirus; idoxuridine vs. vaccinia virus.

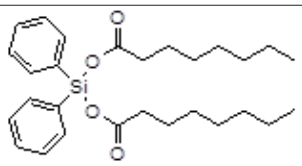
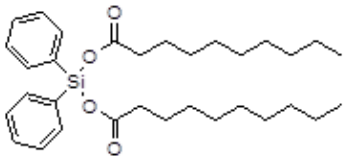
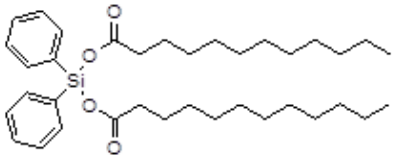
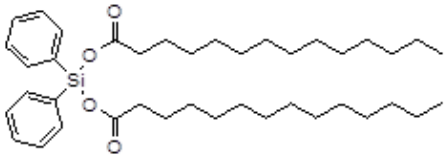
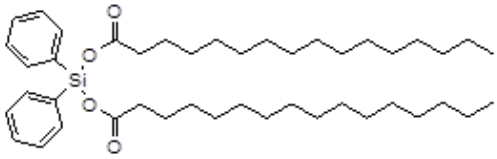
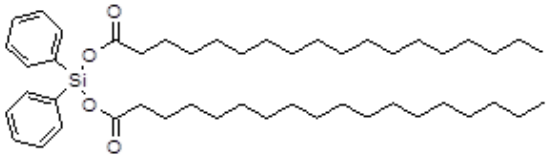
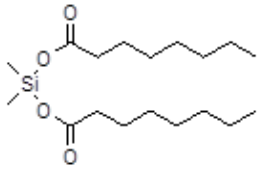
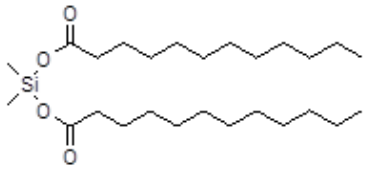
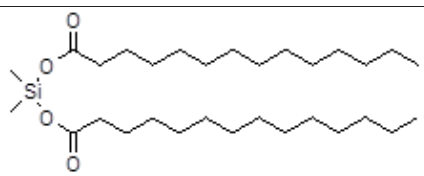
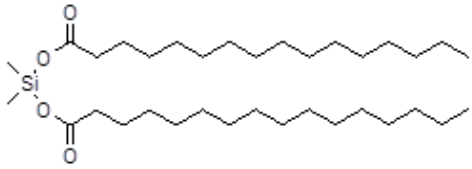
Viruses

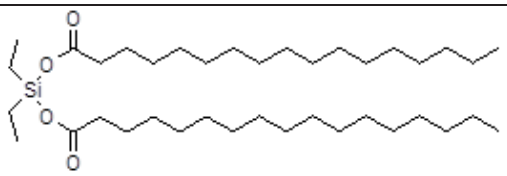
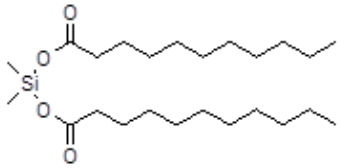
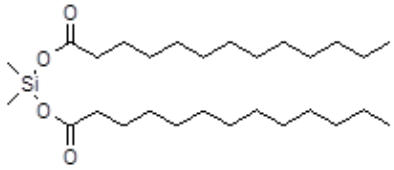
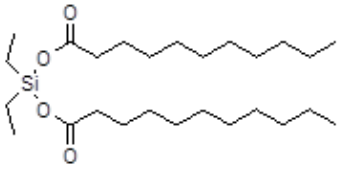
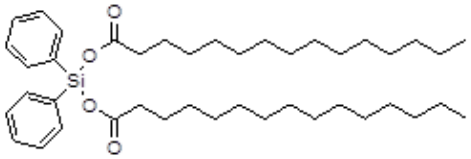
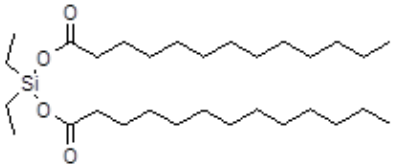
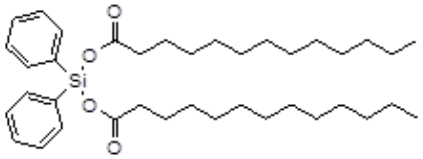
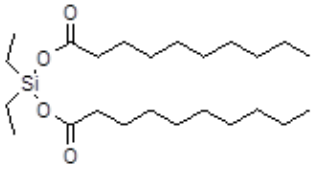
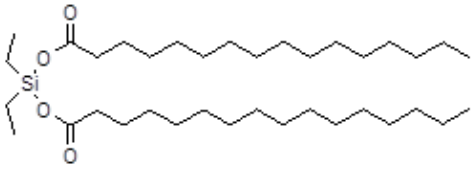
Poliovirus 1 (LSc-2ab strain) [PV1] from the collection of the Stephan Angeloff Institute of Microbiology, BAS (Sofia, Bulgaria), grown in HEp-2 cells (maintenance solution Dulbecco's modified Eagles's medium DMEM (Gibco, Invitrogen, Pais-

Compound	Structure	Name	Formula	Mol. weight
Sil 1		2-(Dimethyloctylsilyl)ethyl-b-D-glucopyranosid	C18H38O6Si	378,59
Sil 2		2-(Dimethyldecylsilyl)ethyl-β-D-glucopyranosid	C20H42O6Si	406,64
Sil 3		2-(Dimethyldodecylsilyl)ethyl-β-D-glucopyranosid	C22H46O6Si	434,69
Sil 4		2-(Dimethyloctadecylsilyl)ethyl-β-D-glucopyranosid	C28H58O6Si	518,86
Sil 5		2-(Dimethyldodecylsilyl)ethyl-β-D-galactopyranosid	C22H46O6Si	434,69
Sil 6		2-(Dimethyloctadecylsilyl)ethyl-β-D-galactopyranosid	C28H58O6Si	518,86
Sil 7		Butyldimethylsilyl-α-D-galactopyranosid	C12H26O6Si	294,42
Sil 8		Decyldimethylsilyl-α-D-galactopyranosid	C18H38O6Si	378,59

Sil 9		Dodecyldimethylsilyl-α-D-glucopyranosid	C20H42O6Si	406,64
Sil 10		Butyldimethylsilyl-α-D-glucopyranosid	C12H26O6Si	294,42
Sil 11		Dimethyl-isopropylsilyl-α-D-glucopyranosid	C11H24O6Si	280,40
Sil 12		1-*O*-Dioctylsilyl-di(2,3,4,6-*O*-tetraacetyl-β-D-glucopyranosid)	C44H72O20Si	949,14
Sil 13		1-*O*-Dioctylsilyl-di(2,3,4,6-*O*-tetraacetyl-β-D-galactopyranosid)	C44H72O20Si	949,14
Sil 14		1-*O*-Dioctadecylsilyl-di(2,3,4,6-*O*-tetraacetyl-β-D-glucopyranosid)	C64H112O20Si	1229,68
Sil 15		1-*O*-Dioctadecylsilyl-di(2,3,4,6-*O*-tetraacetyl-β-D-galactopyranosid)	C64H112O20Si	1229,68

Sil 16		Dodecylsilyl-tris(2,3,4,6-*O*-tetraacetyl-β-D-glucopyranosid)	C54H82O30Si	1239,32
Sil 17		Didoceclylsilyl-di(2,3,4,6-*O*-tetraacetyl-β-D-glucopyranosid)	C52H88O20Si	1061,36
Sil 18		Tridodecylsilyl-(2,3,4,6-*O*-tetraacetyl-β-D-glucopyranosid)	C50H94O10Si	883,39
Sil 19		1-*O*-Dimethyl(dodecyl)silyl-(2,3,4,6-*O*-tetraacetyl-β-D-glucopyranosid)	C28H50O10Si	574,79
Sil 20		1-*O*-Dimethyl(octadecyl)silyl-(2,3,4,6-*O*-tetraacetyl-β-D-glucopyranosid)	C34H62O10Si	658,95
Sil 21		Di(decanoyloxy)dimethylsilan	C22H44O4Si	400,68
Sil 22		Di(octadecanoyloxy)dimethylsilan	C38H76O4Si	625,11

Sil 23		Di(octanoyloxy)diphenylsilan	C28H40O4Si	468,71
Sil 24		Di(decanoyloxy)diphenylsilan	C32H48O4Si	524,82
Sil 25		Di(dodecanoyloxy)diphenylsilan	C36H56O4Si	580,93
Sil 26		Di(tetradecanoyloxy)diphenylsilan	C40H64O4Si	637,04
Sil 27		Di(hexadecanoyloxy)diphenylsilan	C44H72O4Si	693,15
Sil 28		Di(octadecanoyloxy)diphenylsilan	C48H80O4Si	749,26
Sil 29		Di(octanoyloxy)dimethylsilan	C18H36O4Si	344,57
Sil 30		Di(dodecanoyloxy)dimethylsilan	C26H52O4Si	456,79
Sil 31		Di(tetradecanoyloxy)dimethylsilan	C30H60O4Si	512,90
Sil 32		Di(hexadecanoyloxy)dimethylsilan	C34H68O4Si	569,00

Sil 33		Di(heptadecanoyloxy)diethylylsilan	C38H76O4Si	625,11
Sil 34		Di(undecanoyloxy)dimethylsilan	C24H48O4Si	428,73
Sil 35		Di(tridecanoyloxy)dimethylsilan	C28H56O4Si	484,84
Sil 36		Di(undecanoyloxy)diethylylsilan	C26H52O4Si	456,79
Sil 37		Di(pentadecanoyloxy)diphenylsilan	C42H68O4Si	665,09
Sil 38		Di(tridecanoyloxy)diethylylsilan	C30H60O4Si	512,90
Sil 39		Di(tridecanoyloxy)diphenylsilan	C38H60O4Si	608,99
Sil 40		Di(decanoyloxy)diethylylsilan	C24H48O4Si	428,73
Sil 41		Di(hexadecanoyloxy)diethylylsilan	C36H72O4Si	597,06

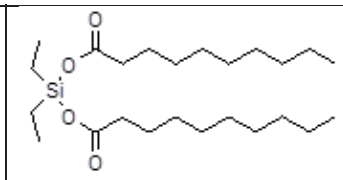
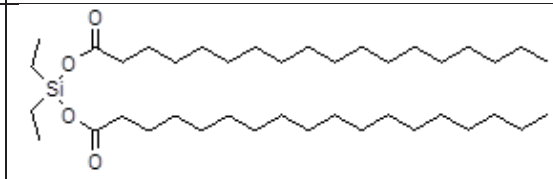
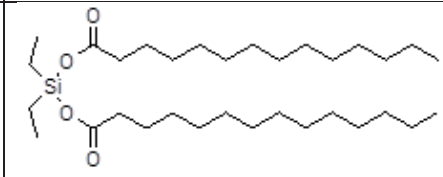
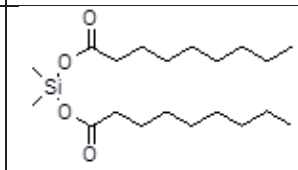
Sil 42		Di(decanoyloxy)diethylylsilan	C24H48O4Si	428,73
Sil 43		Di(octadecanoyloxy)diethylylsilan	C40H80O4Si	653,17
Sil 44		Di(tetradecanoyloxy)diethylylsilan	C32H64O4Si	540,95
Sil 45		Di(nonanoyloxy)dimethylsilan	C20H40O4Si	372,63

Fig. 1. Structural formulas of tested silanes.

ley, Scotland, UK), supplemented by 0.5% bovine fetal serum (Gibco, Invitrogen, Paisley, Scotland, UK), 10 mmol HEPES buffer (AppliChem GmbH, Darmstadt, Germany) and antibiotics (penicillin, 100 U/ml, streptomycin, 100 mg/ml. Infectious titer $10^{6.98\pm0.42}$ $CCID_{50}$/ml.

Bovine viral diarrhea virus (TVM strain) [BVDV], cultivated in calf trachea cell line [CT cells] (maintenance solution DMEM Gibco, Invitrogen, Paisley, Scotland, UK, plus 0.5% fetal bovine serum, Gibco, Invitrogen, Paisley, Scotland, UK, 10 mmol HEPES buffer, AppliChem GmbH, Darmstadt, Germany, and antibiotics -penicillin, 100 U/ml, streptomycin, 100 mg/ml); infectious titer $10^{7.0}$ $CCID_{50}$/ml.

Influenza A virus [Aichi/2/68 (H3N2)] [IAV/H3N2], from the collection of the Stephan Angeloff Institute of Microbiology, BAS (Sofia, Bulgaria). The stock virus represented allantois fluids of virus-inoculated 10-days-embryonated eggs, cultivated at 37°; infectious titer $10^{7.5}$ $CCID_{50}$/ml. The maintenance solution was DMEM Invitrogen, Paisley, Scotland, UK, 3 mg/ml trypsin Gibco BRL, Invitrogen, Paisley, Scotland, UK, 10 mmol HEPES buffer, AppliChem GmbH, Darmstadt, Germany, and antibiotics -penicillin, 100 U/ml, streptomycin, 100 mg/ml.

Human respiratory syncytial virus A2 [HRSV-A2], kindly supplied by the District Center of Hygiene and Epidemiology, Plovdiv (Bulgaria). The virus was grown in HEp-2 cells (maintenance solution Dulbecco's modified Eagles's medium DMEM (Gibco, Invitrogen, Paisley, Scotland, UK), supplemented by 0.5% bovine fetal serum (Gibco Invitrogen, Paisley, Scotland, UK), 10 mmol HEPES buffer (AppliChem GmbH, Darmstadt, Germany) and antibiotics (penicillin, 100 U/ml, streptomycin, 100 mg/ml). Infectious titer $10^{5.67\pm0.15}$ $CCID_{50}$/ml.

Human adenovirus type 2 (adenoid 6) [HAdV2], kindly supplied by District Center of Hygiene and Epidemiology, Plovdiv (Bulgaria). The virus was grown in HEp-2 cells (maintenance solution Dulbecco's modified Eagles's medium DMEM (Gibco, Invitrogen, Paisley, Scotland, UK), supplemented by 0.5% bovine fetal serum (Gibco Invitrogen, Paisley, Scotland, UK), 10 mmol HEPES buffer (AppliChem GmbH, Darmstadt, Germany) and antibiotics (penicillin, 100 U/ml, streptomycin, 100 mg/ml). Infectious titer $10^{5.1\pm0.49}$ $CCID_{50}$/ml.

Herpes simplex virus type 1 (strain Victoria) [HSV-1] was received from Prof. S. Dundarov, National Center of Infectious and Parasitic Diseases, Sofia. The virus was cultivated in cell culture

of Madin–Darby bovine kidney (MDBK) (maintenance solution DMEM Gibco, Invitrogen, Paisley, Scotland, UK, plus 0.5% fetal bovine serum Gibco Invitrogen, Paisley, Scotland, UK, 10 mmol HEPES buffer AppliChem GmbH, Darmstadt, Germany, and antibiotics - penicillin, 100 U/ml, streptomycin, 100 mg/ml). The stock virus infectious titer was $10^{7.0}$ $CCID_{50}$/ml.

Human cytomegalovirus (AD-169 strain) [HCMV], kindly supplied by Prof. Alain le Faou Medical Faculty, University of Nancy, France, grown in MRC-5 cells (maintenance medium DMEM Gibco, Invitrogen, Paisley, Scotland, UK, with 2% fetal bovine serum Gibco Invitrogen, Paisley, Scotland, UK, 10 mmol HEPES buffer AppliChem GmbH, Darmstadt, Germany, and antibiotics - penicillin, 100 U/ml, streptomycin, 100 mg/ml). Infectious titer $10^{4.5}$ $CCID_{50}$/ml.

Vaccinia virus (Elstree strain) (VV), was received from the National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria. VV strain was cultivated on the chorioallantoic membrane of 11-day-old chick embryos (200 $CCID_{50}$/0.2 ml). The embryos were incubated at 37° C for 5 days. After several passages in chick embryos virus strain underwent 2 passages in Vero cells. The stock virus infectious titer was 10^8 $CCID_{50}$ in Vero cells. The maintenance medium was DMEM Gibco, Invitrogen, Paisley, Scotland, UK, with 2% fetal bovine serum Gibco, Invitrogen, Paisley, Scotland, UK, 10 mmol HEPES buffer AppliChem GmbH, Darmstadt, Germany, and antibiotics - penicillin, 100 U/ml, streptomycin, 100 mg/ml).

Cells and media

HEp-2 cells (National Bank for Industrial Microorganisms and Cell Cultures, No. NBIMCC-95, Sofia, Bulgaria) were cultivated in plastic vessels Corning Inc. (Corning, NY, USA), grown in medium consisting of Dulbecco's modified Eagles's medium DMEM (Gibco Invitrogen, Paisley, Scotland, UK), supplemented by 5% bovine fetal serum (Gibco, Invitrogen, Paisley, Scotland, UK), 3.7 mg/ml sodium hydrogen carbonate, 10 mmol HEPES buffer (AppliChem GmbH, Darmstadt, Germany) and antibiotics (penicillin, 100 U/ml, streptomycin, 100 mg/ml). Cells are incubated at 37° C in a humidified atmosphere with 5% CO_2. They were used for cultivation and experiments with PV1, HRSV and HAdV2.

CT cells (calf trachea cell line) from the cell culture bank of the Stephan Angeloff Institute of Microbiology, BAS (Sofia, Bulgaria), were cultivated in plastic vessels Corning Inc. (Corning, NY, USA) with DMEM (Gibco, Invitrogen, Paisley, Scotland, UK), containing 10 mmol HEPES buffer (AppliChem GmbH, Darmstadt, Germany), 10 % fetal bovine serum (Gibco, Invitrogen, Paisley, Scotland, UK) and anibiotics (penicillin 100 U/ml, and streptomycin, 100µg/ml). The cells were used for cultivation and experiments with BVDV.

MDCK (Madin-Darby canine kidney) cells (NBL-2) (ATCC No. CCL-34, USA) were cultivated in plastic vessels Corning Inc. (Corning, NY, USA), and grown in medium DMEM (Gibco, Invitrogen, Paisley, Scotland, UK), containing 10% fetal bovine serum in DMEM (Gibco, Invitrogen, Paisley, Scotland, UK), supplemented with 10 mmol/l HEPES buffer (AppliChem GmbH, Darmstadt, Germany), and antibiotics (penicillin, 100 U/ml, streptomycin, 100 mg/ml). The cells were employed for experiments with IAV/H3N2.

MDBK (Madin-Darby bovine kidney) cells (National Bank for Industrial Microorganisms and Cell Cultures, Sofia) were cultivated in plastic vessels Corning Inc. (Corning, NY, USA), and grown in DMEM medium (Gibco, Invitrogen, Paisley, Scotland, UK), containing 10% fetal bovine serum (Gibco, Invitrogen, Paisley, Scotland, UK), supplemented with 10 mmol HEPES buffer (AppliChem GmbH, Darmstadt, Germany) and antibiotics (penicillin 100 IU/ml, streptomycin 100 µg/ml) in CO_2 incubator (HERA cell 150, Heraeus, Germany) at 37° C/5% CO_2. The cells were employed for experiments with HSV-1.

Vero cells (kind gift of Prof. Syed Sattar, University of Ottawa Medical Faculty) used for VV cultivation in plastic vessels Corning Inc. (Corning, NY, USA), and were grown in DMEM medium (Difco, Invitrogen, Paisley, Scotland, UK), containing 10% fetal bovine serum (Gibco, Invitrogen, Paisley, Scotland, UK), supplemented with 10 mmol HEPES buffer (AppliChem GmbH, Darmstadt, Germany) and antibiotics (penicillin 100 IU/ml, streptomycin 100 µg/ml) in CO_2 incubator (HERA cell 150, Heraeus) at 37° C/5% CO_2. The cells were used for experiments with VV.

MRC-5 cells (human embryo lung diploid cells), supplied by Prof. Alain le Faou, Medical Faculty, University of Nancy, France, were cultivated in plastic vessels Corning Inc. (Corning, NY, USA) with growth medium DMEM (Gibco, Invitrogen, Paisley, Scotland, UK), containing 10 % fetal bovine serum (Gibco, Invitrogen, Paisley, Scotland, UK), 10 mmol HEPES buffer (AppliChem GmbH, Darmstadt, Germany), and antibiotics (penicillin 100 U/ml, and streptomycin, 100 µg/ml). The cells were used for experiments with HCMV.

Table 1a. Antiviral activity against RNA viruses and cytotoxicity of the tested silanes

Sil	PV-1		RSV		Influenza A (H3N2)			BVDV	
No.	HEp-2 CC_{50}	IC_{50}	HEp-2 CC_{50}	IC_{50}	MDCK cells CC_{50}	IC_{50}	SI	CT cells CC_{50}	IC_{50}
1	69.8±10.4	–	25.9±5.5	–	30.0	–	–	6.8	–
2	68.7±21.3	–	21.9±2.3	–	18.0	7.4	2.4	18.9	–
3	73.8±17.0	–	43.4±2.9	–	19.2	6.6	2.9	20.2	–
4	134.4	–	75.9	–	367.5	–	–	280.0	–
5	33.6	–	22.9	–	239.0	–	–	21.0	–
6	201.2	–	179.0	–	–	–	–	593.2	–
7	≈ 1000.0	–	644.9	–	444.2	–	–	551.8	–
8	85.5±32.6	–	15.0±8.5	–	23.5	–	–	57. 9	–
9	≈ 1000.0	–	50.3±0.4	–	444.2	–	–	917.1	–
10	≈ 1000.0	–	182.7±96.4	–	156.0	–	–	210.4	–
11	755.5±50.0	–	494.9±41.3	–	444.2	–	–	695.1	–
12	201.2	–	76.8	–	60.0	–	–	462.1	–
13	84.1	–	30.1	–	134.2	–	–	844.2	–
14	486.9	–	297.1	–	106.0	–	–	642.6	–
15	80.0	–	57.8	–	170.5	–	–	156.7	–
17	180.0	–	165.5	–	166.7	–	–	607.1	–
19	391.8±70.3	–	58.4±11.0	–	65.5	–	–	56.6	–
20	>1000.0	–	≥1000.0	–	2533.0	–	–	2660.5	–
21	69.1±11.4	–	21.2±0.7	–	21.0	–	–	60.5	–
22	58.7±7.3	–	47.5±5.1	–	42.9	–	–	20.1	–
23	20.9	–	21.5	–	93.2	–	–	70.1	–
24	20.7	–	22.6	–	32.0	–	–	35.1	–
25	77.8±7.1	–	64.7±2.1	–	55.9	9.6	5.8	18.3	–
26	79.3±9.2	–	19.8±1.1	–	19.0	–	–	365.2	–
27	≈ 1000.0	–	39.4±15.0	–	317.0	–	–	429.9	–
28	155.2	–	106.4	–	247.1	–	–	141.2	–
29	52.3	–	40.1	–	232.9	–	–	703.0	–
30	821.8	–	≥1000.0	–	284.0	–	–	1108.4	–
31	57.4	–	59.3	–	52.1	–	–	62.0	–
32	59.1	–	46.3	–	45.2	–	–	28.0	–
33	63.9	–	44.5	–	155.0	–	–	50.4	–
34	769.0	–	≥1000.0	–	2533.0	–	–	857.9	–
35	61.3	–	57.0	–	–	–	–	61.3	–
36	54.4	–	24.5	–	–	–	–	54.4	–
37	33.1	–	22.6	–	–	–	–	33.1	–
38	158.6	–	–	–	–	–	–	158.6	–
39	–	–	–	–	–	–	–	–	–
40	–	–	–	–	–	–	–	–	–
41	–	–	–	–	–	–	–	–	–
42	–	–	–	–	398.0	–	–	516.3	–
43	–	–	–	–	137.0	–	–	50.6	–
44	–	–	–	–	537.0	–	–	26.5	–
45	–	–	–	–	560.0	–	–	1357.7	–
Ref.	50.3±9.2	0.8±0.6	≥4095.0	13.1	94.0	0.03	3133.0	946.6	5.5
	Disoxaril		Ribavirin		Rimantadine			Ribavirin	

Table 1b. Antiviral activity against DNA viruses and cytotoxicity of the tested silanes

Sil	HAdV-2		HSV-1			VV			HCMV						
No.	HEp-2 CC_{50}	IC_{50}	MDBK cells CC_{50}	IC_{50}	SI	Vero cells CC_{50}	IC_{50}	SI	MRC-5 cells CC_{50}	320 $CCID_{50}$		32 $CCID_{50}$		3.2 $CCID_{50}$	
										IC_{50}	SI	IC_{50}	SI	IC_{50}	SI
1	16.0±4.3	–	16.2	–	–	115.9	–	–	31.7	–	–	–	–	–	–
2	18.0±1.4	–	12.4	6.9	1.8	50.5	–	–	26.7	15.5	1.7	2.5	10.8	2.1	13.0
3	14.2±6.1	–	17.7	–	–	48.1	–	–	31.7	–	–	–	–	31.7	–
4	45.1	–	296.0	–	–	170.1	–	–	19.6	–	–	12.4	1.6	n.d.	n.d.
5	18.0±0.5	–	–	–	–	17.7	–	–	31.2	–	–	–	–	–	–
6	60.0	–	552.0	–	–	156.9	–	–	46.7	–	–	–	–	–	–
7	454.0±51.5	–	656.0	–	–	538.8	–	–	18.8	15.9	1.2	3.1	6.0	n.d.	n.d.
8	16.4±2.2	–	42.3	–	–	151.4	–	–	316.4	–	–	–	–	100.7	3.1
9	50.3±0.4	–	582.9	–	–	48.7	14.2	3.4	63.8	–	–	–	–	–	–
10	164.2±59.6	–	343.6	–	–	354.4	–	–	23.0	–	–	–	–	–	–
11	520.4±87.4	–	503.9	–	–	401.8	–	–	250.1	–	–	–	–	–	–
12	40.6	–	385.0	–	–	126.5	–	–	32.0	–	–	32.0	–	32.0	–
13	15.1	–	650.0	–	–	128.9	–	–	31.2	–	–	–	–	–	–
14	169.9	–	476.0	–	–	438.6	–	–	173.6	–	–	–	–	–	–
15	20.6	–	187.0	–	–	54.4	–	–	31.2	–	–	10.1	3.1	n.d.	n.d.
17	58.5	–	516.0	–	–	168.7	–	–	203.0	–	–	31.1	8.5	–	–
19	48.1	–	77.8	–	–	156.7	–	–	266.9	250.2	1.1	–	–	31.1	8.5
20	665.9	–	–	–	–	>2000.0	–	–	>320.0	–	–	–	–	10.0	≥32.1
21	14.2	–	56.4	–	–	13.2	–	–	32.0	–	–	–	–	6.2	5.1
22	4.2	–	56.0	–	–	117.5	–	–	8.2	–	–	–	–	–	–
23	17.9	–	–	–	–	28.4	–	–	27.1	–	–	–	_	–	–
24	17.2	–	–	–	–	26.4	–	–	3.1	–	–	–	–	–	–
25	30.1	–	63.7	–	–	54.4	–	–	31.7	–	–	–	–	31.7	–
26	18.6	–	47.6	–	–	_	–	–	–	–	_	–	–	–	–
27	24.4	–	768.9	–	–	87.6	–	–	31.2	–	–	1.0	30.9	n.d.	n.d.
28	64.1	–	184.0	–	–	152.4	–	–	31.2	–	–	–	–	–	–
29	18.6	–	859.0	–	–	20.5	–	–	31.2	–	–	–	–	–	–
30	1000.0	–	765.0	–	–	553.0	–	–	>320.0	–	–	320.0	–	202.2	1.6
31	57.0	–	–	–	–	123.0	–	–	–	_	–	–	–	–	–
32	35.7	–	–	–	–	53.4	–	–	3.2	_	–	–	–	2.8	1.1
33	45.7	–	–	–	–	40.1	–	–	130.8	–	–	–	–	100.7	1.3
34	≥1000.0	–	–	–	–	362.7	–	–	>320.0	–	–	107.7	≥3.0	n.d.	n.d.
35	42.2	–	55.0	–	–	52.4	–	–	31.7	–	–	–	–	–	–
36	16.7	–	21.0	–	–	19.6	–	–	31.7	–	–	–	–	–	–
37	32.6	–	33.0	–	–	20.9	–	–	31.7	–	–	–	–	31.7	–
38	55.5	–	247.0	–	–	40.4	–	–	31.7	–	–	–	–	–	–
39	–	–	49.0	–	–	–	–	–	31.7	–	–	–	–	–	–
40	–	–	589.0	–	–	–	–	–	185.4	–	–	160.8	1.1	24.7	7.5
41	–	–	213.0	–	–	–	–	–	31.2	–	–	–	–	–	–
42	–	–	–	–	–	–	–	–	26.4	–	–	10.0	2.6	n.d.	n.d.
43	–	–	–	–	–	–	–	–	56.0	–	–	–	–	–	–
44	–	–	–	–	–	–	–	–	31.7	–	–	–	–	–	–
45	–	–	–	–	–	–	–	–	205.8	–	–	114.5	1.8	12.4	16.6
Ref.	≥4 000.0	131.0	1296.0	1.5	881.6	100.0	0.6	172.4	1000.0	2.4	420.2	1.2	854.7	0.3	3125
	Ribavirin		Acyclovir			IUdR			Gancyclovir						

Determination of cytotoxicity

The neutral red uptake assay based on the initial protocol described by Borenfreund and Puerner (1984) was used. Monolayer cells in 96-well plates are inoculated with 0.1 ml of the tested solution in several serial dilutions performed in a maintenance medium. Cells inoculated with 0.1 ml maintenance medium (no compound in the medium), serve as a control. Each tested dilution is inoculated in 6 wells of the cell culture plate. Then, the cells were incubated at 37° C in a humidified atmosphere with 5% CO_2 and the cell vitality (neutral red uptake) at the 48th, 72nd or 96th h was estimated (following light microscopy observation) using ELISA reader at $OD_{540nm.}$. The 50% cytotoxic concentration (CC_{50}) is calculated in comparison to the cell control by applying the regression analysis with the help of Origin 6.1 computer program.

Antiviral activity testing

The cytopathic effect (CPE) inhibition test is used for measuring the antiviral effect. Monolayer cells in 96-well plates are inoculated with 0.1 ml virus suspension containing 100 $CCID_{50}$. After an hour for virus adsorption (two hours in the case of HRSV-A2) in a humidified atmosphere at 37° C and 5% CO_2, excessive virus is discarded and cells are inoculated with 0.2 ml of maintenance medium containing serial 0.5 lg dilutions of the tested preparation. Mock-infected cells are left for cell and toxicity controls. The virus CPE is scored daily by inverted light microscope (Olympus CK40, Japan) at 125x and 400x magnification on a 0-4 basis (4 representing total cell destruction) till the appearance of its maximum in the virus control wells (with no compound in the maintenance medium) – the 48th h p.i. for PV1, 72nd h for HRSV-A2 and the 4th day (96th h) p.i. for HAdV-2. When maximum CPE in the virus control wells is reached, cells are processed according to the neutral red procedure described above. The percent of virus CPE protection is calculated by the following formula (Pannecouque *et al.,* 2008):

$$\frac{meanOD_{Test} - meanOD_{VC}}{meanOD_{TC} - meanOD_{VC}} x100$$

$(OD_{Test} – OD_{VC})/(OD_{TC} – OD_{VC})$ x 100 (%), where OD_{Test} is the mean optical density (OD) of the test sample, OD_{VC} – the absorbance of the virus-infected control (no compound in the maintenance medium), and OD_{TC} – the OD of the mock-infected control (toxicity control).

The 50% virus inhibitory concentration (IC_{50}) is determined by applying the regression analysis with the help of Origin 6.1 computer program and it is expressed as the concentration that achieves 50% protection of virus-infected cells. The selectivity index (SI) is evaluated as the ratio between CC_{50} and IC_{50} ($SI = CC_{50}/IC_{50}$).

Each of the tests described above was done in triplicate to quadruplicate, with four cell culture wells per test sample.

Antiviral activity testing against HCMV

The cytopathic effect (CPE) inhibition test is used for measuring the antiviral effect. Monolayer cells in 96-well plates are inoculated with 0.1 ml virus suspension containing 320, 32 and 3.2 and 0.32 $CCID_{50}$. After an hour for virus adsorption in a humidified atmosphere at 37° C and 5% CO_2, excessive virus is discarded and cells are inoculated with 0.1 ml of maintenance medium containing serial 0.5 lg dilutions of the tested preparation. Mock-infected cells are left for cell and toxicity controls. The virus CPE is scored daily by inverted light microscope (Olympus CK40, Japan) at 400x magnification on a 0-4 basis (4 representing total cell destruction = CPE 100%) till the appearance of its maximum in the virus control wells (with no compound in the maintenance medium). The 50% virus inhibitory concentration (IC_{50}) is determined by applying the regression analysis with the help of Origin 6.1 computer program and it is expressed as the concentration that achieves 50% protection of virus-infected cells. The selectivity index (SI) is evaluated as the ratio between CC_{50} and IC_{50} ($SI = CC_{50}/IC_{50}$).

Results

Effects against poliovirus 1 (PV1), respiratory syncytial virus (RSV) and human adenovirus 2 (HAdV2) in HEp-2 cells

None of the tested silanes showed activity against PV1(LSc-2ab strain) replication in HEp-2 cells. Only the reference substance, disoxaril, demonstrated a pronounced activity: CC_{50} (μM) – 56.8 and 43.8, CC_{50} (μM) – 1.2 and 0.4, and SI – 48.1 and 104.3, respectively.

Table 1 presents the data for cytotoxicity for HEp-2 cells.

All tested Silanes were inactive vs. RSV strain Long in HEp-2 cells. Ribavirin as a reference antiviral manifested a strong inhibitory effect: CC_{50} (μM) = ≥4095.0; IC_{50} (μM) = 13.1; SI = ≥312.5.

Figure 2 illustrates an example of both antiviral and cytotoxicity testing in HEp-2 cells using the method of Borenfreund and Puerner (1984).

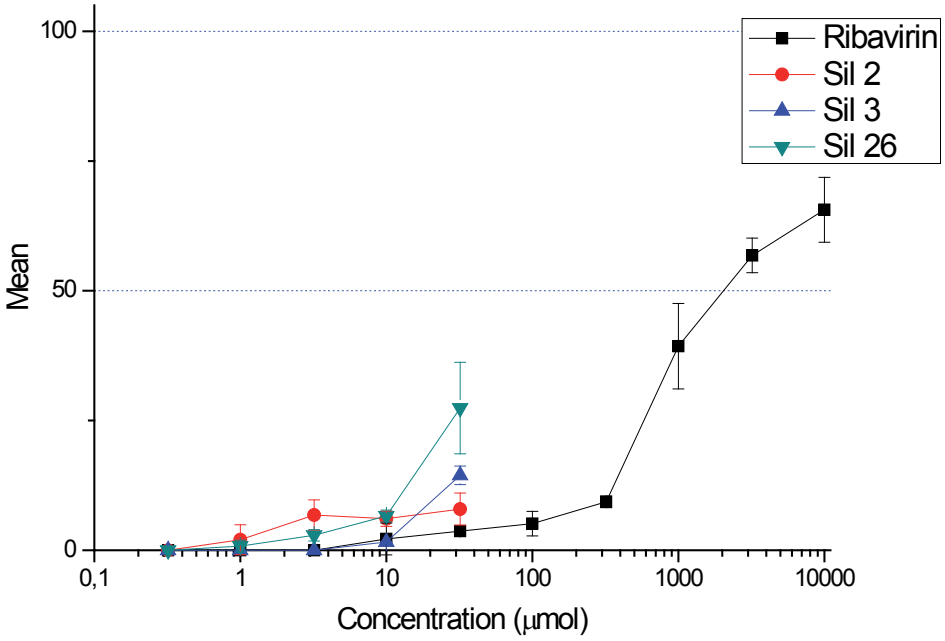


Fig. 2. Testing of silane 2, silane 3 and silane 26 for activity against respiratory syncytial virus (RSV) and cytotoxicity in HEp-2 cells. None of the tested silanes succeeded to inhibit virus replication by 50% even at the highest tolerated nontoxic concentration. So, IC_{50} cannot be determined.

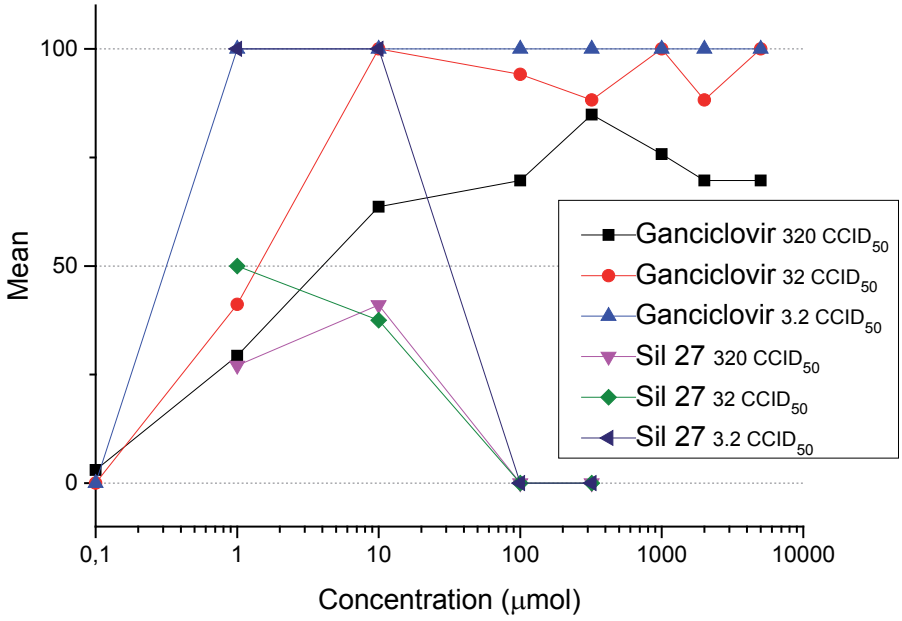


Fig. 3. Testing of silane 27 activity against human cytomegalovirus (HCMV) and cytotoxicity in MRC-5 cells.

Silanes were inactive toward human adenovirus (type 2) as well, the reference compound ribavirin shown a marked effect: CC_{50} (μM) = ≥4 000.0; IC_{50} (μM) =131.0; SI ≥30.5.

Effect against bovine viral diarrhea virus (BVDV)

No one of the tested compounds showed activity on replication of viral diarrhea virus (BVDV) TVM strain in CT cells. A marked effect was established with the reference substance ribavirin: CC_{50} = 946.6, IC_{50} (μM) = 5.5, SI = 171.5. The compounds' cytotoxicity data is given in Table 1a.

Effect against influenza virus A (H3N2)

As seen in Table 1a, one of the tested compounds (Sil 25) manifested a moderate effect manifested a borderline effect on replication of influenza virus A/Aichi/68/2 (H3N2) in MDCK cells, and other 2 (Sil 2 and Sil 3) – a borderline activity. The reference substance, rimantadine. HCl was very highly active.

Effect against herpes simplex virus type 1

Only one of the studied silanes showed an effect versus replication of HSV-1 in MDBK cells; this compound was Sil 2 (Table 1b). The reference antiviral acyclovir was highly active.

Effect against human cytomegalovirus (AD-169) in MRC-5 cells

Completely other results were obtained in the testing of silanes vs. human cytomegalovirus (AD-169 strain) replication in MRC-5 cells (Table 1b). It was interesting that several compounds manifested a marked activity, being tested at three different m.o.i., namely 320 $CCID_{50}$ 32 $CCID_{50}$ and 3.2 $CCID_{50.}$ Table 1b summarizes the results obtained. As seen, the effects registered were strongly depended on the m.o.i. values. At the highest m.o.i., 320 $CCID_{50}$ the silanes were inactive. At the 10-fold lower m.o.i., 32 $CCID_{50}$ one compound, Sil 27, manifested a marked antiviral effect (Fig. 3), three compounds were with a moderate activity Sil 2, Sil 19 and Sil 7, and other two with a weak (borderline) activity ─Sil 15 and Sil 34. At the lowest m.o.i. , 3.2 $CCID_{50}$, seven silanes showed anti-HCMV effect: one of them, Sil 20 – a marked activity, two, Sil 45 and Sil 2 – a moderate one, and four, Sil 40, Sil 19, Sil 21 and Sil 8 – a weak activity.

Effect against vaccinia virus in Vero cells

The study of anti-poxvirus activity of silanes on the model of vaccinia virus replication in Vero cells resulted in only one compound (Sil 9) with a small effect (Table 1b).

Discussion

The screening carried out for antiviral activity *in vitro* (in cell culture experiments) of 43 silanes embraced eight viruses belonging to taxonomic entities (families) including causative agents of infections to which chemotherapy is indicated. The results obtained demonstrated a marked activity of di(hexadecanoyloxy)diphenylsilan (compound 27) only against human cytomegalovirus: SI = 30.9. A marked activity toward this virus at a low m.o.i. (3.2 $CCID_{50}$ per microplate well) was recorded by 1-o-dimethyl(octadecyl)silyl-(2,3,4,6-o-tetraacetyl-β-D-glucopyranosid) (compound 20). Small to borderline effect against this virus was found by silanes 2, 7, 15, 19 and 34, at silanes 2, 3 and 25 toward influenza virus A(H3N2) and at silane 9 versus vaccinia virus. No one of the compounds did manifested activity towards PV1, BVDV, RSV, HuAdV2 and HSV type 1.

As concerns the cytotoxicity it was established a strong variation towards different cell cultures used. Higher cytotoxicity values (CC_{50} <20 μM) were recorded as follows: silanes 1, 2, 5, 21, 22, 23, 24, 26 and 36 towards HEp-2 cells, silanes 1, 2, 3, 5 and 25 – toward CT cells, silanes 2, 3 and 26 toward MDCK cells, 1, 2 and 3 toward MDBK cells, silanes 4, 7, 22, 23, 24 and 32 toward MRC-5 cells, and silanes 5, 21 and 36 vs Vero cells. Summarizing the cytotoxicity data it could indicate several compounds possessing wider toxicity, on more than one cell culture: silane 1 – on HEp-2 cells, CT cells and MDBK cells; silane 2 – on HEp-2, CT and MDCK cells; silane 5 – on HEp-2, CT, MDBK and Vero cells; silanes 22, 23 and 24 – on HEp-2 and MRC-5 cells; silane 26 – on HEp-2 and MDCK cells; silane 36 – on HEp-2 and Vero cells. Compound 21 manifested a marked cytotoxicity only on HEp-2 cells. Comparing the cytotoxicity susceptibility of the different cell cultures species it could marked the higher susceptibility of the HEp-2 cells.

Evidently, the realization of quantitative structure-activity relationship (QSAR) of the silanes included in this study would contribute for the further planned synthesis of active antiviral compounds, especially directed against HCMV.

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