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Review article

Dispirotripiperazine-core compounds, their biological activity with a focus on broad antiviral property, and perspectives in drug design (mini-review)

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ABSTRACT

Viruses are obligate intracellular parasites and have evolved to enter the host cell. To gain access they come into contact with the host cell through an initial adhesion, and some viruses from different genus may use heparan sulfate proteoglycans for it. The successful inhibition of this early event of the infection by synthetic molecules has always been an attractive target for medicinal chemists. Numerous reports have yielded insights into the function of compounds based on the dispirotripiperazine scaffold. Analysis suggests that this is a structural requirement for inhibiting the interactions between viruses and cell-surface heparan sulfate proteoglycans, thus preventing virus entry and replication. This review summarizes our current knowledge about the early history of development, synthesis, structure-activity relationships and antiviral evaluation of dispirotripiperazine-based compounds and where they are going in the future.

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Contents

1. Introduction

Viruses are the most prevalent infectious pathogens worldwide,

and unknown viruses including new serotypes or mutants of known viruses constantly appear. Viral disease outbreaks quite often occur on all continents. For example, the recent rapid spread of a coronavirus-caused (the family *Coronaviridae*) respiratory illness COVID-19, which began in Wuhan, China in December 2019, has killed at least 1,2 M people and infected more than 31 M

worldwide (World health Organization data available on Oct 25, 2020) − and the pandemic is still ongoing [1]. In the beginning of 2019, a measles epidemic caused by the measles virus (the family *Paramyxoviridae*) broke out in the Democratic Republic of the Congo, which by January 2020 resulted in nearly 6000 fatalities and 310,000 confirmed cases of infection [2]. In 2015−2016, an outbreak of Zika fever caused by the Zika virus (the family *Flaviviridae*) quickly spread over Brazil and then to other parts of South and North America [3]. In 2013−2015, the World Health Organization reported that the epidemic of an ebolavirus-caused hemorrhagic fever (the family *Filoviridae*) in West Africa resulted in more than 28,700 cases and about 11,300 deaths [4] and the most recent outbreak in the Democratic Republic of the Congo resulted in over 2200 deaths [5]. All of these recent viral outbreaks point to the fact that even though they stimulated increased drug discovery efforts, none have yet to bear fruit in the form of an approved antiviral likely due to limited and inconsistent funding over the years. There is also a need to apply different computational technologies as early as possible in an attempt to speed up the process of antiviral drug discovery [6,7].

Different antiviral compounds, alone or in combination, have been used to treat several viral diseases, for example, HIV/AIDS, hepatitis B and influenza. Currently approved pharmacotherapies suffer from three major problems: (i) the emergence of resistance, (ii) low bioavailability and (iii) severe toxicity [8]. In addition, many of these drugs share the same target (e.g. RNA polymerase, reverse transcriptase etc); the incidence of cross-resistance is a growing concern [9]. However, antiviral drugs are generally specific, and cross-resistance usually occurs for antivirals targeting the same virus or viruses in the same family. Due to the many problems caused by the currently available drugs, the development of new antiviral compounds with different modes of action is urgently needed.

All drugs that are effective against viruses must target some features of viral replication in the host, which can be efficiently inhibited without causing toxicity. Consequently, to broaden therapy of viral infections and to circumvent mechanisms of resistance, an inhibitor targeting the early process of infection will would be of great value. A promising direction in searching for novel effective antiviral compounds is to find those with a narrow specificity of the action on the components of the viral life cycle. One of the unique mechanisms of action concerning viral invasion is the prevention of the virus adsorption through specific blockage of heparan sulfate (HS) receptors/attachment factors [10].

The first key step in viral replication is entry into the host cell, which requires the binding of the virion to the cell surface. Binding is mediated by various typical cell-surface proteins, for example, glycoconjugates (glycoproteins, glycolipids) or proteoglycans, which are a prerequisite for their functions as receptors or attachment factors. The most common cell-surface structures are HS proteoglycans [10]. In the last decade, this mechanism of binding has been discovered in several viruses (alpha-, flavi-, herpes-, parvo-, papilloma-, picorna-, and retroviruses), and this number is constantly growing [11]. Moreover, besides viruses, various bacterial pathogens also use interaction with HS proteoglycans to penetrate the host cell [12].

The development of antiviral agents targeting inhibition of viral attachment via HS glycosaminoglycans binding has several advantages, including (i) complete prevention of viral infection, (ii) unnecessary intracellular drug delivery, and (iii) treatment of a wide range of different viruses that use the same entry mechanism. There are several different therapeutic classes of drugs that interfere with HS glycosaminoglycan (HSGAG) functions − GAG mimetics that compete with endogenous GAGs, enzymes that remove or modify HSGAGs, and cationic polymers or small molecules that

bind to and inhibit HSGAGs [13]. Among the classes of small molecules, catechines (epigallocatechin gallate), aminoquinolines (surfen), and finally, dispirotripiperazines (DSTP27) seem to be attractive for further development as antivirals with a mechanism of action aimed at inhibiting HSGAG [13].

Dispirotripiperazines belong to the class of dispiro compounds, i.e. tricyclic molecules with two shared atoms (or "spiro atoms"), and they can be conditionally divided into three types depending on their component composition: a) a dispiro system with three six-membered cycles − piperazines − or "6-6-6", b) a dispiro system with two six-membered cycles and one seven-membered cycle − homopiperazine − or "6-7-6" and c) a dispiro system with one six-membered cycle and two seven-membered cycles or "7-6-6" (a dispiro system with three seven-membered cycles is highly unstable and thus not shown) (Fig. 1). A unique feature of dispirotripiperazines is the presence of two quaternary positively charged nitrogen atoms in the structure, which are possibly responsible for electrostatic interactions with negatively charged HSGAGs. Initially, dispirotripiperazines were investigated as antitumor drugs (prospidine, spirobromine) [14,15]; however, in the following studies, it was found that these compounds also have good antiviral activity [16].

In this review, we provide an overall review of the status of dispirotripiperazine derivatives and their evolution as broad-spectrum antivirals. General information on the role of HS glycosaminoglycans in the entry of different viruses is described in the first section. The second section introduces the history of dispirotripiperazine chemistry, the discovery of the first antitumor drugs based on dispirotripiperazine pharmacophore, and the recent studies in this area. Finally, in the third section, we provide an overview of current knowledge on the development of dispirotripiperazines with a heparan-binding mechanism, an emphasis on their antiviral activity and future potential.

1. The role of heparan sulfate proteoglycans in the first step of viral entry into the host cell

Infection of enveloped and non-enveloped viruses occurs in several steps. The viral replication cycle is divided into attachment, penetration, uncoating, nucleic acid synthesis, translation of viral proteins, virion assembly and release. The first stage of viral replication − attachment − implies that the virus comes into close contact with the host cell, and this is a prerequisite for the following receptor binding. Viral adsorption is mediated by the interactions between viral envelope proteins and receptors or attachment factors of host cells. These surface molecules include ligand-binding receptors, for example, ion channels, enzymes, glycoconjugates (glycoproteins and glycolipids) and proteoglycans [17]. It is important to emphasize that many viruses may use multiple attachment factors and receptors in parallel or sequential interactions with them, or use different receptors or co-opt cellular processes to penetrate into different cell types [17].

It is well known that one of the cell-surface molecules, HS proteoglycans (HSPGs), are the major component of the extracellular matrix and are located on the plasma membrane of all mammalian cells [18]. Each HSPG consists of one or more HS glycosaminoglycan chain, long linear polysaccharides (40−300 sugar residues) containing repeating disaccharide residues covalently bound to the central core protein via a tetrasaccharide bridge (Fig. 2A) [19].

Cell-surface HSPGs can be divided into two main groups depending on the nature of the core protein (Fig. 2B). Syndecans are transmembrane proteins structurally composed of an N-terminal signal sequence, an ectodomain and a short C-terminal cytosolic tail. They are anchored to the cell surface by a hydrophobic
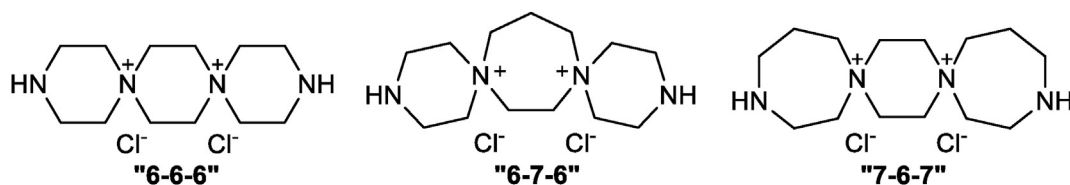
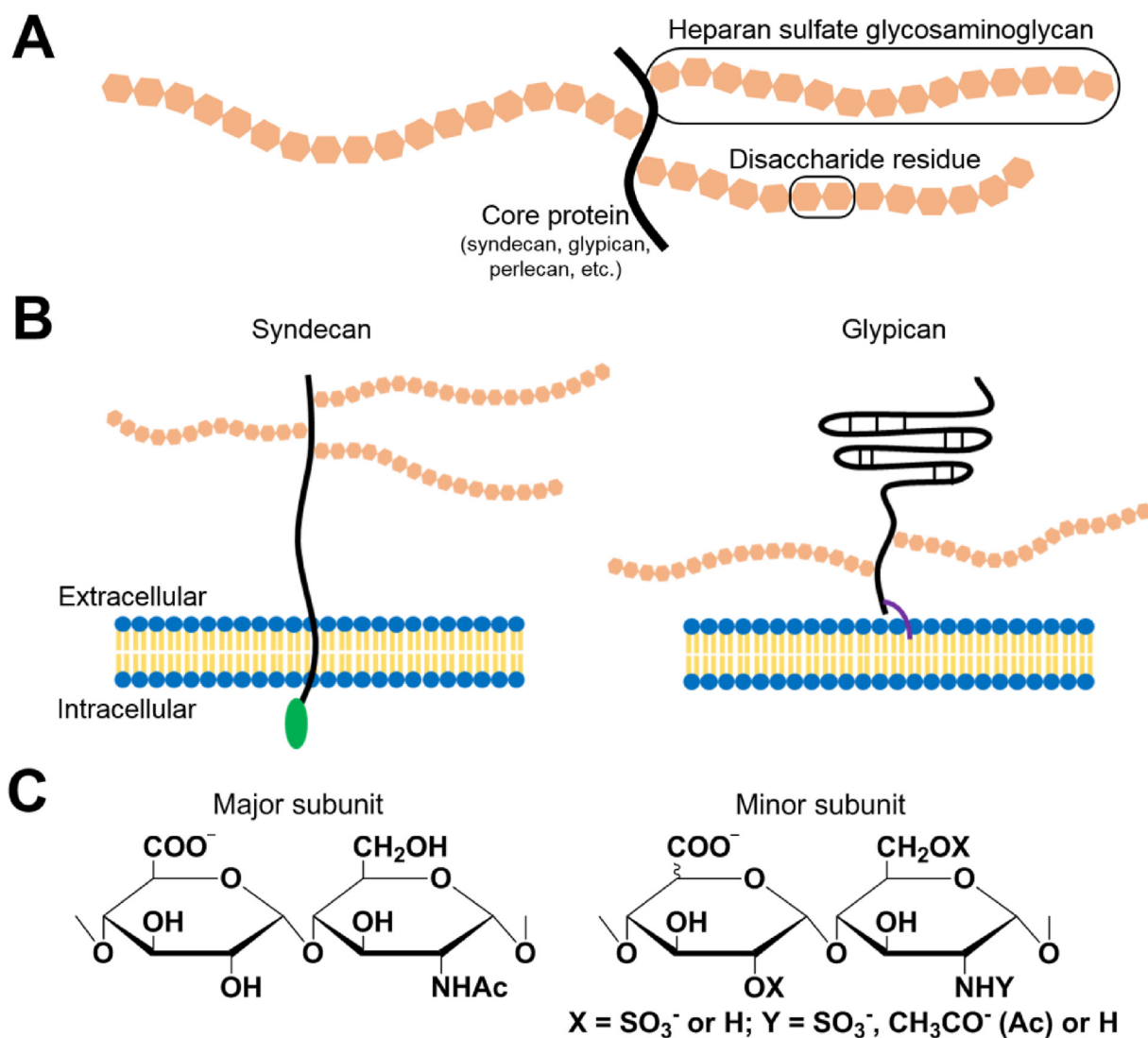**Fig. 1.** Three types of dispirotripiperazines.

Fig. 2. A) General structure of HSPGs; B) Overall structure and location of two main classes of cell-surface HSPGs; C) Chemical structure of the major and minor disaccharide subunits of HSGAGs.

transmembrane domain [20]. Glypicans are not transmembrane glycoproteins but are extracellularly bound to the plasma membrane via a C-terminal lipid moiety, known as a glyco-sylphosphatidylinositol linkage. They contain a large globular domain stabilized by di-sulfide (S—S) bonds at their C-terminal part. In contrast to the syndecans, HSGAGs of glypicans are covalently bound near the *C*-end, and thus are very closely located to the cell membrane [21].

The most common disaccharide unit of HSGAGs is composed of glucuronic acid linked to *N*-acetylglucosamine, and the minor disaccharide units include 2-*O*-sulfated glucuronic acid or 6-*O*-sulfated glucosamine, and the *N*-position of glucosamine can also be sulfated or unmodified (Fig. 2C). The presence and specific location of negatively charged sulfate groups and the orientation of carboxyl groups in HSGAGs determine the position of binding sites with ligands [22].

HSPGs interact with a wide range of proteins (at least 300), such as growth factors, cytokines, chemokines and coagulation factors, leading to involvement in many important normal biological processes. The physiological function of HS binding proteins varies from cell adhesion, migration and differentiation, blood coagulation to wound healing, immune response, synapse organization

and so on [13,19]. At the same time, HSPGs are involved in causing cancer and neurodegenerative disorders [13].

In addition, HSPGs also provide binding sites for many human bacterial and viral pathogens [23,24]. It was found that herpes simplex virus (HSV), human papillomavirus (HPV), human immunodeficiency virus (HIV), and hepatitis virus all use HSGAG as a receptor or attachment factor for entry into the host cell [25−29]. Initial contact with host cells via HSGAG is also required for some echo- and rhinoviruses, yellow fever virus, some coronaviruses, cytomegalovirus, foot-and-mouth disease virus and filoviruses [30−39]. However, different biological studies of the role of HSPGs in the entry of such viruses as respiratory syncytial virus (RSV) and Zika virus indicate different outcomes, and more clear evidence of their role is needed [40−42].

HSGAG can be classed as an anionic molecule due to the presence of sulfate and carboxyl groups and it is widely used as a binding site for many viruses from different classes which also makes HSGAG a potential target for the prevention of viral infection and pathogenicity. It is reasonable to hypothesize that cationic antiviral agents will effectively block the electrostatic interactions between HSGAG and viral envelope glycoproteins by temporary competitive binding to the negatively charged HS. In this context, we now consider the research and development of potential antivirals in the class of cationic small molecules, such as dispirotripiperazines.

2. The past of dispirotripiperazines: a brief background of their discovery as effective antitumor drugs and recent research in this area

Research into the chemistry of dispirotripiperazines started with the discovery of spirazidine in a project for a new antitumor agent based on 1,4-di-(β-chloroethyl)-piperazine by Soviet researchers (only one compound from this chemical class was previously known [43]) [44,45]. It was established that 1,4-di-(β-chloroethyl)-piperazine rapidly dimerized into N,N'''-di-(β-chloroethyl)-N',N''-dispirotripiperazinium dichloride **6** in the presence of alcohol solution at reflux. The structure of **6** was identified by the following synthesis. According to Scheme 1, the substitution of the initial 1-benzoyl-4-(β-oxyethyl)-piperazine hydrochloride **1** with thionyl chloride led to 1-benzoyl-4-(β-chloroethyl)-piperazine hydrochloride **2**. Base of **2** dimer in the presence of MeOH to N,N'''-dibenzoyl-N',N''-dispirotripiperazinium dichloride **3**. N',N''-Dispirotripiperazinium dichloride **4**, or dispirotripiperazine "6-6-6",

was obtained by removal of the benzoyl groups of **3** in refluxing hydrochloric acid and subsequent treatment with lithium hydroxide. Dispiropiperazine **4** was successfully reacted with ethylene oxide, N,N'''-di-(β-oxyethyl)-N',N''-dispirotripiperazinium dichloride **5** was isolated and subsequently converted into N,N'''-di-(β-chloroethyl)-N',N''-dispirotripiperazinium dichloride **6** or spirazidine [44,45].

In the following studies, it was found that spirazidine had a wide spectrum of anti-cancer activity with relatively low toxicity. This compound was highly effective in the treatment of malignant neoplasms of the larynx or nasopharynx and cancer of the lungs or bladder. Moreover, it was noted that there was no negative effect on hematopoiesis [46].

However, spirazidine has some shortcomings such as low chemical stability, which was an obstacle to industrial development. Using 3,12-diaza-6,9-diazoniadispiro[5.2.5.2]hexadecane dichloride **4** as a key intermediate in the synthesis of compounds with this heterocyclic system [47], Mikhalev et al. in 1965−1976 developed several new dispirotripiperazine derivatives, including prospidine **7** and spirobromine **8** (Scheme 2), which are still used as antitumor drugs [15,46,48]. 3,12-Bis(3-chloro-2-hydroxypropyl)-3,12-diaza-6,9-diazoniadispiro[5.2.5.2]hexadecane dichloride **7** (prospidine) was obtained by reaction of **4** with epichlorohydrin, and 3,12-bis(3-bromopropanoyl)-3,12-diaza-6,9-diazoniadispiro[5.2.5.2]hexadecane dichloride **8** (spirobromine) was synthesized by treatment of dispirotripiperazine **4** with 3-bromopropanoyl chloride (Scheme 2).

Clinical studies of prospidine demonstrated efficacy in the treatment of patients with laryngeal, or lung, or bladder cancers [14]. In addition, this drug has also been studied in the therapy of rheumatoid arthritis [49−51] as it inhibits proinflammatory cytokine production [49]. Spirobromine, unlike prospidine, had a different spectrum of antitumor action as it is more active towards sarcoma 45 and 536 in rats and sarcoma 180 in mice [52]. Spirobromine possesses antileukemic activity, whereas prospidine did not influence the development of the leukemia process in mice [52]. It was established that the chemotherapeutic index of spirobromine on Jensen sarcoma was two-fold greater than that of prospidine. Moreover, spirobromine was less toxic than prospidine ($LD_{50} = 1924$ mg/kg vs. $LD_{50} = 100$ mg/kg) after a single intraperitoneal injection in mice [52]. Prospidine and spirobromine are still used by Russian physicians in oncology practice as cytostatic antitumor chemotherapeutic drugs with an alkylating mode of action while little is known of their use in the west.
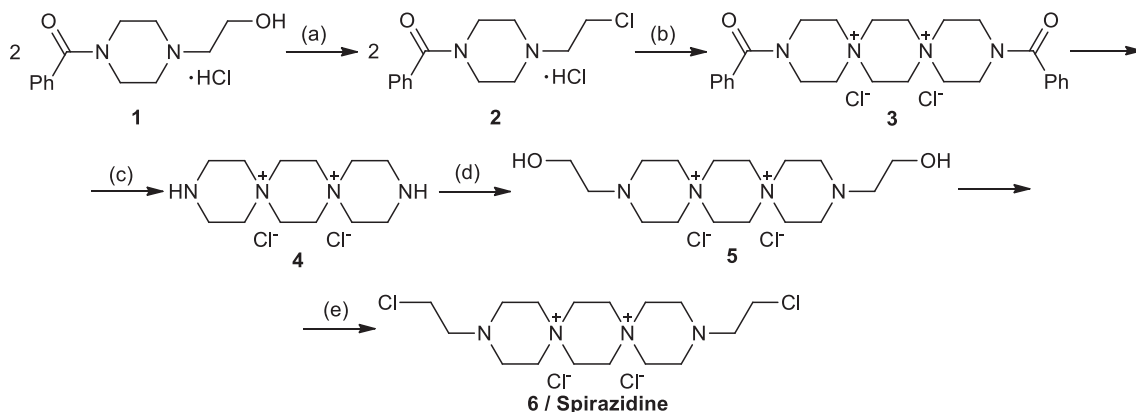
Reagents and conditions: (a) SOCl$_2$; (b) NaOH, MeOH; (c) 1. HCl, H$_2$O, 2. LiOH, MeOH; (d) epoxide; (e) 1. SOCl$_2$, 2. LiOH, MeOH

Scheme 1. Spirazidine synthesis.
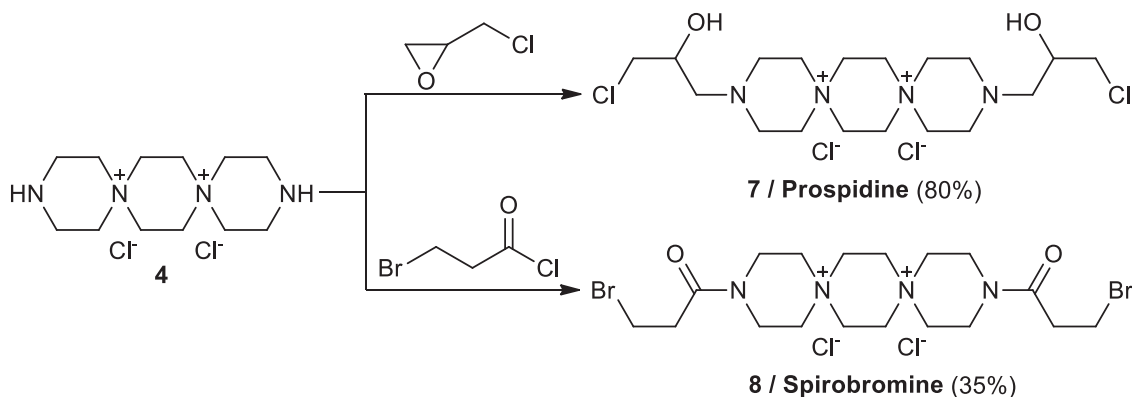
Scheme 2. One-step synthesis of prospidine and spirobromine.

Prospidine and spirobromine may be considered bis(β-chloroethyl)amine derivatives. While prospidine has γ-chloro-β-hydroxypropyl groups on the terminal nitrogen atoms, which are required for its alkylating ability, spirobromine contains β-bromopropionyl residues at the same site, and this structural distinction influences their different spectrum of antitumor activity [53]. The tricyclic system also contributes to the activity, whereas the compounds with an "open" central ring of the system are inactive [54]. However, the role of such a positively charged system on antitumor capacity is unclear, Dorokhova et al. proposed that the dispiro system serves as a carrier for the functional groups [54]. Pol'shakov et al. found that the guanine residues may be the main binding sites for prospidine to DNA, in contrast to spirobromine, which does not bind to them [55]. Moreover, Geodakyan and Chernov suggested that the effect of prospidine on intracellular processes in tumor cells may be mediated by the effect of the drug on the permeability of their plasma membrane [56,57]. But these studies were carried out many years ago and have not yet been confirmed with modern methods.

Further investigations to identify spirobromine analogues with similar antitumor activity failed as all of the synthesized compounds were found to be less active than spirobromine [58].

In 2002, Safonova et al. [59] synthesized a series of 3,12-diaza-6,9-diazoniadispiro[5.2.5.3]heptadecane bisquaternary salts in the continuation of the search for novel antitumor drugs based on the dispirotripiperazine scaffold. The formation of this heterocyclic spiro system was initiated with the cycloquaternization of the parent di(benzoylpiperazinyl)propane **10** with ethylene dibromide, which provided the key intermediate **11** (Scheme 3). Hydrolysis of *N*-benzoyl groups by the boiling of dibenzoyl dibromide **11** in HBr solution led to dihydrobromide. The following reaction with LiOH in aqueous solution produced 3,12-diaza-6,9-diazoniadispiro [5.2.5.3]heptadecane dibromide **12**, or dispirotripiperazine "6-7-6". The functionalization of **12** resulted in the target derivatives **13a-e**, **14a,b**. Treatment of dibenzoyl dibromide **11** with picric acid in the presence of NaHCO₃ provided dipicrate **15**. The acidic degradation of this product yielded dichloride **16**. Hydrolysis of *N*-benzoyl groups of **16** under acidic conditions and following reaction with LiOH led to 3,12-diaza-6,9-diazoniadispiro[5.2.5.3]heptadecane dichloride **12b** (another salt of dispirotripiperazine "6-7-6"). The functionalization of **12b** resulted in the key compounds **17a-c**.

Among the synthesized derivatives, only the dispirotripiperazines **13a** with the chloroethylcarbamoyl side chain and **17a** with the β-bromopropanoyl side chain inhibited Jensen's sarcoma, sarcoma M − 1 and melanoma B-16, whereas several other compounds **13b-e**, **14a,b** and **17b,c** did not show antitumor activity. However, the antitumor activity of **13a** and **17a** did not

exceed the activity of spirobromine and prospidine, thus further studies were discontinued [59].

In 2009, Liu et al. [60] described compound **19** (Scheme 4) which was designed based on the structure of cyclophosphamide, a common alkylating agent in anticancer therapy, and spirobromine with the key dispirotripiperazine moiety. This compound was synthesized by a one-step synthesis from the reaction of freshly prepared 2-chloro-1,3,2-oxazaphosphinane 2-oxide **18** with previously known 3,12-diaza-6,9-diazoniadispiro[5.2.5.2]hexadecane dichloride **4** in the presence of NaHCO₃ (Scheme 4). Unfortunately, no further results of biological evaluation were reported, and it is possible to suppose that the data were unsatisfactory, as further studies focused only on the synthesis of cyclophosphamide spiropiperazinium salts [61].
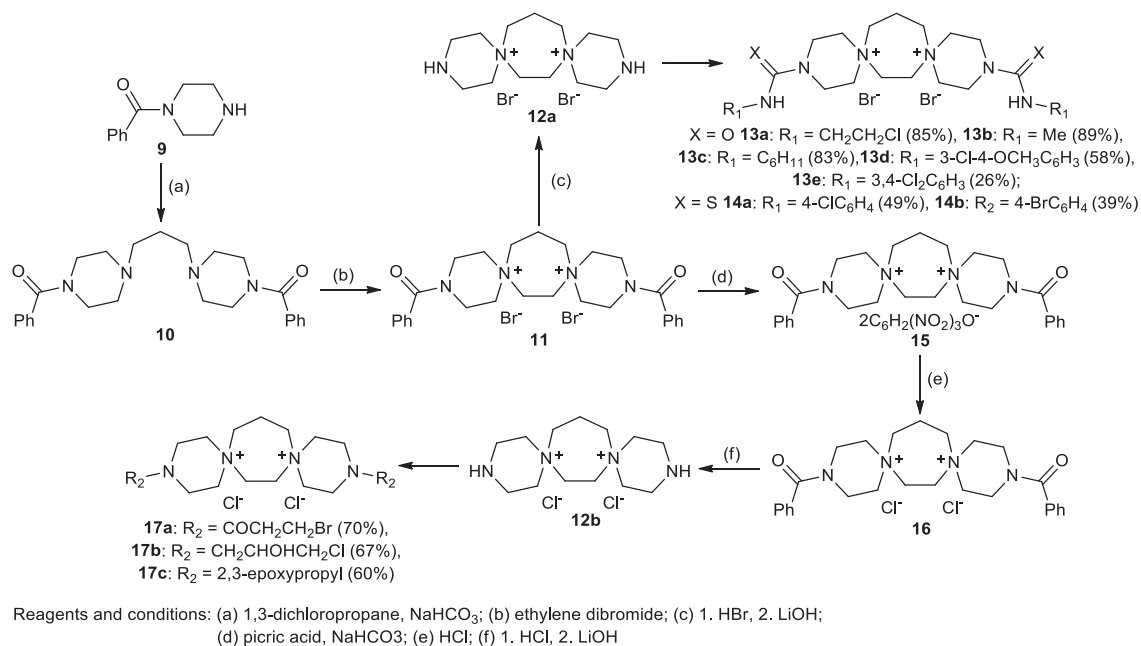
2. The present of dispirotripiperazines: progress as compounds with HS-binding mechanism of action

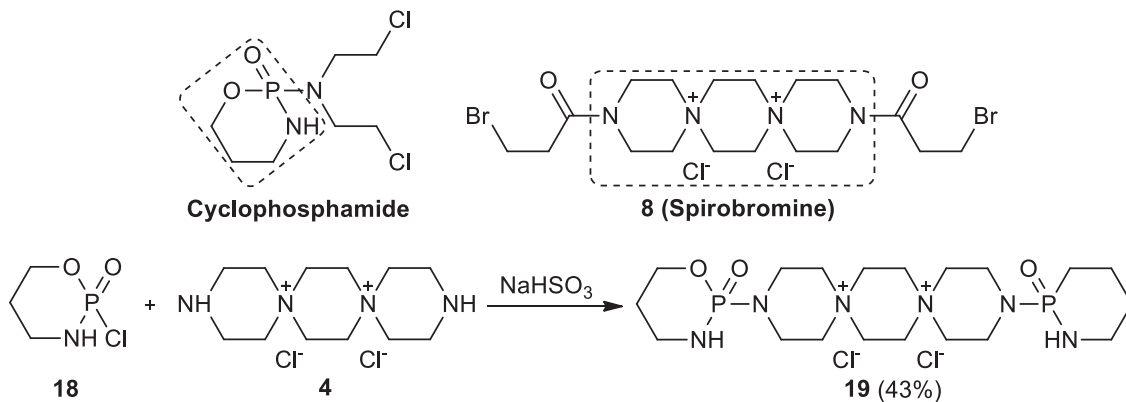### 2.1. From anticancer spirobromine to antiviral PDSTP, DSTP27 and their analogues

In order to study a wide spectrum of biological activity of spirobromine, Levkovskaya et al. [62] evaluated spirobromine **8** and bisquaternary salts of 3,12-bis(3′-haloacyl)-3,12-diaza-6,9-diazoniadispiro[5.2.5.2]-hexadecane **20a-c** (Fig. 3) against herpesviruses (e.g. HSV type 1 [L₂ strain] and 2 [Tᵣ and VN strains]) *in vitro* and *in vivo*. A structure-activity relationship (SAR) study revealed that the presence of a specific halogen atom in the position Y or X is critical for the antiviral activity. For example, spirobromine **8** with a bromine atom as Y and a chlorine atom as X inhibited the replication of both HSV-1 and HSV-2, whereas compound **20b** with Y = Cl and X = Br selectively inhibited only HSV-1 and derivative **20c** with both chlorine atoms in positions only inhibited HSV-2. At the same time, derivative **20a** with two bromine atoms was completely inactive against these viruses. Spirobromine **8** and compound **20c** reduced the mortality of herpetic encephalitis in mice by up to 35−40%. However, these compounds were found to be ineffective for the treatment of influenza virus pneumonia in mice [62].

As a next step, Fomina et al. [63] synthesized a structurally related analogue of spirobromine **24** and evaluated the *anti*-HSV activity of spirobromine and this derivative. The synthesis of analogue **24** is described in Scheme 5 and begins with the reduction of previously synthesized diazoniadispiro[5.2.5.2]hexadecane dichloride **21** [47] by zinc (Scheme 5).

It was found that both spirobromine **8** and its analogue **24** with the insertion of NH-fragment in the side chain of the molecule inhibited the replication of the HSV-1 L₂ strain at equal

Reagents and conditions: (a) 1,3-dichloropropane, NaHCO$_3$; (b) ethylene dibromide; (c) 1. HBr, 2. LiOH; (d) picric acid, NaHCO3; (e) HCl; (f) 1. HCl, 2. LiOH

Scheme 3. Synthesis of dispirotripiperazine "6-7-6" derivatives.

Scheme 4. One-step synthesis of a hybrid molecule includding cyclophosphamide and dispirotripiperazine moieties.

8: Y = Br, X = Cl (**Spirobromine**);
20a: Y = Br, X = Br; **20b**: Y = Cl, X = Br; **20c**: Y = Cl, X = Cl
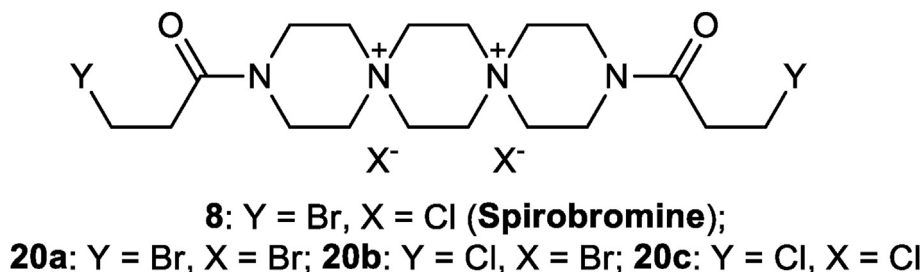
Fig. 3. 3,12-Bis(3′-haloacyl)-3,12-diaza-6,9-diazoniadispiro[5.2.5.2]-hexadecanes **20a-c**.

concentrations, but at the same time, spirobromine **8** was more active towards the HSV-2 T$_r$ strain. A brief summary of the initial studies of the SAR based on the structure of spirobromine is shown in Fig. 4.

Further studies of antiviral dispirotripiperazine derivatives were continued for a decade. For a detailed analysis of the SAR,
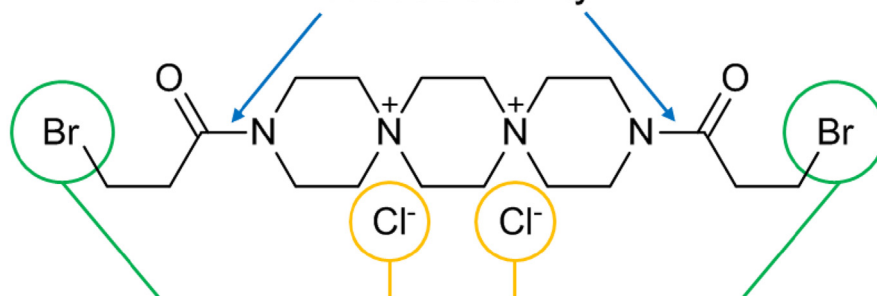
Schmidtke et al. [16] synthesized novel dispirotripiperazine-based compounds as well as a number of previously known compounds and tested them against a coxsackievirus B3 (CVB3) strain Nancy, an influenza virus A (IVA) strain Hong Kong and a HSV type I (HSV-1) strain Kupka. Interestingly, the synthesized compounds efficiently inhibited HSV-1-induced cytopathic effect, but at the same
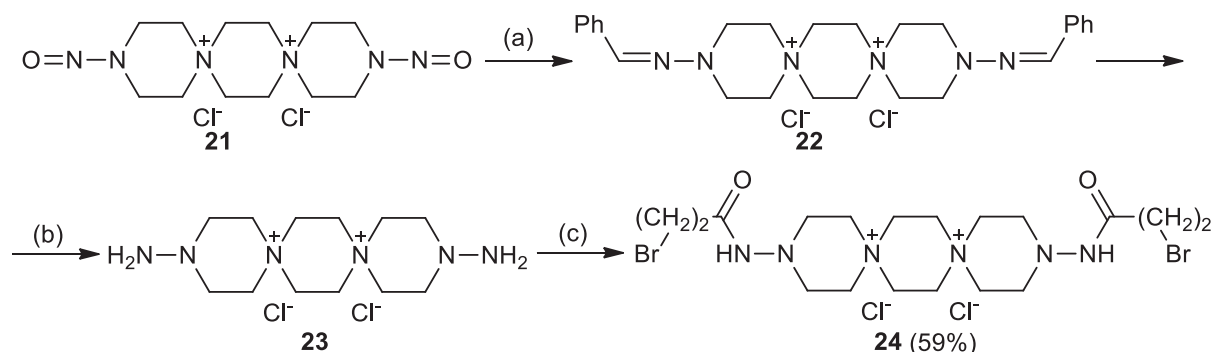
Reagents and conditions: (a) 1. Zn, CH$_3$COOH, 2. Benzaldehyde; (b) HCl, 120 °C;
(c) 1. LiOH, H$_2$O, 2. 3-Bromopropanoyl chloride, LiOH, CH$_3$COOH

Scheme 5. Synthesis of dispirotripiperazine "6-6-6" derivatives.

Fig. 4. SAR studies of dispirotripiperazines as antiviral agents based on the structure of spirobromine by Fomina et al. [63].
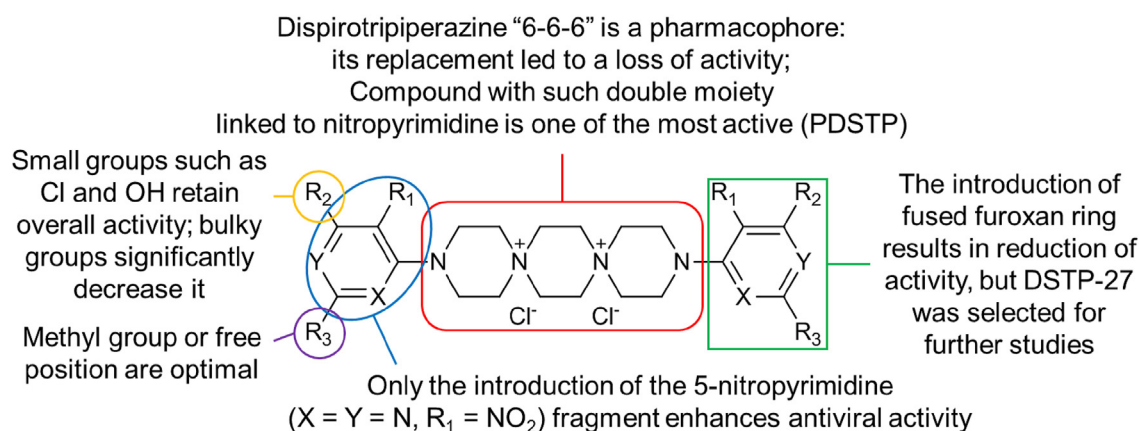
time, no antiviral activity was seen against CVB3 and IVA. Spiro-bromine **8** was used as a reference compound but did not exhibit any antiviral activity in contrast to previous studies [62,63]. However, prospidine **7**, the second reference compound, weakly inhibited HSV-1 replication with an IC$_{50}$ value of 62.47 μM. The structurally related analogue of spirobromine (structure not shown) was found to exhibit antiviral activity similar to prospidine (IC$_{50}$ = 66.10 μM). SAR studies also revealed that the dispirotripiperazine with two closely located quaternary positively charged nitrogen atoms in its spiro system is one of the very important features for demonstrating *anti*-HSV-1 activity, as the compounds with the two positively charged piperazines far away from each other or with a disrupted dispirotripiperazine moiety are inactive (Fig. 5A). It was also observed that 5-nitropyrimidine derivatives have stronger activity against HSV-1 (IC$_{50}$ values 0.90–2.02 μM), and the replacement of one or both nitrogen atoms of the pyrimidine ring with carbon atom results in an inability to block viral replication. Another SAR finding is the necessity for a free position 2 of the pyrimidine ring — compounds with

substituents were significantly less active than compounds without substituents in this position (the exception is a compound with a methyl group). The incorporation of a furoxan ring to pyrimidine leads to a decrease in the overall activity of the molecules, but one such compound, DSTP27 **25** (Fig. 5B), was chosen for further detailed studies. Another promising derivative from this chemical library is compound PDSTP **26** (Fig. 5B) with a double dispirotripiperazine moiety linked to nitropyrimidine.

Schmidtke et al. [64] also reported that DSTP-27 **25** moderately inhibits several human herpesviruses, including acyclovir/foscarnet-resistant strains of HSV-1/2 and human cytomegalovirus (HCMV), (IC$_{50}$ range 8.4–20.4 μM). In an attachment assay, preincubation of gradient-purified radiolabeled PrV-1112 (pseudorabies virus with a proven use of HSPG as a receptor) with DSTP27 resulted in a concentration-dependent inhibition of virus binding with a maximum of 95% at 80.0 μg/mL. It was also found that the compound was active towards HIV (IC$_{50}$ range of 1.4–33.7 μM, except for HIV-1 RF, IC$_{50}$ = 150 μM), but at the same time, was completely inactive against varicella zoster virus and

A Structure-activity relationships:

Dispirotripiperazine "6-6-6" is a pharmacophore:
its replacement led to a loss of activity;
Compound with such double moiety
linked to nitropyrimidine is one of the most active (PDSTP)

Small groups such as Cl and OH retain overall activity; bulky groups significantly decrease it

The introduction of fused furoxan ring results in reduction of activity, but DSTP-27 was selected for further studies

Methyl group or free position are optimal

Only the introduction of the 5-nitropyrimidine
(X = Y = N, R_1 = NO_2) fragment enhances antiviral activity

B Selected examples of dispirotripiperazine-based compounds:
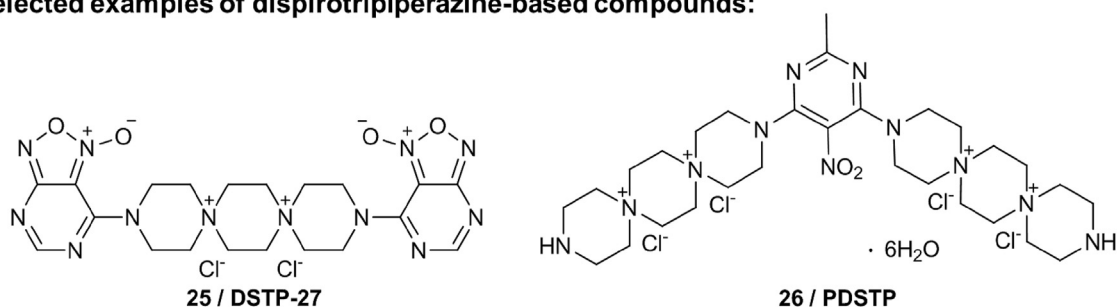

25 / DSTP-27

26 / PDSTP

Fig. 5. A) SAR studies of antiviral dispirotripiperazines by Schmidtke et al. [16] and B) selected examples of dispirotripiperazine-based compounds.

Epstein-Barr virus (EBV), which also use HSPG as a receptor [64]. VZV infection requires cell-to-cell fusion, which is mediated by gB/gH-gL. In addition, this fusion induces syncytium formation, which is a characteristic of VZV in the skin and sensory ganglia. EBV entry depends on the cell type. The attachment of EBV to B cells requires gp42 interaction with HLA class II, while entry into epithelial cells is mediated through the docking of gHgL KGD motif with αvß6

integrin [65]. So, DSTP-27 simply cannot bind to the surface of these specific cells, and this may serve to explain why the compound is inactive against these viruses. DSTP-27 also inhibits human papillomavirus types 16 and 18 (HPV16 and HPV18) and bovine papillomavirus type 1 (BPV1) infection with the IC_{50} values of 1.4, 0.7, and 2.6 μM, respectively (Fig. 6) [66].

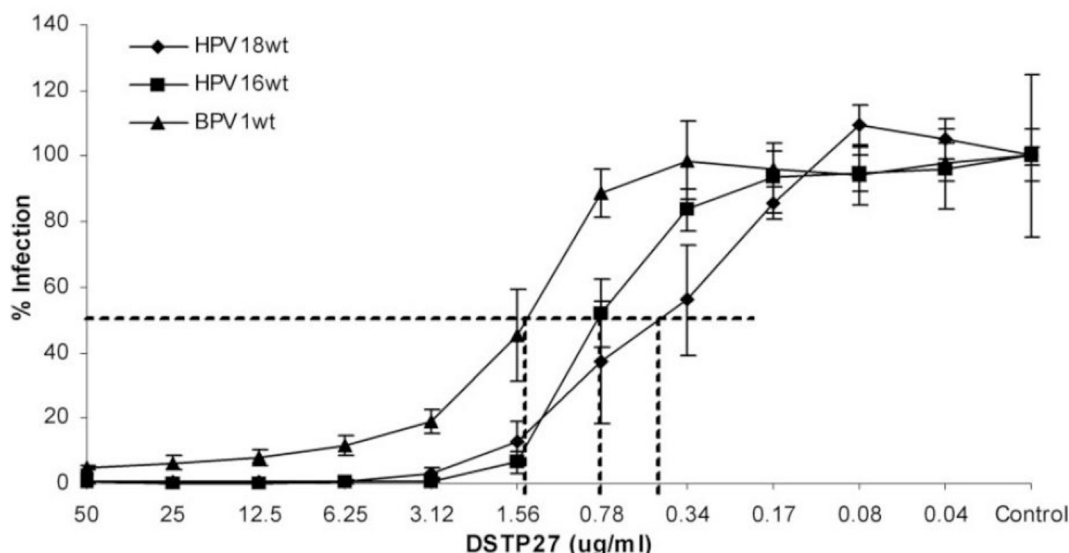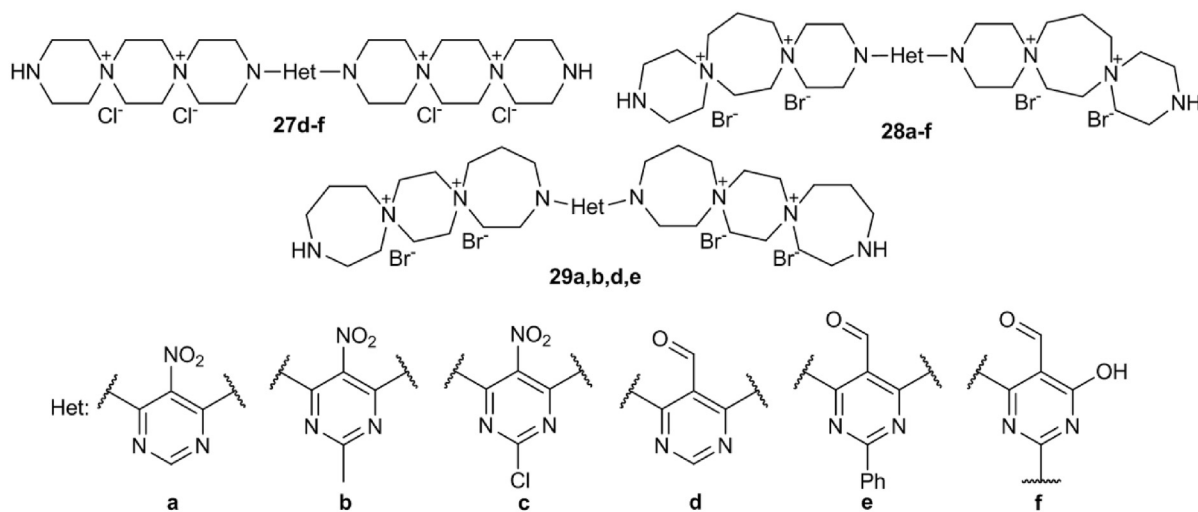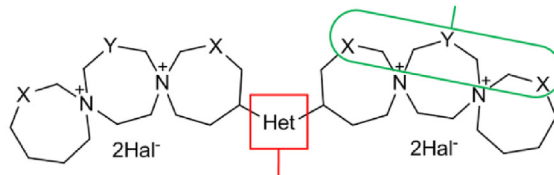Paeschke et al. [67] demonstrated the potent *in vitro* antiviral

Fig. 6. Determination of IC_{50}: HEK 293 TT cells were infected with HPV16, HPV18, and BPV1 pseudovirions in the presence of decreasing concentrations of DSTP27 [66].
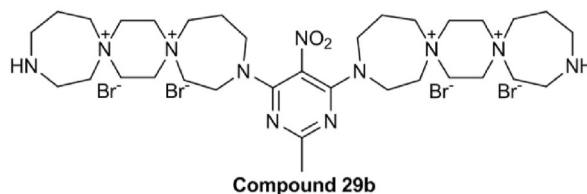
Fig. 7. The structurally related derivatives of PDSTP: A) Their library; B) SAR studies; and C) selected example of the class of dispirotripiperazines.

activity of DSTP27 against two laboratory HCMV strains as well as ganciclovir-sensitive and -resistant clinical isolates (IC_{50} range of $0.15-0.85~\mu M$). Based on the results of time-of-addition assays, immunofluorescence and Western blot analysis, the authors suggested that the inhibitory effect was associated with the block of early steps of the viral replication cycle, but not attachment. It was also observed that DSTP27 is metabolically unstable due to its ability to release nitric oxide [68]. Taken together, these issues prevented further development of DSTP27.

Another dispirotripiperazine-based compound, PDSTP **26**, 3,3'-(2-methyl-5-nitropyrimidine-4,6-diyl)3,12-bis-6,9-diaza-diazo-niadispiro[5.2.5.2]hexadecane tetrachloride dihydrochloride, was selected as a potentially metabolically stable derivative for detailed biological evaluation. PDSTP exhibits good *in vitro* activity towards HSV-1 (IC_{50} value of $1.48~\mu M$) [16]. However, PDSTP has no significant effect on a herpetic encephalitis mouse model when used as monotherapy, only in combination therapy with acyclovir is it effective, which suggests that a combination of the antiviral agents

with different modes of action might resolve the problem of emergent resistance [69]. At the same time, PDSTP was found to be more effective compared to acyclovir in a guinea pig model of genital herpes, reducing symptom intensity scores, mean duration of illness, and viral titers [70].

Target-based drug discovery was used to propose several structurally related PDSTP derivatives, containing different combinations of piperazine/homopiperazine cycles **27d-f**, **28a-f**, **29a,b,d,e** (Fig. 7A) which were synthesized and tested for activity against HSV-1 [71]. During SAR studies, it was additionally confirmed that only the 2-methyl-5-nitropyrimidine moiety is associated with high *anti*-HSV-1 activity of dispirotripiperazines: as compounds **28b**, **29b** were the most active in the series and their IC_{50} values were comparable to PDSTP 26 (1.97 and $1.12~\mu M$ vs. $1.48~\mu M$, respectively), while compounds **28a**, **28c**, **29a** with 2-chloro or 2-free position in the 5-nitropyrimidine and compounds **27d-f**, **28d-f**, **29d,e** with 5-formylpyrimidine were significantly less active. Modification of the piperazine/homopiperazine
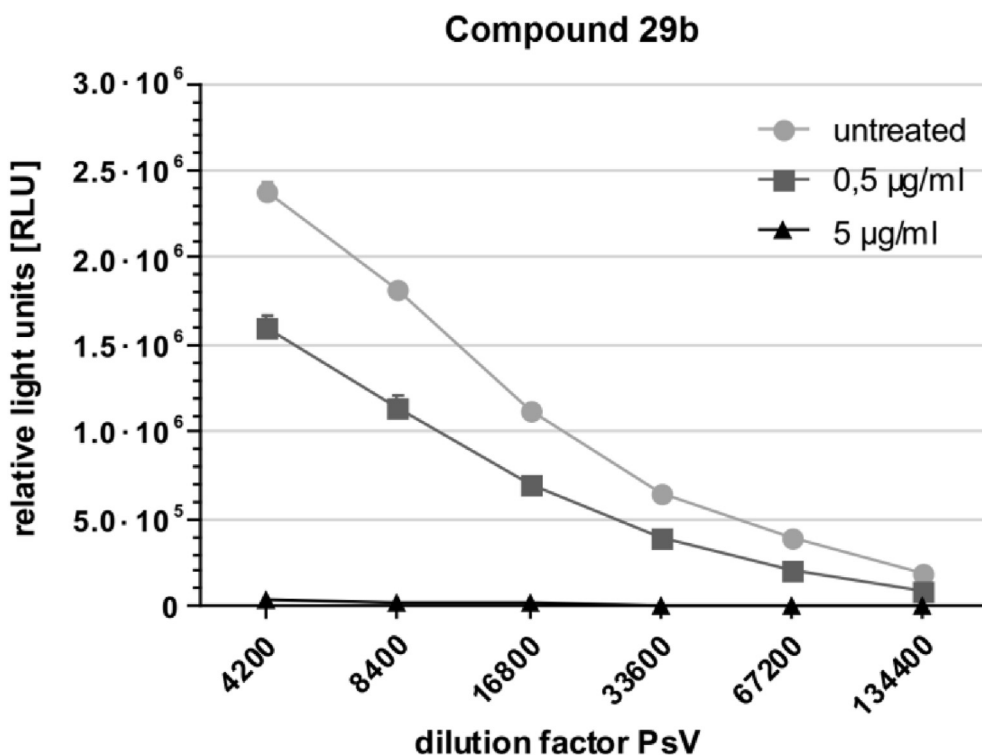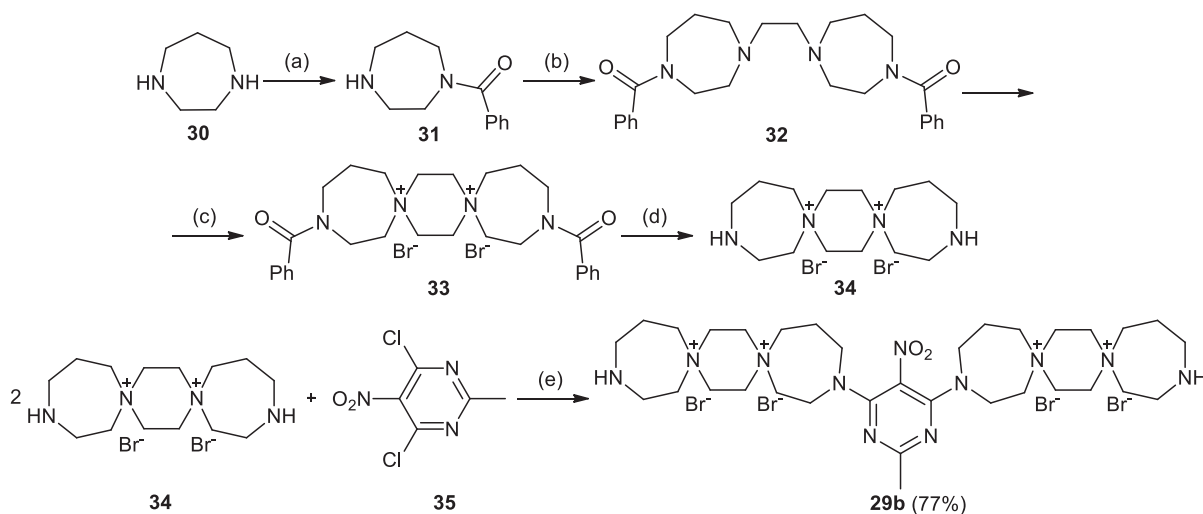
Fig. 8. The activity of **29b** against HPV16 pseudovirions (Makarov, unpublished results).

Reagents and conditions: (a) benzoyl chloride, CH₃COOH; (b) ethylene dibromide, NaHCO₃;
(c) ethylene dibromide; (d) 1. HBr, 2. LiOH, H₂O; (e) 1. Et₃N, EtOH/H₂O

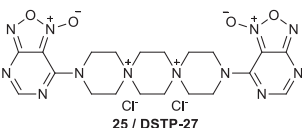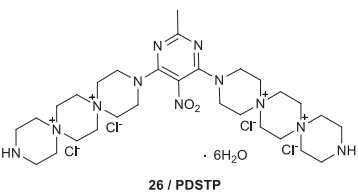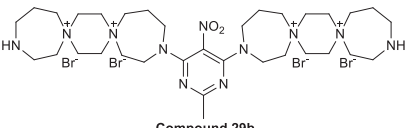**Scheme 6.** Synthesis of dispirotripiperazine "7-6-7" derivative 29b.

cycle combinations had almost no effect on the antiviral activity against HSV-1, however, compound **29b** was selected as a new lead dispirotripiperazine.

The antiviral activity of the lead molecule **29b** towards HPV16 pseudovirions was evaluated using a neutralization assay (Fig. 8). It was found that the compound reduces the infectivity of HPV16 pseudovirions and completely inhibits virus penetration at a concentration of 5.0 μg/mL (Makarov, unpublished results).

Synthesis of **29b** began with the formation of a novel spiro system — 3,13-diaza-7,10-diazoniadispiro[6.2.6.2]octadecane dibromide **34**,

or dispirotripiperazine "7-6-7", – according with Scheme 6 [72]. Treatment of 1,4-diazepane **30** by benzoyl chloride in acetic acid led to benzoyl-homopiperazine **31**. N-alkylation of **31** with 1,2-dibromoethane in the presence of NaHCO₃ in refluxing ethanol successfully provided ethane-diylbis(benzoyl-homopiperazine) **32**, which then was heated with 1,2-dibromoethane to obtain dibenzoyl-dispirotripiperazine **33**. Finally, work-up of **33** by 10% HBr at first, and then by LiOH yielded 3,13-diaza-7,10-diazoniadispiro[6.2.6.2]octadecane dibromide **34**. The reaction between this original spiro-system **34** and 4,6-dichloro-2-methyl-5-nitropyrimidine

Table 1
Key dispirotripiperazines and their *in vitro* activity against viruses which use HSPG as an attachment factor.

Compound	50% Inhibitory concentration (IC$_{50}$), μM											
	RSV	HSV-1	HSV-2	HCMV	HIV	HPV16	HPV18	BPV1	CVB3	IVA	VZV	EBV
25 / DSTP-27	7.5	8.1—9.8	10.2—20.4	0.15—0.85	1.4—150.0	1.4	0.7	2.6	NA	NA	NA	NA
26 / PDSTP		1.48							NA	NA		
Compound 29b	>100	1.97			4.0—15.0							

NA — not active.
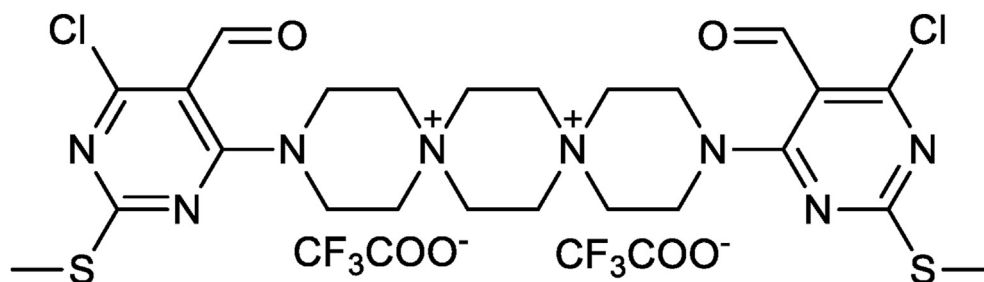
Adhesamine 36

Fig. 9. Chemical structure of adhesamine.

35 in the presence of triethylamine resulted in the target derivative **29b**.

There are some key structural requirements of dispiro tripiperazine-core compounds for their antiviral activity, such as.

1. As with anticancer activity, the presence of two quaternary positively charged nitrogen atoms in the dispirotripiperazine system is critical for inhibitory activity against viruses. In the case of antivirals, charged molecules are likely to interact electrostatically with the HSPGs on the cell surface;
2. The nitropyrimidine moiety was found to strongly contribute to the antiviral effect of the molecules, which suggests that dispirotripiperazines bind to HSPGs not only through electrostatic interactions, but also through hydrogen-bonding of the nitro group and two nitrogen atoms of the heterocycle with HSPG's functional groups, but this statement needs to be clarified;
3. Dispirotripiperazines exhibit antiviral activity regardless of the piperazine/homopiperazine combination in their structure;

Finally, in this section, we summarize the main representatives of the dispirotripiperazine class and their *in vitro* antiviral activity observed to date in Table 1.

2.2. Discovery of adhesamine, a small molecule that promotes cell adhesion

While discussing the dispirotripiperazine derivatives as heparan sulfate binding compounds, it is also worth mentioning one more dispirotripiperazine derivative namely adhesamine. In contrast, this molecule acts as an agonist of the heparan sulfate proteoglycans, boosting cell adhesion and growth, so the compound may potentially be used as a cell-attaching reagent for cell therapy [72]. Adhesamine **36**, chemically known as 3,12-bis[6-chloro-5-formyl-2-(methylsulfanyl)-4-pyrimidinyl]-3,12-diaza-6,9-diazoniadispiro[5.2.5.2]hexadecane ditrifluoroacetate (Fig. 9), was found during screening of chemical library by Yamazoe et al. [72]. Cell biology assays showed that adhesamine binds to selective cell-surface glycosaminoglycans, especially heparan sulfate, increasing cell adhesion and growth. Additionally, it was found that this small synthetic molecule induces normal cell adhesion similar to fibronectin, a general cell adhesion protein.

Takemoto et al. [73] synthesized several adhesamine analogues for detailed SAR investigation and confirmed the earlier findings that two positively charged nitrogen atoms and their close proximity to each other in the molecule are critical for the heparan sulfate binding and the accessible biological activity. Pilot animal
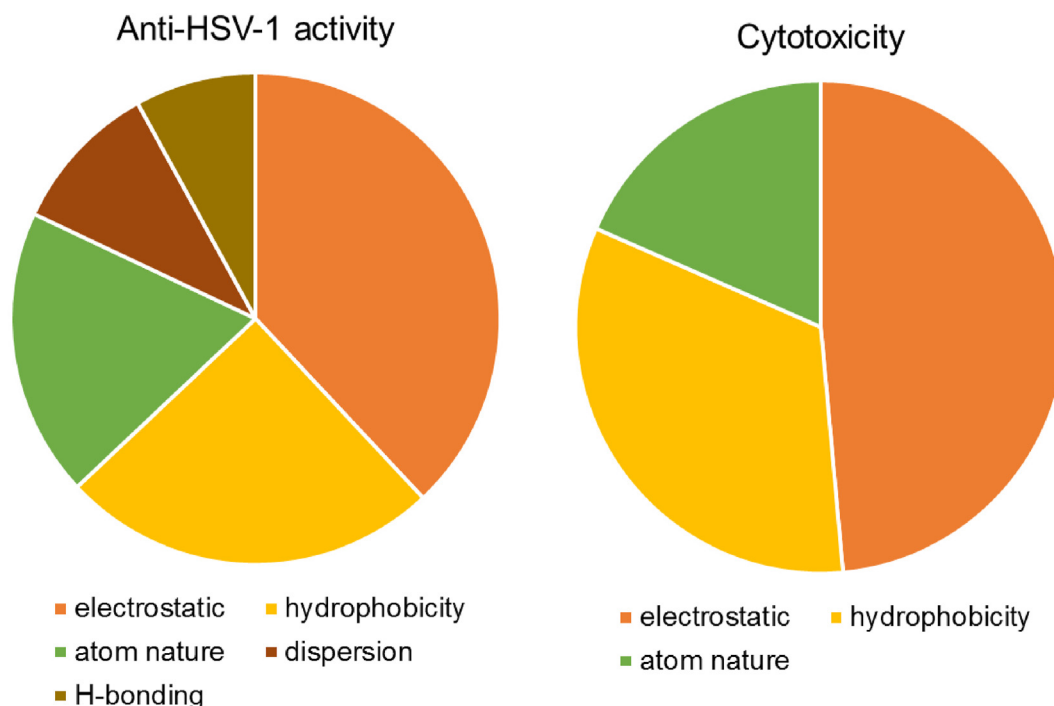
Fig. 10. Prediction of the relative influence of some physicochemical factors on *anti*-HSV-1 activity and cytotoxicity of dispirotripiperazines.

studies have shown that adhesamine improves the viability and attachment of transplanted cells in mice. However, as noted by researchers [74], adhesamine has some problems for cell therapy applications: low solubility in water, relatively low potency (compared to an endogenous cell-attaching protein, fibronectin) and limited effect (adhesamine binds only to heparan sulfate and does not affect integrin sulfate).

4. The future of dispirotripiperazines: through chemoinformatics to new applications

Artemenko et al. [75] evaluated the influence of the structure of 48 dispirotripiperazines on their antiherpetic activity and cyto-toxicity by using the quantitative structure activity relationship approach based on the simplex representation of molecular structure (SiRMS) in order to provide an in-depth understanding of the chemico-biological interactions governing their activities. The SiRMS method has also been previously described with advantages including consideration of the different physical chemical properties of atoms and good interpretability of models [76].

It was established that the antiherpetic activity and cytotoxicity of molecules predominantly depend on electrostatic factors (38% or 50%, respectively), hydrophobicity (25% and 34%, respectively), atom nature (19% and 16%, respectively), dispersion (10%) and H-bonding (8%) also have some effect on the activity (Fig. 10).

The importance of data curation and validation for such computational models is paramount and the reader is referred to earlier articles on the best practices for such modeling and the discussion of the benefits of making such models available for others [77,78]. We have now analyzed the broad set of dis-pirotriperazines with HSV-1 inhibition data at a threshold of $IC_{50} < 10 \mu M$ as active (N = 28) using the Assay Central® machine learning software as previously described [79–83]. A high-quality structure-activity dataset was first created (i.e. structures are de-salted and neutralized, finite activities merged) then a Bayesian algorithm was applied utilizing extended-connectivity fingerprints

(ECFP6) as molecular descriptors [79,80]. Due to the limited data availability for these compounds, we set a binary threshold so that an $IC_{50} < 10 \mu M$ was considered active (N = 28), and those without IC_{50} data at the time were considered inactive. The model can be used to predict new molecules from chemical structure alone (Fig. 11A). We also curated 70 HSV-1 inhibitors from the ChEMBL database (target id CHEMBL613200) to build a separate model with a calculated threshold of 10.42 μM (Fig. 11B). This model was then used to score 39 dispirotriperazines with IC_{50} values at this same threshold, with reasonable performance (Fig. 11C). Finally a model was built which combined the 70 ChEMBL compounds and the 39 compounds with IC_{50} data herein to total 109 molecules, resulting in a calculated threshold of 10.42 μM (Fig. 11D); this model expands the chemical space to more specifically target the dis-pirotriperazines and was utilized to score prospective molecules and prioritize synthesis in this series. These efforts demonstrate that public HSV-1 data can to some degree rank the HSV-1 activity for the dispirotriperazines and that a combination of datasets may aid us in predicting this activity for future analogues.

3. Summary and outlook

As illustrated by the recent SARS-CoV-2 outbreak, viruses continue to represent a great threat to humanity. Broad spectrum vaccines or synthetic drugs against a large number of viruses are still not available to tackle this virus at the time of writing. There are different strategies for the development of broader spectrum an-tivirals, and the search for small molecules, which could prevent the initial interactions between the virus and the host cell, is certainly worth pursuing for the future. Viral adsorption is there-fore the earliest infectious event in the viral life cycle and is one of the most promising target for the development of new antiviral drugs.

As we have described, in order to gain access to the cell interior, viruses attach to the cell-surface receptor and many viruses from different classes have evolved to use cell-surface HSPGs as either
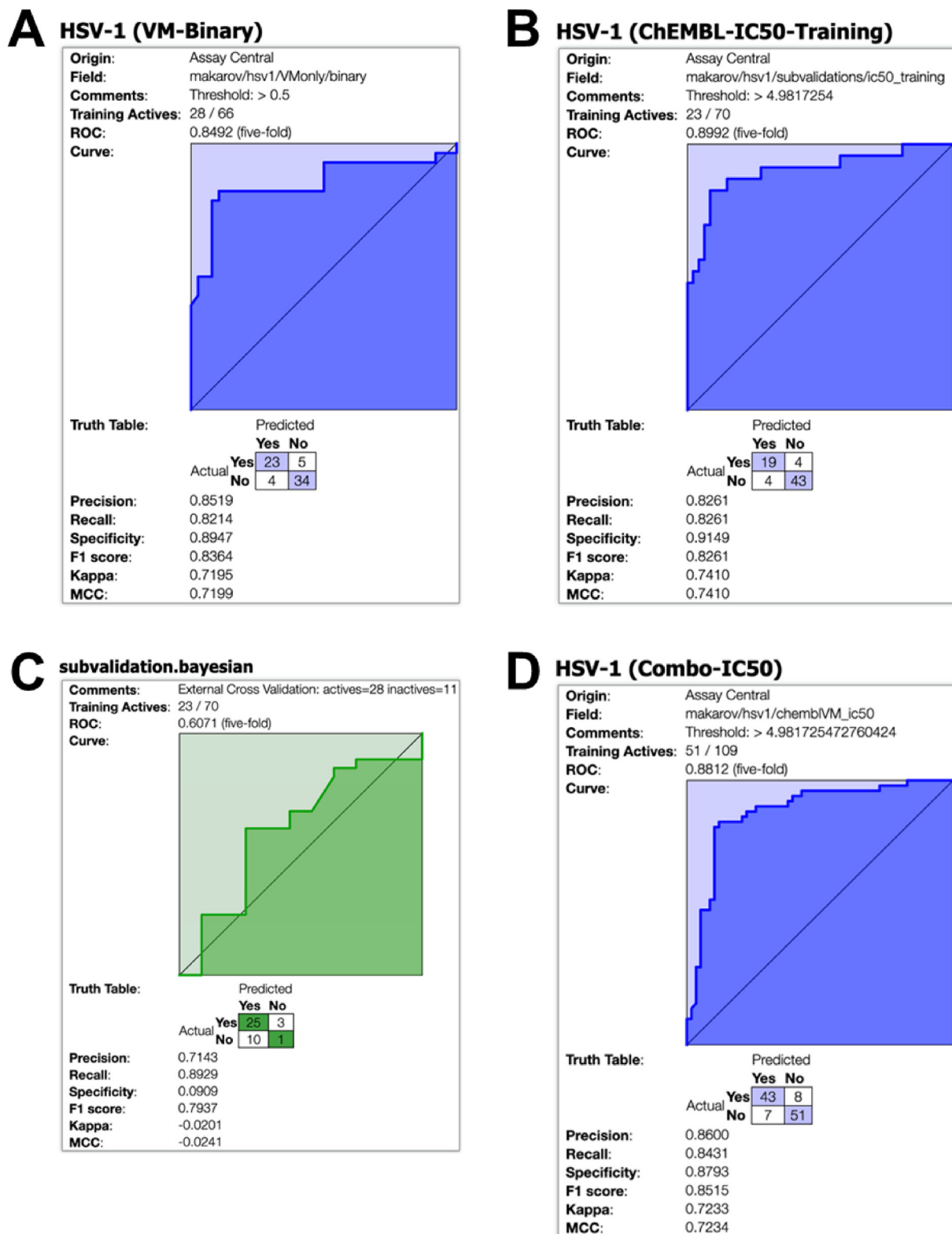
Fig. 11. Five-fold receiver operator characteristic plots for A) dispirotriperazines and their *anti*-HSV-1 activity, B) ChEMBL HSV-1 inhibitors, C) test set statistics for the dispirotriperazines predicted by the ChEMBL model, D) Combined model using the dispirotriperazines and ChEMBL HSV-1 inhibitors.

direct receptors, co-receptors or attachment factors for penetration into the cell. However, the contradictory data of the role of heparan sulfate in virus entry for several viruses has been reported (e.g. RSV and Zika virus [40−42]), thus more biological investigations are required.

Several different strategies are currently being studied to target HSPGs including their inhibition by dispirotripiperazine-based compounds [13]. According to *in vitro* studies, a representative of

the class of dispirotripiperazines, DSTP-27, binds to heparan sulfate proteoglycans [57]. It may be assumed that this binding occurs through electrostatic interactions between the negatively charged heparin/heparan and the positively charged dispirotripiperazine fragment of the molecules and also through hydrogen-bonding of nitro-pyrimidines with functional groups of HSPG. Based on data available to date on the various SARs with different viruses, greater insights of the key structural features of dispirotripiperazine-based compounds for antiviral activity and HS binding were provided. Compounds from this class, PDSTP and compound 29b, were previously found to be active against strains of herpesvirus and human immunodeficiency virus and may be further investigated against other viruses that use HSPGs.

Thus, the synthesis of compounds in this class of cationic dispirotripiperazines could be continued to provide further more in-depth views on the SARs of binding to HSGAGs in order to find the most active broad spectrum antiviral agent amongst them with enhanced stability, solubility etc. We will also need to pay special attention to drug safety issues, since HSPGs are involved in many different physiological processes [13,19]. With a growing database of information for this class of compounds it may open up the potential for further computational analyses. It is certainly worth considering that the antiviral activity of dispirotripiperazines may not be limited to targeting HSPGs alone. Moreover, it is worth mentioning the potential of dispirotripiperazines not only as antivirals, but also as agents in other pathophysiological processes associated with the negatively charged HS involvement, such as antibacterials, anticancer drugs, antiparkinsonian agents, and others.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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