Plant and Animal Steroids a New Hope to Search for Antiviral Agents

V. Castilla*[,1], J. Ramírez[2] and C.E. Coto[1]

[1]*Laboratorio de Virología, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina*

[2]*Departamento de Química Orgánica and UMYMFOR (FCEyN – CONICET), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina*

Abstract: Scientific literature provides evidence about the use of steroids as an adjunct treatment to antiviral therapies. Immunomodulatory activity of some steroids would account for the recovery in patients with herpetic and other viral infections. However, *in vitro* studies have demonstrated a direct antiviral effect of this kind of molecules. In this review we discuss recent reports about the mechanism of antiviral action of steroids from animal and plant origin. Chemical structures of most active compounds are also provided.

Keywords: Steroids, virus, antiviral activity, progestagen, glucocorticoid, dehydroepiandrosterone, brassinosteroid, secopregnane.

1. STEROIDS AND THEIR POTENTIAL ANTIVIRAL ACTIVITY

In the search for antineoplasic drugs, a family of synthetic nucleoside analogs emerged like powerful antiviral agents at the middle of the 20th century. From that time, hundreds of chemical compounds of different structure were isolated from natural sources or were laboratory synthesized. Despite that, only a handful of drugs are in clinical use due mainly to side undesirable effects like toxicity and virus resistance mutation.

Starting at the last decade of previous century a new family of chemical structural compounds was tried to reveal potential antiviral activities: the steroids.

Steroids are compounds possessing the skeleton of cyclopenta[*a*]phenanthrene or a skeleton derived from that by one or more bond scissions or ring expansions or contractions. Methyl groups are normally present at C-10 and C-13. An alkyl side chain may also be present at C-17. Sterols are steroids carrying a hydroxyl group at C-3 and most of the skeleton of cholestane. Additional carbon atoms may be present in the side chain. Hundreds of distinct steroids are found in plants, animals, and fungi. Besides many sterol derivatives were obtained by chemical synthesis and assayed *in vivo* and *in vitro* against several pathogenic viruses.

Steroid hormones have been known to play important roles in various phyla. These include sex hormones, glucocorticoids and mineralocorticoids in animals and moulting hormones in arthropods [1, 2].

In this review we will mainly focused on the antiviral activity of human steroid hormones, steroid plant hormones and chemical derivatives. Results obtained with other plant steroids will be also discussed.

*Address correspondence to this author at the Laboratorio de Virología, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, Piso 4. C1428EGA Buenos Aires, Argentina; Tel: 054-011-45763334; Fax: 054-011-45763342; E-mail: viviana@qb.fcen.uba.ar

2. MAMMALIAN STEROID HORMONES

Mammalian steroid hormones are generally synthesized from cholesterol in the gonads and adrenal glands and have similar chemical skeletons. These molecules can be grouped into five groups by the receptors to which they bind: glucocorticoids, mineralocorticoids, androgens, estrogens and progestagens. Steroids exert a wide variety of effects mediated by slow genomic as well as by rapid nongenomic mechanisms. They bind to nuclear receptor in the cell nucleus for genomic actions. Because steroids and sterols are lipid soluble, they can diffuse fairly freely from the blood through the cell membrane and into the cytoplasm of target cells. In the cytoplasm the steroid may or may not undergo an enzyme-mediated alteration such as reduction, hydroxylation, or aromatization. The steroid binds to the specific receptor, a large metalloprotein, within the cytoplasm and upon binding, many kinds of steroid receptor dimerize: two receptor subunits join together to form one functional DNA-binding unit that can enter the cell nucleus. In some of the hormone systems known, the receptor is associated with a heat shock protein that is released on the binding of the ligand, the hormone. Once in the nucleus, the steroid receptor-ligand complex binds to specific DNA sequences and induces transcription of its target genes. Membrane-associated steroid receptors activate intracellular signaling cascades involved in nongenomic actions [3- 6].

Here we will discuss about the antiviral properties of progestagens, glucocorticoids and dehydroepiandrosterone (DHEA), a precursor of estrogens and androgens. The antiviral activity of several synthetic derivatives will be also commented.

2.1. Progestagens

Retroviruses

Despite the existence of a successful retrovirus antiviral therapy, many questions concerning human immunodeficiency virus (HIV) infection remain to be answered. Recently, questions were addressed to the role of progesterone and estradiol on HIV replication. Progesterone (**1**), naturally produced in the ovary and placenta, is the major naturally occurring human progestagen (Fig. (**1**)) involved in the fe-

0929-8673/10 $55.00+.00 © 2010 Bentham Science Publishers Ltd.

male menstrual cycle, pregnancy and embryogenesis of humans and other species. It is essential for pregnancy to reach full term and its physiological effects are mediated by the progesterone receptor (PR) a member of nuclear receptor superfamily of transcription factors [3]. This steroid inhibits T lymphocyte proliferation and functions, as well as also inhibits the expression of inducible nitric oxide synthase (iNOS) in activated macrophages and natural killer cells. Cytotoxic T lymphocyte function and tumor necrosis factor (TNF) synthesis are also inhibited by progesterone (**1**) [7, 8].

Vertical transmission of HIV-1 is a serious public health worldwide specially in developing countries when infected mothers did not received antiretroviral treatment.

Data gathered from UNICEF, WHO and UNAIDS indicate that in 2007, in particular in African countries, the number of pregnant women who did not receive antiretroviral drug treatment is overwhelming. Thus, in order to reveal the mechanism involved in virus transmission from mother to infant in uterus through the placenta a group of investigators proposed to study the effect of **1** on HIV-1 replication in trophoblast cells and placental cell lines and they also investigated the cellular and molecular mechanisms involved [9]. Using a concentration of **1** present during pregnancy they found that replication of HIV-1 is inhibited in infected or transfected placental cells. The investigators demonstrated that the inhibitory effect observed is not due to a direct antiviral action produced by the hormone, it is an indirect one, since **1** reduces the levels of TNF which is required for optimal HIV replication [9]. Another approach to evaluate the role of **1** in the replication of HIV in peripheral blood mononuclear cells (PMBCs) was investigated by Asin *et al.* [10]. To perform the experiments these authors used a combination of **1** and estradiol, a female sex hormone that it is also present in males and it represents the major estrogen in humans. Like **1**, estradiol acts *via* a nuclear receptor, estrogen receptor (ER) and binds to specific response elements located in the promoters of target genes. ERs can also regulate gene expression in two other forms: through protein-protein binding interactions with other DNA-binding transcription factors in the nucleus or by interaction with a membrane associated ER that mediates nongenomic actions [11]. Based on the fact that endogenous levels of estradiol and progesterone (**1**) fluctuate in PMBCs premenopausal women during the menstrual cycle, Assin *et al.* [10] compared the effect on HIV replication under two hormone concentrations present in mid-secretory phase (high concentration) and mid-proliferative phase (low concentration) in PMBCs obtained from uninfected women. They also studied the effect of these sex hormones on HIV-1 reverse transcription, integration and transcription, as well as on surface expression of the HIV-1 coreceptor CCR5. The results obtained were contrasting since at high hormone concentration virus replication was inhibited whereas at low concentration HIV replication increased. In relationship with hormone action on specific stages of the viral life cycle they found decreased levels of HIV-1 integration in the mid-secretory phase and increased levels of viral transcription in the mid-proliferative phase. Similar results were obtained when they studied the activity of long terminal repeat (LTR) promoter in the absence of the regulatory Tat protein. The investigators concluded that progesterone-estradiol acts as regulators of HIV-1 replication likely by directly altering HIV-1 transcriptional activators.

Two recent reports intended to understand the effect of progestin-based contraceptive medroxyprogesterone acetate, known as Depo-Provera (**2**) (Fig. (**1**)), in monkeys infected with simian immunodeficiency virus (SIV) or the chimeric virus SHIV. Trunova *et al.* [12] demonstrated that a single dose of **2** administered to rhesus macaques 5 weeks before challenge with a mixture of pathogenic strains of SHIV produced an immunosuppressive effect, thus impeding that animals mounted an antiviral cellular immune response and as a consequence viral burden increased [12]. Furthermore, the effect of **2** on protection afforded by attenuated strain of SIV on male rhesus macaques was also studied [13]. It was proved that administration of **2** eliminates live-attenuated lentivirus vaccine efficacy and postulated that the mechanism involved could be due trough systematic effects on antiviral immunity and /or viral replication.

Another concern about administration of **2** arises from the fact that million of women worldwide use hormonal contraception. A multicenter prospective cohort study among African women concluded that there is not association between hormonal contraceptive use and HIV acquisition in settings of high HIV prevalence. An unexplained finding was that herpes simplex virus type 2 (HSV-2) seronegative women present an increased risk of HIV acquisition [14]. Another study performed among Kenyan women reported contradictory results than those described above. The authors concluded that hormonal contraception and HSV-2 infection were both associated with increased risk for HIV-1 acquisition [15]. In relation with these observations a recent study proved that progesterone (**1**) impairs innate antiviral defenses blocking toll-like receptor mediated and virus-induced interferon α (IFN-α) production by plamacytoid dendritic cells [16]. Finally, we will mention the results obtained in a population of 4200 HIV-negative South African women receiving treatment with **2**. After a follow up of 24 months they conclude that hormonal contraceptive use is not associated with increased risk of HIV acquisition [17].

Herpesviruses

It has been proposed that the effect of progesterone (**1**) on natural immunity in mice genital herpes virus infection masked the potential antiviral activity of this molecule against herpesviruses. However, it is interesting to note that progesterone (**1**) and Depo-Provera (**2**) exhibit different effects in this animal model. Mice immunized with an attenuated strain of HSV-2 two weeks after treatment with **2** failed to develop protection when challenged intravaginally with wild-type HSV-2. In contrast, mice treated with **1** were completely protected from intravaginal challenge indicating that treatment with 2 changes susceptibility and local immune responses to genital HSV-2 infection [18].

Experiments performed in our laboratory with several synthetic progesterone derivatives against HSV replication in Vero cells demonstrated that some derivatives, compounds **3**, **4** and **5** (Fig. (**1**)), were very active as inhibitors of both HSV-1 and HSV-2 multiplication [19]. These are interesting results since it has been shown that related 6, 19-bridged steroids are devoid of progestational activity which might be

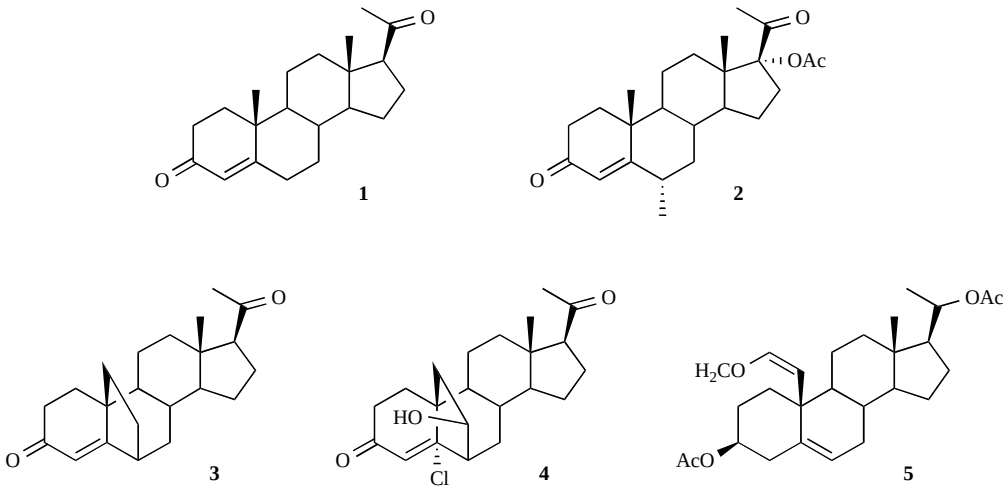

Fig. (1). Natural and synthetic progestagens.

related with an increased susceptibility to genital herpes infection as was discussed above.

2.2. Glucocorticoids

Early reports about *in vitro* antiviral activity of glucocorticoids described that treatment of L929 cells with dexamethasone (**6**), a synthetic glucocorticoid, inhibited plaque formation by vesicular stomatitis virus (VSV), encephalomyocarditis virus, or vaccinia virus. The inhibitory action of **6** against VSV was attributed to inhibition of viral protein synthesis and was not associated to interferon induction [20].

Respiratory Viruses

Glucocorticoids are part of the standard treatment for acute asthma attacks, which are associated with a viral infection in as many as 20–50% of the cases and rhinoviruses (RVs) are responsible for 80–85 and 45% of the asthma flairs in 9- to 11-yr-old children and adults, respectively. It was demonstrated that dexamethasone (**6**) and hydrocortisone (**7**) (Fig. (**2**)) reduced viral titers of RV14 in cultured human tracheal epithelial cells indicating that glucocorticoids might be able to inhibit RV infection of the respiratory tract epithelium, a primary target for respiratory viruses [21]. The results obtained suggest that **6** may inhibit RV14 infection by reducing the surface expression of ICAM-1, a major RV receptor, and treatment in these primary cultures also inhibits production of cytokines interleukin 1β (IL-1β), IL-6, IL-8 and TNF-α induced by virus infection. However, a previous study consisting in a randomised controlled trial of glucocorticoid prophylaxis against experimental RV infection in healthy subjects showed that there was no difference in the rate of infection or the level of viral shedding between patients receiving glucocorticoids or a placebo [22]. In another study 25 patients with mild asthma who underwent experimental RV16 infection, received double-blind, placebo-controlled treatment with the inhaled corticosteroid budesonide (**8)** (Fig. (**2**)) [23]. It was concluded that RV16 infection by itself induces only subtle worsening of airway inflammation in asthma, which is not improved (or worsened) by inhaled corticosteroids. Thus further investigations are needed to clarify an antiviral action of glucocorticoids in RV *in vivo* infection.

Other respiratory viruses have proved to be inhibited by glucocorticoid treatment in animal models. Kimsey *et al*. [24] reported that pretreating hamsters with steroids prevents subsequent infection with parainfluenza virus (Sendai). Hamsters exposed to virus after pretreatment with a single subcutaneous injection of the corticosteroid methylprednisolone acetate (**9**) developed no antibodies to virus, had no virus detectable by plaque assays or immunocytochemistry and had no pulmonary lesions. Although the mechanism of this effect is not known, the authors speculate that decreased viral receptors on epithelial cells might be responsible. On the other hand, intranasal experimental infection of guinea pigs with Sendai virus was also used as an *in vivo* model to analyze the antiviral effect of **6** against parainfluenzavirus [25]. This study showed that a high-dose of **6** decreased both viral titers and virus-induced inflammation whilst a low-dose of **6** treatment decreased viral content in the lungs without inhibiting virus induced inflammation, suggesting that under certain conditions treatment with glucocorticoids actually reduces viral replication in the lungs without hamper the body's ability to restrain the infection.

SARS CoV

The application of steroids in other infections like severe acute respiratory syndrome (SARS) originated by an animal coronavirus (SARS CoV) is controversial [26]. SARS emerged in 2003 as a new human disease entity [27] caused by a novel emergent coronavirus identified in clinical specimens from patients with SARS with the use of cell cultures and molecular techniques [28]. The overall global case fatality of SARS during the 2003 pandemic was 9.6% (World Health Organization Summary of probable SARS cases with onset illness from 1 November 2002 to 31 July 2003. Available at: http://www.who.int/csr/sars/country/ table 2004_04_21) and acute respiratory failure occurs in 20% to 25% of SARS patients. SARS suddenly broke into a health care institution in Hong Kong and rapidly spread among patients and health workers. Out of one SARS patient a cohort of one hundred and thirty eight persons acquired the disease [29]. Since there was not an effective specific antiviral therapy, the treatment of SARS remained supportive and under those conditions 90% of patients survived. However,

as discussed by Tai *et al*. [30], in the case of severe ill or deteriorating patients, the administration of an antiviral agent plus an anti-inflammatory therapy to prevent inflammatory pneumonitis and irreversible pulmonary fibrosis was proposed. In consequence, many patients were treated with ribavirin and corticosteroids. However, at high concentration, ribavirin therapy was associated with many adverse effects, among others hemolytic anemia, hypocalcaemia and hypomagnesaemia [31, 32]. Fortunately there were not new epidemics of SARS-CoV after 2004, although nobody can predict a new reappearance of the infection among humans. If this event takes place different treatment regimens are recommended, all include intravenous administration of **7** or **9**, followed by oral prednisolone (**10**) (Fig. (**2**)) for varying periods and doses as per clinical evaluation [30]. After the results obtained in prospective and retrospective studies the treatment recommendations consider that steroids should not be used in the early phase of SARS, but rather as rescue therapy, as it may impair host viral clearance [29]. To conclude SARS-Cov infections are treated with glucorticoids to depress the high inflammatory response mounted by the host upon infection; these steroids are not used as adjunct therapy and they have no effect on virus replication.

Herpesviruses

HSV is a frequent human pathogen, most commonly associated with orolabial, genital, and ocular infections. HSV causes fever blisters or genital lesions that are the result of both a viral cytopathic effect and a massive inflammatory response. Although bothersome, HSV primary infection, in immunocompetent individuals, is usually mild or even asymptomatic and results in lifelong latent infections in sensory ganglia and the central nervous system (CNS) [33]. Paradoxically, several antiviral drugs in clinical use are specifically active against HSV-1 and HSV-2 replication but do not counteract the painful inflammation, neither avoid virus entrance in episomal latency in neurons. Combination of antiviral drugs with glucocorticoids that depress innate immune inflammatory response is not recommended for HSV-1 therapy of mild cases because several publications suggest that the treatment prolongs and increases viral yields [34].

Varicella Zoster virus (VZV), other member of the *Alphavirinae* subfamily of herpesviruses, causes in childhood a characteristic infection (Varicella) and like HSV establishes a life long latency state. Upon reactivation, generally, a new clinical entity known as herpes zoster appeared which is sensitive to acyclovir (ACV).

Certain severe uncommon reactivated herpes infections are regularly treated with corticosteroids such as herpes simplex virus encephalitis (HSE), Bell's palsy, vestibular neuritis and Ramsay Hunt syndrome.

Bell's palsy is an idiopathic acute peripheral unilateral facial paralysis that is the second most common acute facial paralysis with an incidence of 20-30 per 100.000 people annually [35]. HSV-1 and VZV are implicated in the disease; by means of polymerase chain reaction (PCR) performed in the saliva of patients within 7 days after the onset of the paralysis, DNA of HSV-1 was detected in 40% of the patients and DNA of VZV in 7% of the patients [36]. Ramsay Hunt syndrome is the second most common acute facial paralysis and is caused by reactivation of latent VZV. It is associated with zoster oticus and often complicated by vestibular cochlear dysfunction [35]. Vestibular neuritis is the second most common cause of peripheral vestibular vertigo and it would be caused by reactivation of HSV-1 infection. Steroids and adjuntive antiviral drugs are used to treat Bell'palsy and Ramsay Hunt syndrome. The combination of choice is valaciclovir and prednisolone (**10**) [37]. In cases that treatment started 5 days after the paralyses onset, prednisolone (**10**) alone is prescribed [35]. Studies on the benefits of using a combination of valacyclovir and methyprednisolone (**9**) to treat vestibular neuritis concluded that the administration of the antiviral is not necessary and patients improved with steroid treatment alone [38].

HSE is a life-threatening consequence of HSV infection of the CNS. Mortality rate reaches 70% in the absence of

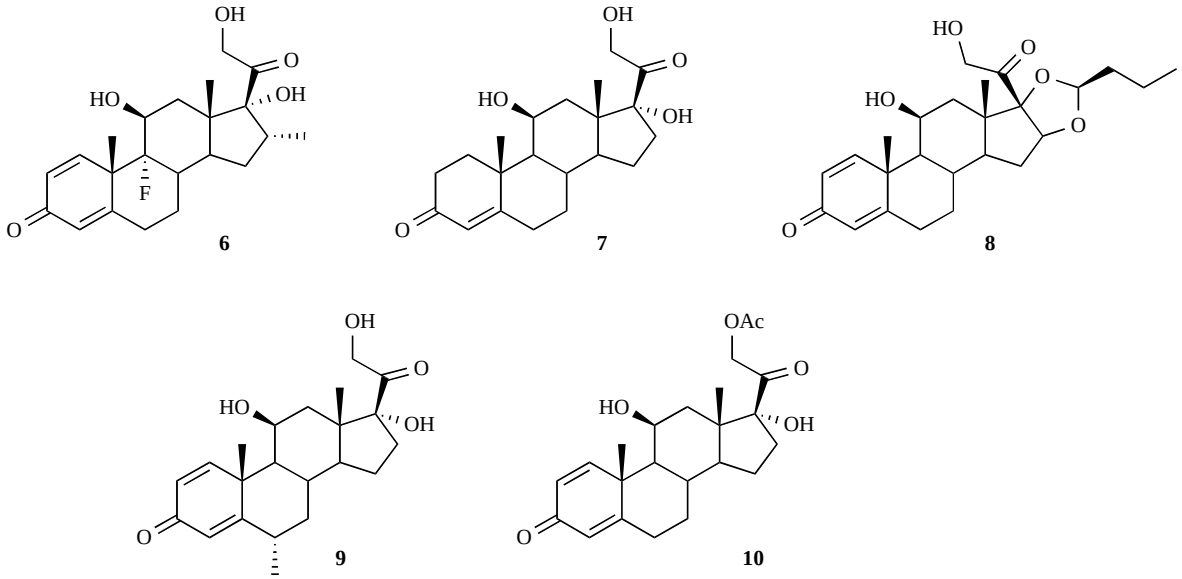

Fig. (2). Natural and synthetic glucocorticoids.

therapy and only a minority of affected individual returned to normality [39]. HSE is the result of HSV primary infection or virus reactivation from latent state. Evidence that supports the use of steroids to treat HSE came initially from animal models. The efficacy of corticosteroid treatment with ACV therapy in HSE patients, was assessed for the first time by Kamei *et al*. [40]. For treatment they use ACV by intravenous route and prednisolone (**10**) or dexamethasone (**6**) administered at the same time with the antiviral. They concluded that combination therapy represents one of the predictors of outcome of HSE. Concern about the proper time administration of corticosteroids arose from studies performed in a mice model of HSE [41]. Experimental results indicated that delayed time administration of glucocorticoids during viral infection is associated with neuroprotection and host survival. In a very recent review [42] authors assessed that systemic steroid treatment is an important aspect of CNS infectious disease treatment.

Controversial results have been obtained concerning *in vitro* anti-herpetic activity of glucocorticoids. In the case of HSV-1 some studies report up-regulation of virus yield while others describe the opposite effect depending on the cell system, the viral strain and the method used [34]. HSV-1 infection has been shown to induce the proinflammatory mediator nuclear factor kappa B (NF-κB), and to increase glucocorticoid receptor (GR) protein levels. Since NF-κB and GR mutually antagonize the effect of one another and considering that as part of an auto-regulation mechanism treatment with glucocorticoids may down-regulate GR expression, Erlandsson *et al*. [43] proposed that modulation of GR and NF-κB levels would explain the stimulatory or inhibitory effect of glucocorticoids on HSV-1 replication.

Finally, Epstein-Barr virus (EBV) is an oncogenic herpesvirus associated with a number of human malignancies, including Burkitt's lymphoma, Hodgkin's lymphoma, and post-transplantation lymphoproliferative diseases. Since the majority of EBV-infected tumor cells carry the EBV genome in a latent form one approach for the antiviral therapy would be to induce EBV lytic infection in tumor cells, which may make the cells susceptible to antiviral drugs, such as ganciclovir (GCV). Using lymphoma Akata cells, that carry the EBV genome but only 1 to 2% of EBV-positive cells express lytic antigens, Daibata *et al.* [44] reported that treatment with dexamethasone (**6)** raised up to 15% the percentage of cells expressing the lytic proteins. Combination of **6** with rituximab, a chimeric anti-CD20 monoclonal antibody, resulted in synergistic induction of lytic EBV infection in latently EBV-infected B-lymphoma cells, suggesting that treatment with dexamethasone/rituximab in combination with GCV could be a potential virally targeted therapy for EBV associated B-cell lymphoma [44].

HIV

Glucocorticoids have also been shown to have an effect on HIV-1 infection, however, as for HSV-1, some reports showed a slight increase in HIV-1 replication while others reported an inhibitory effect. It is generally not recommended to use corticosteroids as part of long-term HIV-1 treatment due to side effects associated with corticosteroid therapy; however, there are evidences that support the potential benefits of these molecules in short-term treatments. CCL2 is a proinflammatory chemokine induced in HIV-1 infection and a significant correlation of CCL2 gene expression with HIV-1 viremia has been demonstrated. A recent study investigated the effect of prednisolone (**10)** on viral load in an HIV-1-infected patient receiving high-dose of **10** for severe uveitis. A reduction of more than 1 log in viral load was observed on day 3 of prednisolone (**10**) administration and this decrease of viral load was strongly associated with a profound decrease of CCL2 mRNA expression [45]. Previous studies have revealed glucocorticoid-mediated modulation of HIV replication in human cell lines [46]. Suppression of viral load might be a direct consequence of **10** on HIV-1 LTR activity since the HIV-1 LTR promoter contains glucocorticoid-responsive element (GRE)-like sequences that could mediate this effect. Another explanation for **10** activity could be the reported transcriptional activation of IκBα, the cytoplasmic inhibitor of the proinflammatory transcription factor NF-κB and/or the inhibition of NF-κB that also binds to LTR [45].

Dengue Virus

Dengue virus (DENV) infection is amongst the most important emerging viral diseases transmitted by mosquitoes to humans, in terms of both illness and death. DENV most commonly causes a benign febrile illness called dengue fever (DF) and less frequently causes a life threatening illness, dengue hemorrhagic fever/ dengue shock syndrome. Reports concerning corticosteroid beneficial effects in DENV infections, including DF, are yet inconclusive. It was described that during the acute phase of DF circulating peripheral monocytes present DENV antigens indicating their role during natural infection. Taking this fact into account, an *in vitro* model with human monocytes infected with DENV (serotype 2) for evaluating immunomoulatory and antiviral activities of potential pharmaceutical products, was established [47]. Using this approach it was demonstrated that **6** decreased virus titer and inhibited the induction of TNF-α, IFN-α and IL-10 cytokines in the supernatants of DENV-infected cultures. Cytokines play an important role during DF pathogenesis; they can be related to hemorrhagic manifestations, coagulation activation, fibrinolysis and vascular permeability. The observed IFN-α down-regulation did not interfere with virus clearance which can be explained by the antiviral effects of other molecules such as nitric oxide (NO), known to control DENV replication in monocytes [48]. It is well-known that IL-10 exerts blocking effect on STAT-1 and IRF-1, relevant factors of nitric oxide synthase (iNOS) activation, so IL-10 expression induced by DENV infection may result in iNOS inhibition together with reduced NO effect on DENV replication. Therefore, down-regulation of IL-10 by **6** might result in iNOS activation leading to the inhibition of virus multiplication. Studies performed to assess NO as a potential antimicrobial agent against different pathogens suggest that NO would exert its antiviral action *via* S-nitrosylation of cysteine residues contained in viral enzymes (such as proteases, reverse transcriptases, ribonucleotide reductases) or in cell or viral encoded transcriptional factors involved in viral replication [49].

2.3. Dehydroepiandrosterone and Synthetic Derivatives

Dehydroepiandrosterone (DHEA, **11**) **(**Fig. (**3**)) and its sulfate ester are the most abundant circulating steroid hor-

mones in humans. A number of potentially beneficial effects, such as anti-aging, anti-inflammatory, immunomodulatory, antiviral, anticancer and neurotropic effects, have been attributed to replacement therapy with these compounds based on findings in rodent models, *in vitro* studies and human populations [50, 51]. Studies performed to understand the molecular basis of DHEA (**11**) multi-functional properties led to identify genomic and rapid non-genomic mechanisms of DHEA action. A cell membrane receptor for **11** has been proposed and DHEA (**11**) binding to this receptor would trigger non-genomic effects by modulating mitogen-activated protein kinase (MAPK) and the serine/threonine kinase (Akt) cell signaling pathways [4, 52].

There are reasons to believe that **11** may play a role in HIV-1 infection and progression toward acquired immunodeficiency syndrome (AIDS). Epidemiological studies have shown that sera from AIDS patients have lower than normal levels of **11** and that serum level of this steroid may be used as predictor for progression to AIDS in asymptomatic HIV-infected individuals [53]. Protection against disease progression has been ascribed to the ability of **11** to augment Th1 responses as opposed to Th2 responses and to dramatically increase natural killer cell cytotoxicity [54].

On the other hand, *in vitro* antiviral studies show that **11** exhibits a broad spectrum of antiviral action against both RNA and DNA viruses (Table **1**).

Table 1. DHEA (11) *In Vitro* Antiviral Activity

Virus	Family	EC_{50} (μM)	References
HIV	*Retroviridae*	50	54
JEV	*Flaviviridae*	50	59
JUNV	*Arenaviridae*	100	61
TCRV	*Arenaviridae*	204	61
PICV	*Arenaviridae*	110	61
VSV	*Rhabdoviridae*	70	60

EC_{50} values were calculated as the compound concentration that reduced by 50% the viral induced cytopathic effect (HIV and JEV) or compound concentration required to inhibit by 50% infectious virus released to the culture supernatant (arenaviruses and VSV).

Despite the fact that **11** is a native steroid that has been used clinically with minimal side effects, administration of the steroid for extended periods increases circulating testosterone and dihydrotestosterone manifold above normal levels, especially in women, and may cause masculinization [51]. Therefore, the evaluation of the antiviral activity of structural related compounds may be useful in the search of an effective treatment for virus infections. Prior studies demonstrated that **11** and the synthetic compound **12** (Fig. (**3**)), a DHEA analog, inhibit EBV-induced morphologic transformation and stimulation of DNA synthesis in human lymphocytes [55]. *In vitro* studies revealed that treatment with **11** or its synthetic analogs **13** and **14** (Fig. (**3**)) resulted in a modest down-regulation of HIV-1 replication in phytohemagglutinin-stimulated peripheral blood lymphocytes as measured by syncytium formation, release of p24 antigen, and accumulation of reverse transcriptase activity [56]. The initial infection with HIV-1 in most individuals usually results in the establishment of a latent or chronic infection before eventual progression toward AIDS. HIV-1 can establish a latent or persistent infection in some T cell lines, that show minimal constitutive virus expression, and activation leading to enhanced HIV-1 replication can be induced by antigens, mitogens, cytokines and various gene products from other viruses. DHEA (**11**) probed to be effective on impairing HIV-1 reactivation from chronically infected cell lines [57] and this observation is relevant taking into account that reactivation of latent HIV-1 harbored in chronically infected T lymphocytes, monocytes or macrophages plays an important role in the pathogenesis of AIDS. Furthermore, addition of **11** to MT-2 cell cultures infected with either 3'azido-3'deoxythymidine (AZT)-sensitive or AZT-resistant isolates of HIV-1 resulted in dose-dependent inhibition of HIV-1-induced cytopathic effect and suppression of HIV-1 replication [54]. *In vitro* studies performed by Bradley *et al*. [58] with other lentivirus revealed that **11** affects the replication of feline immunodeficiency virus (FIV) in chronically infected cells at levels where cellular viability and DNA synthesis were not affected.

DHEA (**11**) also displays *in vitro* inhibitory action against several RNA viruses (Table **1**) such as the flavivirus Japanese encephalitis virus (JEV) [59], the rhabdovirus VSV [60] and the arenaviruses Junin (JUNV), Tacaribe (TCRV) and Pichinde (PICV) [61].

JEV infection, that commonly affects children, is a major cause of acute encephalopathy in several parts of South-East Asia and there is no specific antiviral therapeutic available for the treatment of this viral pathology. Infection by JEV can cause acute encephalitis with a high mortality rate in humans and increasing evidence suggests that both neuronal destruction and dysfunction might partly explain the manifestation of the disease. Although the mechanisms by which JEV directly induces the death of infected neurons remain largely unknown it has been reported that enforced expression of bcl-2 and activation of MAPK signaling cascades may protect neurons from JEV-induced cell death [62]. Chang *et al.* [69] showed that JEV infection resulted in the alteration of MAPK signaling cascades leading to apoptotic cell death and suggested that the inhibitory effect of DHEA could be attributed to the ability of this compound to restore MAPK signaling functions.

JUNV causes a severe disease in humans known as Argentine hemorrhagic fever (AHF). Other arenaviruses, such as Lassa virus, Machupo virus, Guanarito virus and Sabia virus also cause severe hemorrhagic diseases in man. Although the importance of these viruses as human pathogens and the continuous emergence of new arenaviruses during the last years in North and South America to date ribavirin is the only drug that has shown efficacy for Lassa fever infection and only partial success in treatment of AHF patients. In addition, undesirable side effects were recorded for ribavirin treatment, thus the current therapy for AHF is based upon the early administration of immune plasma. However, a drawback of this treatment is the development of a late neurological syndrome in about 10% of the treated patients. Besides **11**, several synthetic DHEA derivatives with different substituents at positions C-3, C-15, C-16 and C-17 exhibited antiviral activity against JUNV and the non-human pathogen

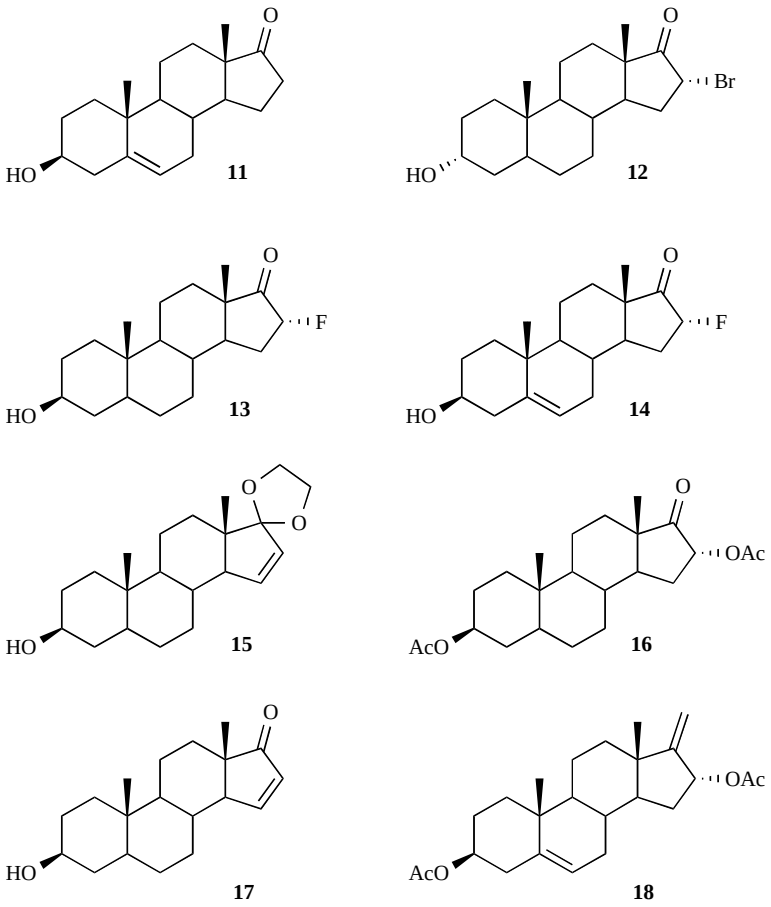

Fig. (3). DHEA (**11**) and synthetic DHEA derivatives.

RNA virus VSV. Fig. (**3**) and Table **2** show the most active compounds (**15-18**) that exhibit better SI than ribavirin used as reference drug [60, 61].

Table 2. Antiviral Activities of DHEA-Derivatives Against JUNV and VSV Replication in Vero Cells

Compound	VSV SI	JUNV SI
15	43.8	121
16	22.6	21
17	ND	24
18	37.2	ND

SI (selectivity index): ratio CC_{50}/EC_{50}. CC_{50}: compound concentration required to reduce cell viability by 50%. EC_{50}: compound concentration required to reduce virus yield by 50% [60, 61]. ND: not done.

The analysis of the effect of DHEA (**11**) on different steps of JUNV and VSV replicative cycle revealed a strong inhibition on virus protein synthesis and infectious virus formation. An important reduction of the synthesis of primary transcripts was detected in VSV-infected DHEA-treated cells [60]. Despite the fact that a more accurate quantification of JUNV transcripts would be required, a slight reduction of JUNV positive strand RNA levels could be observed in DHEA-treated cultures [61]. Though little is known about host components required for JUNV and VSV replication, increasing evidence indicates that successful virus replication relies upon the evolution of strategies that modulate host cell signaling pathways. Many viruses, such as influenza A virus, human cytomegalovirus and coxsackievirus B3 (CV-B3), can trigger PI3K/Akt signaling pathway activation and specific inhibitors of Akt phosphorylation lead to a reduction on virus RNA and protein synthesis [63]. In addition, it has been reported that DHEA impairs Akt phosphorylation in HepG2 cells, and though the effects of this steroid appear to be determined by the cell type, these experimental evidences would support the speculation that the inhibitory effect of DHEA might be related with cell signaling pathway modulation [64].

The experimental evidences described above indicate the need of a more exhaustive investigation of the influence of this type of steroids in the switch from transcription to genome replication in RNA viruses that display distinct replication strategies such as JEV (plus stranded RNA genome), VSV (negative stranded RNA genome) and JUNV (RNA genome with ambisense coding strategy).

Although an inhibitory effect of DHEA (**11**) on JUNV genome synthesis can not be excluded the mode of DHEA antiviral action differs from the inhibitory action of ribavirin, since DHEA exerts a strong antiviral activity even when added late during infection, impairing the formation of infectious virus particles. It was also shown that DHEA partially reduced the expression of JUNV glycoprotein G1 at the sur-

face of treated infected cells [61]. This effect would be related with the capacity of steroid hormones to intercalate into cell membrane bilayer and alter membrane fluidity [65]. This property of the steroid would affect the insertion of viral glycoproteins further inhibiting the assembly of virus particles.

In relation with the effect of steroids on membrane-dependent processes, a DHEA-derivative named immunor (IM 28) inhibited cell-cell fusion mediated by HIV-1 glycoprotein stably expressed in a T cell line [66]. Interestingly, a similar dose dependent cell fusion inhibition was obtained in cultures treated with the glucocorticoid dexamethasone (**6**). The authors proposed that IM28 and **6** would interact with phospholipase A2 (PLA2), which plays an important role in HIV entry into the cell. Binding of envelope glycoprotein to CD4 receptor would activate PLA2 triggering a local membrane-destabilizing effect preparing the cell membrane for fusion with the viral particle.

As was mentioned above for **6**, the antiviral activity of DHEA (**11**) has been also related to its ability to promote NO production. Studies performed in an attempt to understand the role of **11** on the regulation of vascular function showed that DHEA administration to human endothelial cells triggers NO synthesis, due to enhanced expression and stabilization of endothelial nitric oxide synthase (eNOS). Additionally, DHEA rapidly activates eNOS, through a non-transcriptional mechanism that depends on ERK1/2 MAPK [67]. Oral DHEA administration to ovariectomized Wistar rats dose-dependently restores aortic eNOS levels and eNOS activity, suggesting that NO synthesis could also explain some of the *in vivo* biological properties of **11** [67].

A protective effect of DHEA (**11)** was demonstrated in studies of two lethal viral infection models in mice: systemic CV-B4 and HSV-2 encephalitis. Histopathological analysis, leukocyte counts, and numbers of spleen antibody forming cells in the CV-B4 model suggest that **11** functions by up-regulating the immune competence of mice. DHEA does not affect virus tissue titers and *in vitro* antiviral activity against these viral infections has not been demonstrated [68]. *In vivo* studies performed with HSV-1 [69], West Nile virus, Sindbis virus neurovirulent and Semliki Forest virus [58] also support the idea of **11** as an immunostimulator rather than a molecule with direct antiviral activity.

In contrast with these studies Pedersen *et al*. [70] demonstrated that the analog **12** (Fig. (**3**)) modulates acute FIV infection in laboratory cats. FIV infected cats that were treated with the synthetic derivative exhibited an enhanced initial viremia, however, the subsequent levels of virus in the blood were significantly lower in treated versus untreated animals. In addition, CD4 T cells were decreased in FIV-infected cats treated with **12** compared to their untreated cohort, while CD8 T cells tended to be higher in treated animals.

3. PLANT STEROIDS

Plants possess the ability of synthesize a large variety of steroids, and hormonal function was postulated for plant steroids also. Brassinolide (**19**) was the first identified plant steroid with hormonal activities (Fig. (**4**)). Since then, many others structurally related steroidal compounds with growth-promoting activities have been isolated from plants; some 60 are known [71] and they are collectively referred to as brassinosteroids (BRs). BRs contain the typical 5α-cholestan steroidal skeleton with fused rings A, B, C, and D and an alkyl side chain at C-17 not found in mammalian steroidal hormones.

BRs function as signaling molecules in plants and are involved in processes such as stem elongation, vascular differentiation, male fertility, timing of senescence and flowering, leaf development, and resistance to biotic and abiotic stresses [72]. BRs are perceived at the plasma membrane by direct binding to the extracellular domain of a transmembrane protein, BRI1, that has a serine–threonine protein kinase activity. BR binding initiates a signaling cascade and further kinases and phosphatases determine the phosphorylation state and stability of nuclear transcription factors. These factors mediate major BR effects in various plant physiological processes [73].

Plants have also evolved constitutive and inducible defense mechanisms by producing a vast array of secondary metabolites against various microbial pathogens [74]. It is therefore probable that antiviral compounds would occur in plants as part of their innate defense, thus secondary metabolites constitute a promissory group of compounds for screening of new antiviral agents.

3.1. Hormones: Brassinosteroids (BRs)

In the last years our laboratory has described the inhibitory action of several natural and synthetic BRs against different animal viruses [75-79]. It was first demonstrated that two natural BRs, brassinolide (**19**) and 28-homocastasterone (**20),** display antiviral activity against poliovirus, HSV-1, VSV and the arenaviruses JUNV and TCRV [79], with higher inhibition values than the reported for other natural steroidal molecules tested before [80-82].

After these preliminary results we decided to evaluate the cytotoxic and antiviral activity of synthetic derivatives of BRs (**21-29**) (Fig. (**4**)) obtained by chemical synthesis from stigmasterol, a natural plant sterol.

Most of the assayed compounds exhibit better SI than 28-homocastasterone (**20**) which possesses the basic steroidal skeleton of all the synthetic compounds (Table **3**). Compounds **21**, **28** and **29** were active against all the viruses assayed: HSV-1, HSV-2, measles virus (MV) and JUNV. Compound **21** was the most active one, although it presents lower activity than ACV against HSV-1 and HSV-2 [77, 79]. By contrast, compound **21** showed 3.5 to 18 fold higher activity than ribavirin against MV and JUNV, respectively [76, 79]. BR-derivatives were highly effective to inhibit JUNV *in vitro* infection, with SI values higher than 100 for compounds **21-26**. Similar results were obtained with TCRV and PICV, indicating that arenaviruses were very sensitive to the antiviral activity of this type of compounds [75]. The bioactivity of more than 30 analogues was evaluated in order to develop a preliminary structure-activity relationship.

It is known that natural BRs (for example 28-homocastasterone (**20**), a very active phytohormone, posses a diol with a *22R, 23R* configuration at the side chain. Our findings

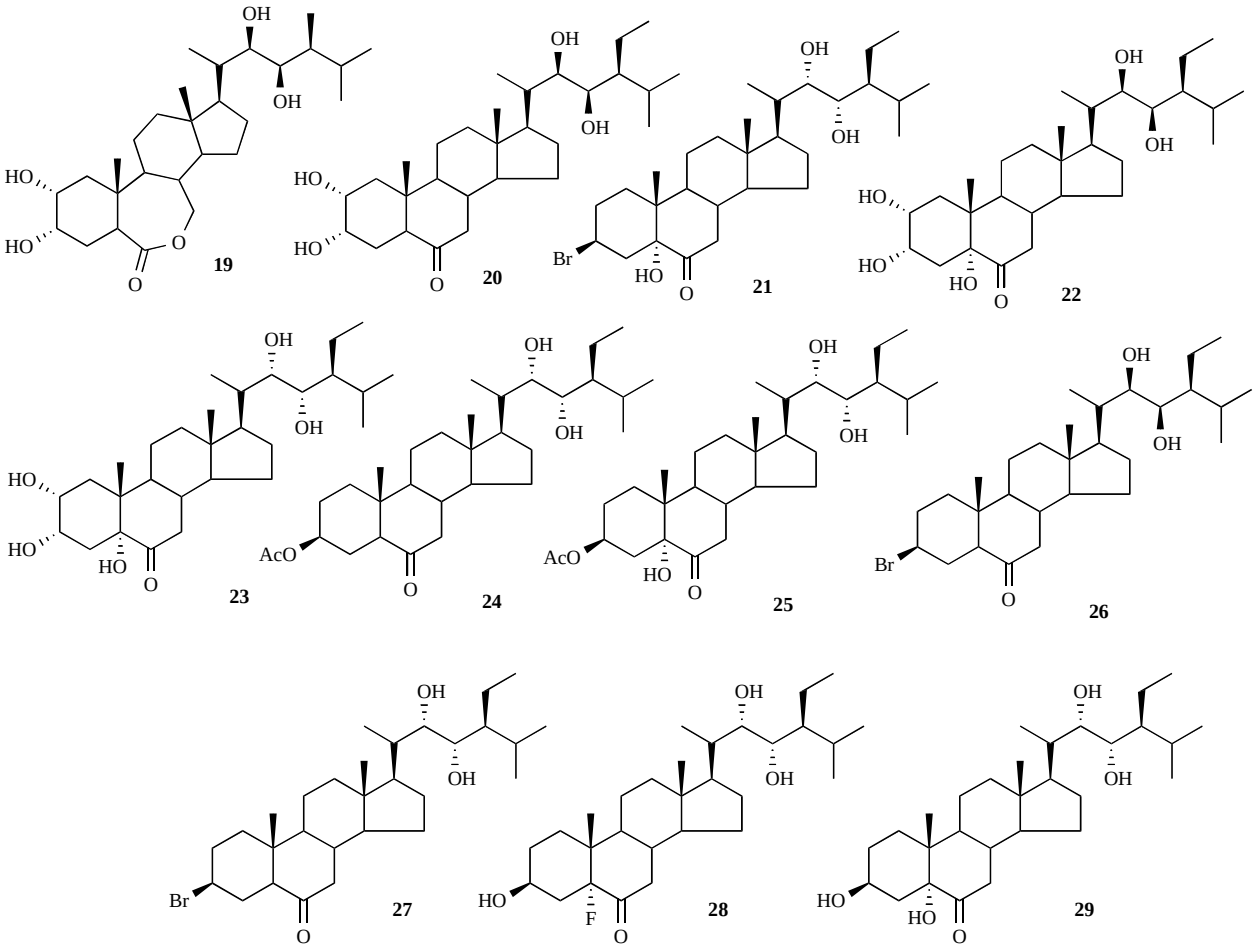

Fig. (4). Natural and synthetic BRs.

Table 3. Antiviral Activity of Brassinosteroids (BRs)

Compound	CC50 (μM)	SI			
		HSV-1	HSV-2	MV	JUNV
20	63	2	ND	2	18
21	277	100	71	44	693
22	263	1	ND	2	200
23	502	16	ND	6	228
24	226	10	ND	8	232
25	462	17	ND	20	231
26	114	4	ND	2	310
27	152	6	ND	4	72
28	160	109	27	54	27
29	1044	80	40	40	40

CC_{50}: compound concentration required to reduce viability of Vero cells by 50% of the untreated control. SI: selectivity index or ratio CC_{50}/EC_{50}. EC_{50}: compound concentration required to reduce virus yield by 50% of the untreated control [79].

indicate that analogues having the opposite unnatural configuration at these positions usually show an enhanced activity (compare **26** to **27**, or **22** to **23**). On the other hand, introduction of an electronegative group at C-5 (fluorine in **28** and hydroxyl in **29**) leads to more active compounds against HSV-1.

None of the tested compounds exhibited direct inactivating effect indicating that they inhibit a step of the virus replicative cycle. Attempts to disclose the mode of action of **21** against HSV-1 indicated that in the presence of the BR virus late protein synthesis was severely diminished [78]. Since late protein synthesis is dependent on viral DNA replication,

studies of drug-drug combination with inhibitors of viral DNA synthesis such as ACV and foscarnet (FOS) were performed. These assays demonstrated that **21** would act synergistically with low concentrations of ACV and moderate concentrations of FOS against HSV [83]. These results suggest that the mechanism of antiviral action of **21** differs from the antiviral mode of action of nucleoside analogues.

The inhibitory action of **21** against JUNV replication in Vero cells was also investigated [84]. Time of addition experiments revealed that **21** was most effective the earlier it was added to the cells after infection and that **21** prevents the formation of mature viral particles rather than the release of progeny virus to the extracellular medium. In accord with the results previously obtained with HSV-1 neither adsorption nor internalization of viral particles was the target of the inhibitory action. The effect of **21** on viral RNA synthesis was investigated. Two single stranded RNA fragments with ambisense coding strategy, called S and L, compose virus genome. The S fragment, which encodes the nucleocapsid protein, N, and the precursor of virus glycoproteins, is transcribed to two antigenomic forms: the 1.8 kb N mRNA and the 3.4 kb full length antigenomic S (a similar transcriptional strategy has been described for L fragment). The analysis by RT-PCR showed that at 6h post-infection only N mRNA was detected in infected cultures treated with **21** indicating that **21** prevents the synthesis of full length antigenomic RNA. It has been proposed that the switch from transcription to replication during arenavirus infection might depend on the intracellular level of N protein since at low levels of N protein only N mRNA transcripts are produced. When the N mRNA is translated, the newly synthesized N protein might act as a transcriptional antiterminator factor, allowing the synthesis of full length antigenomic S RNA [85]. Thus **21** might block N protein synthesis or N protein function. However, it is not known whether the arenavirus transcription–replication switch implies a more complex scheme involving other viral or cellular factors. Besides the effect on RNA synthesis, a high inhibition of virus yields and JUNV-mediated cell fusion was also observed when the compound was present during the last steps of virus multiplication cycle. Given these results, an adverse effect of **21** on post-translational processing or proper insertion of JUNV glycoproteins into the cell membrane cannot be ruled out. The effect of **21** on different steps of VSV replicative cycle was also investigated [86]. In this model primary transcription was not inhibited but a high inhibition of viral protein synthesis and virus particle formation was evident. Though the effect of **21** on VSV full lenght RNA synthesis has not been examined yet, these results are in accord with experimental evidences obtained with JUNV. Although **21** shows a broad antiviral spectrum the fact that some viruses are more susceptible to its inhibitory effect than others indicates that 6b could exert its antiviral action by affecting a specific viral factor and to a lesser degree a cellular function required for viral replication.

There is some evidence for the low level of toxicity of natural BRs, being constituents of practically all plants, BRs are usually consumed by mammals and the safety of some natural BRs has been confirmed by toxicological studies in mice and rats (orally and dermally) [87]. *In vivo* studies performed in a murine experimental model have demonstrated a non-toxic effect after topical eye administration of **21** (40μM) three times a day during 3 consecutive days [88].

Evaluation of *in vivo* antiherpetic activity of synthetic BRs was performed in the murine herpetic stromal keratitis (HSK) experimental model. Administration of compounds **21**, **28** or **29** to the eyes of mice at 1, 2 and 3 days post-infection delayed and reduced the incidence of HSK, however, viral titers of eye washes were not diminished in BR-treated mice, suggesting that the compounds do not exert a direct antiviral effect but rather may play a role in immune-mediated stromal inflammation [88]. This hypothesis was further supported by in-vitro studies that demonstrated that the compound **21** modulated the response of epithelial and immune cells to HSV-1 infection, acting as an inductor or inhibitor of cytokine production depending on the cell type involved [89]. Thus, the protective effect in mice could be due to a balance between the immunostimulating and immunosuppressive effects of the derivative. Similar results were obtained with other synthetic stigmastane analogs [90]. In addition, several synthetic stigmastanes were able to inhibit the TNF-α production in L929 cells and some of them resulted as active as dexamethasone (**6**). According to preliminary analysis of the structure–activity relationships, the modulatory effect on the TNF-α production of these synthetic compounds could be related with the presence of a hydroxylated stigmastane side chain, having a 22S, 23S configuration, and with a 3β, 5α-dihydroxy-6-keto moiety in the steroidal ring system [91].

3.2. Secondary Metabolites: Seco-Pregnanes and Steroid-Related Structures

Seco-Preganane Steroids

Recent studies provide evidence that seco-pregnane steroids and its glycosides isolated from the herbaceus plants *Strobilanthes cusia* and *Cynanchum paniculatum* are effective and selective inhibitors of several members of the positive-strand RNA containing alphavirus-like supergroup, including both plant- and animal-infecting viruses [92]. To screen for antiviral compounds, the plant-infecting tobacco mosaic virus (TMV) was used as a model target. This approach allowed the isolation of five seco-pregnane steroids with anti-TMV activity: glaucogenin C (**30**) and cynatratoside A (**31**) (isolated from *S. cusia*) and paniculatumoside C (**32**), D (**33**) and E (**34**) (isolated from *C. paniculatum*) (Fig. (**5**)).

Then, the effect of the compounds on Sindbis virus (SINV) multiplication, an alphavirus that resembles TMV in genome organization and replication strategy, was evaluated. The five compounds could effectively impair SINV-induced cytopathic effect in BHK-21 cells without toxic effects on cell proliferation. Furthermore, the compound **32** proved to be an effective inhibitor of other alphaviruses. On the contrary, this compound did not affect the multiplication of other RNA or DNA viruses (Table **4**) indicating a high specific activity of these inhibitors.

Antiviral activity was similar for all the tested compounds indicating that the aglycon per se is responsible for the inhibitory effect and the linked oligosaccharide chains make little contribution to the antiviral action.

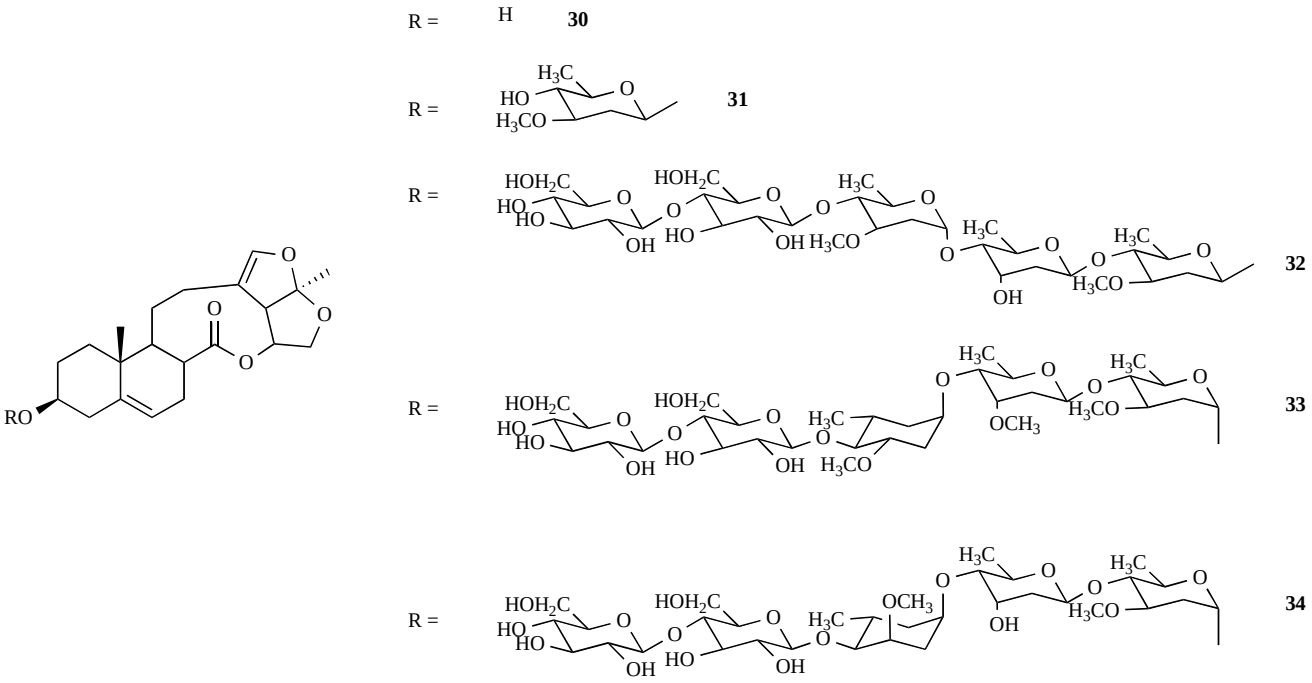

Fig. (5). Natural seco-pregnanes isolated from *S. cusia* (**30** and **31**) and *C. paniculatum* (**32**, **33** and **34**).

Table 4. Antiviral Spectrum of the Seco-Pregnane Steroid Paniculatumoside C (32)

Virus	Family	Cells	EC50 (nM)	SI
SINV	*Togaviridae*	BHK-21	1.5	11,000
GETV	*Togaviridae*	BHK-21	1	16,500
EEV	*Togaviidae*	BHK-21	2	8,250
JEV	*Flaviviridae*	C6/36	>24,000	I
HCV	*Flaviviridae*	B	>24,000	I
HIV	*Retroviridae*	H9	>24,000	I
MV	*Paramyxoviridae*	Vero	>24,000	I
Influenza A virus	*Orthomyxoviridae*	MDCK	>24,000	I
Reovirus	*Reoviridae*	CIK	>24,000	I
Hadv-4	*Adenoviridae*	983A	>24,000	I

CC_{50}: compound concentration that reduced cell viability by 50% with respect to control. EC_{50}: compound concentration required to achieve 50% inhibition of virus-induced cytopathogenicity. SI: ratio CC_{50}/EC_{50}. I: inactive [92].

Northen-blot analysis indicated that these compounds exert their inhibitory effect on TMV and SINV infection through selective suppression of subgenomic (sg) RNA(s) [92]. In the case of SINV, two viral mRNAs are produced in infected cells: the 49S genomic RNA, which is translated into the viral non-structural proteins and the 26S sgRNA which encodes the viral structural proteins, the capsid protein and the two envelope glycoproteins E1 and E2. During the first hours of infection, the level of the 26S sgRNA decreased almost linearly with an increase in the concentration of the seco-pregnane steroids and no changes in the level of genomic RNA were detected even at the highest compound concentration tested. Transcription machinery for expression of the sgRNA mainly consists of a transcriptase complex formed by the viral polymerase, other viral non-structural proteins and some cellular factors. This complex binds to a stretch of nucleotides on the negative-strand genome-length RNA surrounding the beginning of the sgRNA called the sgRNA promoter. It was proposed that the compounds might alter the structure of sgRNA promoter, interfere with binding of transcriptase complex to the sgRNA promoter or even affect the function of cell factors associated to the transcription machinery.

In vivo administration of the seco-pregnane steroid **32** protected newborn BALB/c mice from lethal SINV infection [92]. When compound-treated mice that survived infection were subsequently inoculated with a lethal dose of SINV no mice survived indicating that **32** protected mice from SINV infection by directly inhibiting virus multiplication rather than mounting a protective immune response to viral infection.

Ursolic Acid

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid, **35**), a pentacyclic triterpenoid (Fig. (**6**)) derived from berries leaves, flowers, and fruits of several medicinal plants displays pleiotropic biologic activities, such as antibacterial, hepatoprotective, immunomodulatory, antiproliferative activities and a broad spectrum of antiviral activity.

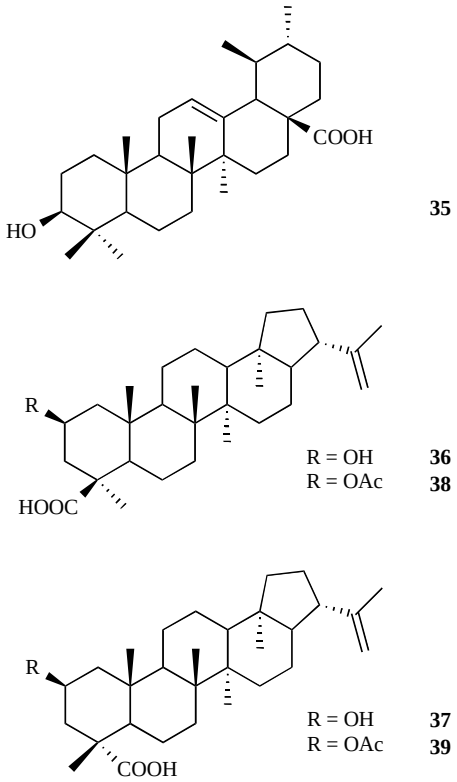

Fig. (6). Ursolic acid (**35**) and related compounds.

Extracts and purified components of *Ocimum basilicum*, a well known medicinal herb in traditional Chinese medicine preparations, were used to identify possible antiviral activities against DNA viruses: HSV-1, adenoviruses (ADV) and hepatitis B virus, and against RNA viruses: CV-B1 and enterovirus 71 (EV71) [93]. Among the active compounds identified, ursolic acid exhibits antiviral activity against HSV-1, ADV-8, CVB1 and EV71 (Table **5**). A modest anti-HIV activity of ursolic acid (**35**) (Table **5**) has been also described [94]. Recent studies demonstrated that two new hopane type triterpenes, named dryopteric acids A (**36**) and B (**37**) (Table **6**, Fig. (**6**)), isolated from the Rhizome of *Dryopteris crassirhizoma* (Aspiadaceae) together with ursolic acid showed potent *in vitro* inhibitory activity against HIV-1 protease. In addition, acetylated compounds (**38** and **39**) appreciably increased their inhibitory activities [95].

It was also reported that ursolic acid suppressed the growth of human papillomavirus (HPV)-positive cervical carcinoma cells. HPV is the most prevalent sexually transmitted infection in the world, occurring in up to 75% of sexually active women and about 99.7% of cervical cancers are directly linked to previous infection with one or more of the oncogenic types of HPV. HPV contains a circular double-stranded DNA genome that encodes eight genes, of which E6 and E7 are necessary for malignant conversion. Association of E6 and E7 proteins with tumor suppressor p53 and pRB, respectively, has been proposed as a mechanism by which these viral proteins induce tumors. Ursolic acid (**35**) -treated HeLa cells, a HPV-18 positive cell line, developed typical apoptotic characteristics and exhibited reduced levels of E6 and E7 gene expression as determined by reverse PCR. By contrast, treatment of HPV-positive lines with the glucocorticoid dexamethasone (**6**) did not affect cell proliferation or viral gene expression [96]. Thus, these findings suggest that **35** might be a useful anticancer drug in treatment of HPV-associated cervical neoplasia. Transcription of HPV E6 and E7 gene is controlled by the upstream regulatory region (URR). Transcriptional factors participate in the positive or negative regulation of URR activity. In addition, the URR has been shown to contain response elements for progesterone (**1**) and glucocorticoids, thus, it can be speculated that response elements for ursolic acid (**35**) in URR might be responsible for the negative regulation of E6/E7 genes interfering with the binding or activity of trans-acting transcriptional factors. Therefore, these studies provide evidence of a novel mechanism of antiproliferative and antiviral effect of a steroidal molecule.

Table 5. ***In Vitro*** **Antiviral Activity of Ursolic Acid (35)**

Virus	EC50 (μM)	SI
HSV-1	14.5	15.2
ADV-8	9.2	23.8
CVB1	0.9	251.3
EV71	1.1	201.0
HIV-1	4.4	3.3

CC_{50} was the compound concentration that reduced cell growth by 50% (BCC-1/KMC cells for HSV-1, ADV-8, CVB1 and EV71 or H9 cells for HIV-1). EC_{50} was defined as the compound concentration that achieved 50% inhibition of virus-induced cytopathic effect for HSV-1, ADV-8, CVB1 and EV71 or 50% inhibition of p24 antigen in culture supernatants for HIV-1 [93, 94].

Table 6. Inhibitory Effects of Compounds Isolated from the Rhizome of *D. crassirhizoma* Against HIV-1 Protease *In Vitro*

Compound	Name	EC_{50} (μM)
35	Ursolic acid	8.9
36	Dryopteric acid A	26.5
37	Dryopteric acid B	44.5
38	2-*O*-Acetyldryopteric acid A	1.7
39	2-*O*-Acetyldryopteric acid A	10.8

The EC_{50} value was defined as the compound concentration that reduced the activity of HIV protease (PR) by 50% with respect to control. The inhibitory activity was determined by an HPLC method using the synthetic peptide [His-Lys-Ala-Arg-Val-Leu-(pNO2-Phe)-Glu-Ala-Nle-Ser-NH2] as a substrate and a fused recombinant HIV-1 PR . The hydrolysate and remaining substrate were quantitatively analyzed by HPLC [95].

4. CONCLUDING REMARKS

Viruses respond to antiviral treatment with a rapid selection of drug resistant mutant particles, compelling virologists to search for new active compounds. Steroids from different

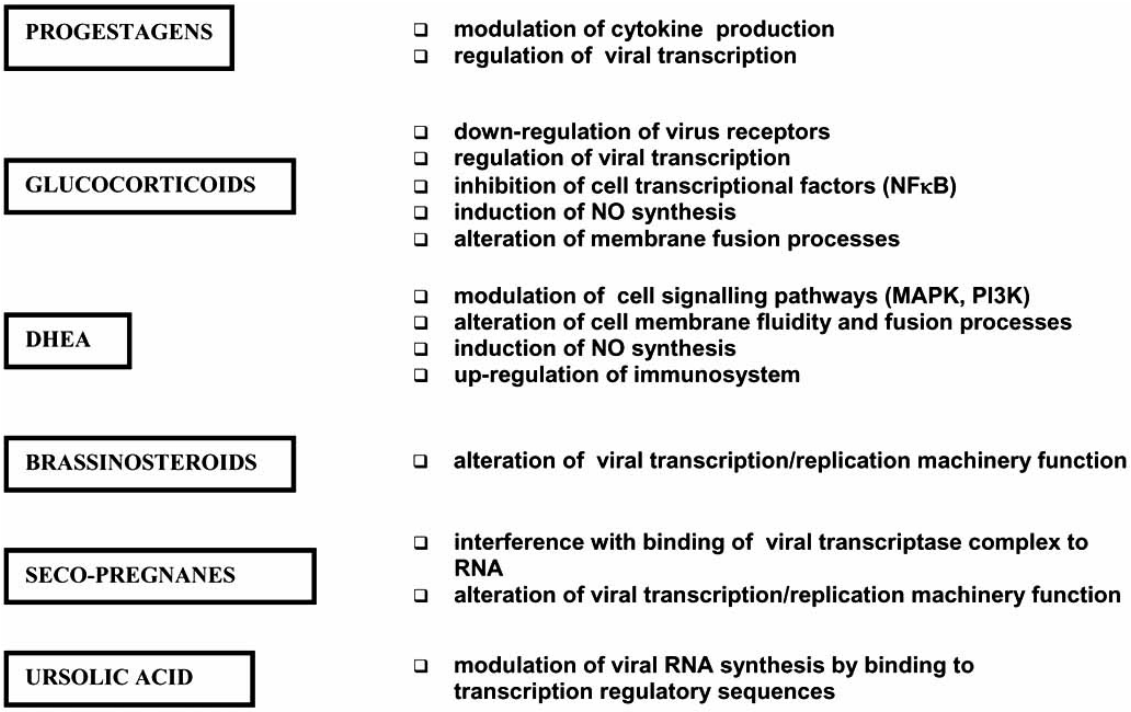

Fig. (7). Diverse mechanisms proposed for the antiviral action of animal and plant steroids.

natural sources offer a great variety of compounds that display a vast diversity of biological functions including modulation of virus replication. Studies focused on the antiviral activity of mammalian steroid hormones disclose a complex and not well- understood scenario with an important number of controversial results. Despite *in vitro* studies show that many of these molecules exhibit direct inhibitory effect on virus replication, *in vivo* assays and clinical trials reveal that steroid actions involve the regulation of distinct cell processes that would finally lead either to an inhibitory or a stimulatory effect on virus replication. For some viral infections the use of natural or synthetic progestagens and glucocorticoids, alone or in combination with antiviral therapy, can be beneficial under certain well-studied conditions, however, several drawbacks of steroid therapy, mainly related with immunosuppressive activity should be considered. Fig. (**7**) summarizes the diversity of the antiviral mechanisms of action that have been proposed for mammalian hormones.

Plant steroids have been less studied; however, *in vitro* assays indicate that despite structural differences, these compounds appear to exert their inhibitory action essentially altering viral transcription/replication processes (Fig. (**7**)). In addition, interesting antiviral properties have been proved for synthetic analogues of plant steroids and although immunomodulating properties were also described for some of these molecules, no correlation between this effect and antiviral action has been demonstrated yet.

Therefore, the actual knowledge about the antiviral action of steroid compounds reveals that cell targets are an important component of this activity and highlights the need for studies to further understand steroid-virus-cell interactions. The development of novel synthetic compounds would also be essential in the search for alternatives to deal with the unwanted effects of natural active compounds.

ACKNOWLEDGEMENTS

This work was supported by grants from the Universidad de Buenos Aires, UBACyT X-206 (08-10) and X-084, and from the Agencia Nacional de Promoción Científica y Técnica (ANPCyT PICT 38285/05).

ABBREVIATIONS

HIV	=	immunodeficiency virus
PR	=	progesterone receptor
iNOS	=	inducible nitric oxide synthase
TNF	=	tumor necrosis factor
PMBCs	=	peripheral blood mononuclear cells
ER	=	estrogen receptor
LTR	=	long terminal repeat
SIV	=	simian immunodeficiency virus
HSV	=	herpes simplex virus
VSV	=	vesicular stomatitis virus
RV	=	rhinovirus
IL	=	interleukin
SARSCoV	=	acute respiratory syndrome caused by coronavirus
CNS	=	central nervous system
VZV	=	Varicella Zoster virus
ACV	=	acyclovir
HSE	=	herpes simplex encephalitis

NF-κB = nuclear factor kappa B
GR = glucocorticoid receptor
EBV = Epstein-Barr virus
GCV = ganciclovir
GRE = glucocorticoid-responsive element
DENV = dengue virus
DF = dengue fever
NO = nitric oxide
DHEA = dehydroepiandrosterone
MAPK = mitogen-activated protein kinase
AIDS = acquired immunodeficiency syndrome
AZT = 3´azido-3´deoxythimidine
FIV = feline immunodeficiency virus
JEV = Japanese encephalitis virus
JUNV = Junin virus
TCRV = Tacaribe virus
PICV = Pichinde virus
AHF = Argentine hemorrhagic fever
CV = coxsackievirus
PLA2 = phospholipase A2
eNOS = endothelial nitric oxide synthase
BR = brassinosteroid
SI = selectivity index
CC_{50} = 50%cytotoxic concentration
EC_{50} = 50% effective concentration
MV = measles virus
HSK = herpetic stromal keratitis
TMV = tobacco mosaic virus
SINV = Sindbis virus
sg = subgenomic
ADV = adenovirus
EV = enterovirus
HPV = human papillomavirus

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Received: December 23, 2009 Revised: March 24, 2010 Accepted: March 26, 2010