Short communication

Synthesis and evaluation of *N*-substituted acridones as antiviral agents against haemorrhagic fever viruses

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Background: In the present study, a series of *N*-substituted acridone derivatives was synthesized and evaluated against two haemorrhagic fever viruses (HFV).
Methods: Compounds were tested against Junin virus (JUNV), an arenavirus agent of Argentine haemorrhagic fever, and dengue virus (DENV), a flavivirus agent of the most prevalent arthropod-borne viral disease in humans.
Results: Among tested compounds, two *N*-allyl acridones (derivatives 3c and 3f) elicited a potent and selective antiviral activity against JUNV (strain IV4454) and DENV-2 (strain NGC) with 50% effective concentration values between 2.5 and 5.5 μM, as determined by virus yield inhibition. No cytotoxicity was detected at concentrations up to 1,000 μM, resulting in selectivity indices >181.8–400.0. Both acridones were effective against a wide spectrum of arenaviruses and the four serotypes of DENV. Furthermore, 3c and 3f failed to inactivate virus before cell infection as well as to induce a refractory state by cell pretreatment, indicating that the inhibitory effect was exerted through a blockade in virus multiplication during the infectious process.
Conclusion: These data are the first demonstration that acridone derivatives have a potent antiviral activity that block *in vitro* multiplication of HFV belonging to *Arenaviridae* and *Flaviviridae*, such as JUNV and DENV.

Introduction

Haemorrhagic fever viruses (HFV) comprise a heterogeneous group of enveloped viruses with RNA genome mainly classified in the *Arenaviridae*, *Bunyaviridae*, *Filoviridae* and *Flaviviridae*. After transmission from their reservoir host or vector to humans, these viruses can often produce a mild febrile syndrome or even a subclinical infection; however, the more severe forms of haemorrhagic disease caused by these agents are associated with extremely high morbidity and mortality. Although the evaluation of different types of compounds for inhibitory activity against HFV has been reported [1–5], no specific and safe chemotherapy for any HFV is currently available. Ribavirin is the only drug that is known to be of any benefit in the treatment of arenavirus and bunyavirus haemorrhagic fevers, but with a high level of undesirable adverse reactions in treated patients [6–8]. Based on the danger of HFV for human health, their increased emergence in recent years and the lack of effective tools for their control or prevention, the search for novel antiviral compounds effective against these pathogenic agents is a continuous demanding effort.

Acridone derivatives of natural and synthetic origin are known to possess a variety of biological activities, including antiviral action. Several studies have shown the inhibitory activity of diverse types of acridones against viruses with DNA genomes, such as herpes simplex virus, human cytomegalovirus [9–11], Epstein–Barr virus [12] and adenovirus [13]. Although certain acridone-based compounds were identified as DNA topoisomerase II inhibitors, the exact target and mode of action of these compounds against DNA viruses is currently unknown [14,15]. Among viruses with an RNA genome, the replication of HIV was inhibited at the transcriptional level by acridone derivatives [16,17]. A recent study also reported the effectiveness of 7-amino-1,3-dihydroxy-10-methyl-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*) acridinone against bovine viral diarrhoea virus, and the authors postulated that viral RNA synthesis may be the

target of the compound [18]. Additionally, in support of this hypothesis, Watterson *et al.* [19] reported the inhibitory activity of certain acridones on inosine monophosphate dehydrogenase, a key enzyme in the *de novo* synthesis of guanosine nucleotides and a potential target for RNA virus chemotherapy [20].

In the present study, a series of acridone derivatives was synthesized and evaluated against two HFV. Junin virus (JUNV), an arenavirus, is the agent of the annual outbreaks of Argentine haemorrhagic fever, an endemoepidemic disease recognized as a major public health problem in agricultural zones of Argentina [2]. Dengue virus (DENV), a member of *Flaviviridae*, is the agent of the most prevalent arthropod-borne viral disease in humans producing about 50 million cases per year of dengue fever and 250,000–500,000 cases of the life-threatening dengue haemorrhagic fever [21].

Methods

Chemistry

Melting points were measured on a Unimelt apparatus (A.H. Thomas Co., Philadelphia, PA, USA) and were uncorrected. The ^{1}H NMR spectra were recorded with a Bruker AC 200 Instrument, (Bruker Corp., Karlsruhe, Germany) at 200 MHz and the ^{13}C NMR spectra were recorded at 50 MHz for solutions in $DCCl_3$ with tetramethylsilane as the internal standard. 2D NMR spectra were recorded with Bruker AM 500 Instrument at 500 MHz. Mass spectra were performed with a Shimadzu QP-5000 instrument (Shimadzu Corp., Tokyo, Japan) by electron impact ionization. Analysis (thin-layer chromatography) was performed on plates coated with silica gel G (Merck, Darmstadt, Germany) using appropriate eluents each time and warm sulphuric acid for detection.

The acridones (**2a–g**) were obtained from the corresponding 2-anilinobenzoic acids (**1a–g**), using Ullman modifications in the presence of water as solvent according to previous reports [22,23]. The compounds **4a** [24], **3a**, **3c**, **3e**, **3f**, **6a**, **6c**, **6e** and **6f** [25] were synthesized as previously reported. The synthetic route is shown in Figure 1.

The structures of the novel compounds **3b**, **3d**, **3g** and **5c** were deduced on the basis of NMR spectra analysis. The ^{13}C NMR assignations were performed using bidimensional techniques. For compounds **3d** and **3g,** the coupling constants were observed in the ^{13}C NMR because of the fluorine substituent.

For the synthesis of the novel derivatives, a stirred mixture of the 9(10*H*)-acridone derivative (0.01 mol), alkenyl bromide (0.8 ml, 9.24 mmol), cetyltrimethylammonium bromide (0.08 g, 0.22 mmol), aqueous 50% potassium hydroxide (50 ml, 0.62 mol) and butanone (75 ml, 0.84 mol) was refluxed for 2.5 h. The butanone phase was separated, dried with sodium sulphate and evaporated *in vacuo*. The residual product was recrystallized. The following derivatives were synthesized using this procedure.

*10-Allyl-7-chloro-9(10*H*)-acridone (**3b**)*

From acridone **2b** we obtained an amorphous solid with 74.0% yield, mp 131–133°C (ethanol). ^{1}H NMR δ: 8.49 (dd, 1H, H-1), 7.28 (dd, 1H, H-2), 7.71 (dd, 1H, H-3), 7.66 (dd, 1H, H-4), 7.31 (dd, 1H, H-5), 7.57 (dd, 1H, H-6), 8.45 (d, 1H, H-8), 4.92 (m, *J*=3.7; 1.9 Hz, 1H, H-1′), 6.12 (ddd, *J*=3.8; 17.2 Hz; 10.5 Hz, 1H, H-2′), 5.06 (dd, *J*=17.2; 1.6 Hz, 1H, H-3′a), 5.31 (ddd, *J*=10.5; 3.8 Hz; 1.6 Hz, 1H, H-3′b); ^{13}C NMR δ: 126.7 (C-1), 122.2 (C-1a), 121.8 (C-2), 133.8 (C-3), 116.9 (C-4), 141.9 (C-4a), 117.6 (C-5), 140.2 (C-5a), 134.1 (C-6), 127.3 (C-7), 127.6 (C-8), 123.2 (C-8a), 177.0 (C-9), 49.4 (C-1′), 130.2 (C-2′), 115.1 (C-3′). Anal. calcd. for $C_{16}H_{12}ClNO$: C, 71.25; H, 4.48. Found: C, 71.50; H, 4.65.

*10-Allyl-2-fluor-9(10*H*)-acridone (**3d**)*

From acridone **2d** we obtained an amorphous solid with 71.0% yield, mp 141–142°C (ethanol); ^{1}H NMR δ: 8.11 (dd, 1H, H-1), 7.70 (ddd, 1H, H-3), 7.66 (dd, 1H, H-4), 7.37 (dd, 1H, H-5), 7.25 (t, 1H, H-6), 7.25 (d, 1H, H-7), 8.47 (dd, 1H, H-8), 4.93 (m, *J*=3.4; 1.9 Hz, 1H, H-1′), 6.11 (ddd, *J*=3.8Hz; 17.2; 14.2 Hz, 1H, H-2′), 5.05 (d, *J*=17.2 Hz, 1H, H-3′a), 5.33 (d, *J*=10.6 Hz, 1H, H-3′b); ^{13}C NMR δ: 112.0/111.6 (d, J_{CF}=20.0 Hz, C-1), 134.1 (C-1a), 160.0/155.2 (d, J_{CF}=248.0 Hz, C-2), 117.5 (C-3), 122.4 (C-4), 138.7 (C-4a), 115.1 (C-5), 141.9 (C-5a), 134.1(C-6), 121.6 (C-7), 127.5 (C-8), 123.2 (C-8a), 177.3 (C-9), 49.5 (C-1′), 130.4 (C-2′), 117.5 (C-3′). Anal. calcd. for $C_{16}H_{12}FNO$: C, 75.88; H, 4.74. Found: C, 76.21; H, 4.92.

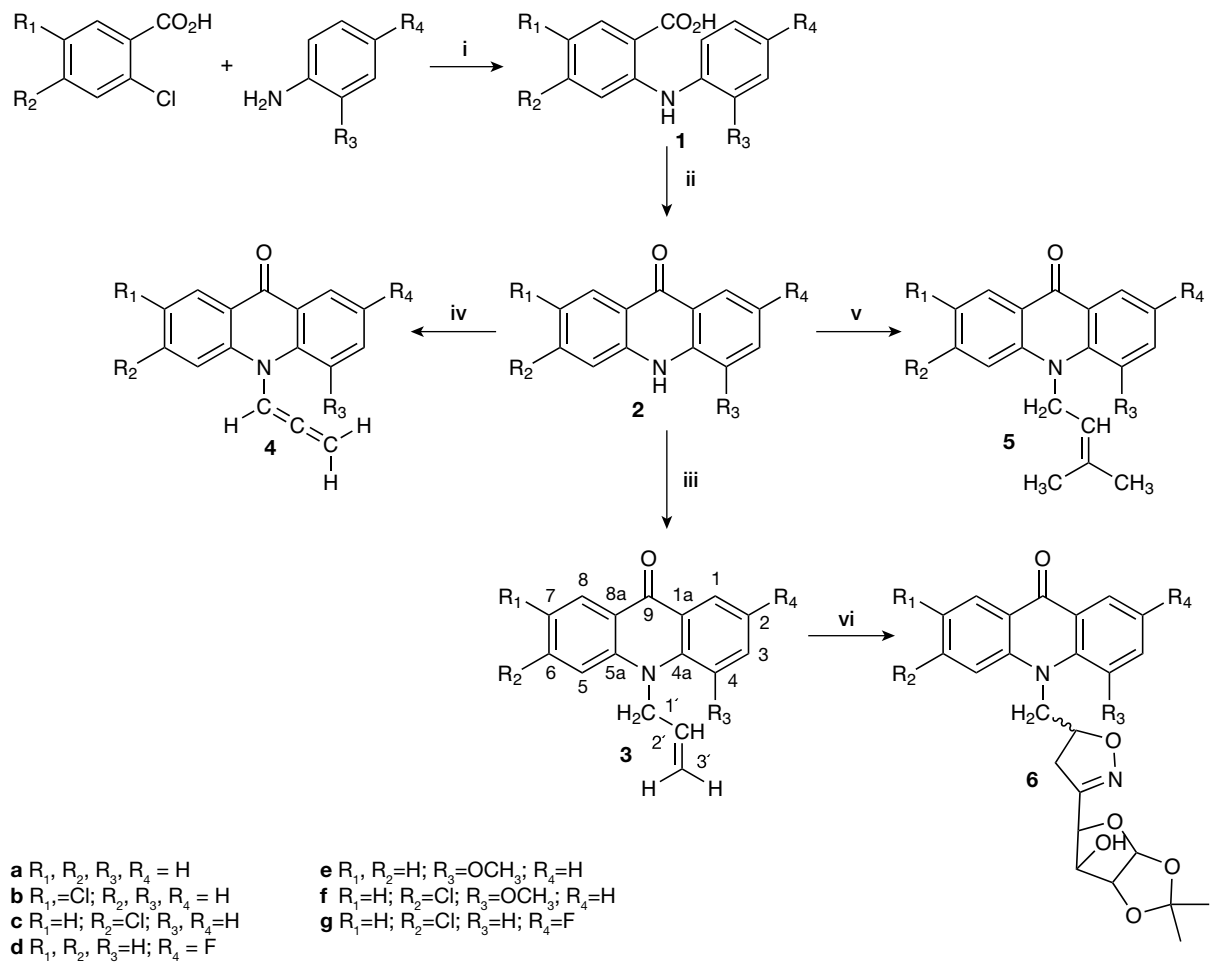
*10-Allyl-6-chloro-2-fluor-9(10*H*)-acridone (**3g**)*

From acridone **2g** we obtained an amorphous solid with 72.0% yield, mp 211–212°C (ethanol). ^{1}H NMR δ: 8.13 (dd, 1H, H-1), 7.48 (dd, 1H, H-3), 7.38 (dd, 1H, H-4), 7.36 (broad signal, 1H, H-5), 7.25 (dd, 1H, H-7), 8.42 (d, 1H, H-8), 4.92 (d, *J*=1.8 Hz, 1H, H-1′), 6.13 (ddd, *J*=3.7; 17.6; 10.6 Hz, 1H, H-2′), 5.09 (d, *J*=17.3 Hz, 1H, H-3′a), 5.36 (d, *J*=10.7 Hz, 1H, H-3′b); ^{13}C NMR δ: 112.3/111.9 (d, J_{CF}=28.0 Hz, C-1), 123.7 (C-1a), 160.4/155.5 (d, J_{CF}=243.0 Hz, C-2), 117.9 (C-3), 122.8 (C-4), 138.7 (C-4a), 114.9 (C-5), 140.7 (C-5a), 142.7 (C-6), 122.3 (C-7), 129.3 (C-8), 120.9 (C-8a), 178.8 (C-9), 49.8 (C-1′), 129.9 (C-2′), 117.5 (C-3′). Anal. calcd. for $C_{16}H_{11}ClFNO$: C, 66.78; H, 3.83. Found: C, 66.5; H, 4.1; N, 5.0.

*6-Chloro-10-(3-methyl-2-butenyl)-9(10*H*)-acridone (**5c**)*

From acridone **2c** we obtained an amorphous solid with 62.0% yield, mp 176-178°C (methanol:water). ^{1}H NMR

Figure 1. Synthetic route of acridones



i. K_2CO_3/Cu/H_2O/ultrasonic 20 min; ii. H_2SO_4/100°C/2 h; iii. allyl bromide/KOH 50%/butanone/2.5 h; iv. propargyl bromide/KOH 50%/ butanone/2.5 h; v. 4-bromo-2-methyl-2-butene/KOH 50%/butanone/2.5 h; vi. 1,2-O-isopropyldene-α-D-xylopentadialdo-1,4-furanose oxime/chloramine-T/60°C/3 h.

δ: 8.50 (dd, 1H, H-1), 7.28 (ddd, 1H, H-2), 7.69 (ddd, 1H, H-3), 7.39 (d, 1H, H-4), 7.37 (d, 1H, H-5), 7.20 (dd, 1H, H-7), 8.45 (d, 1H, H-8), 4.85 (broad d, *J*=5.5 Hz, 1H, H-1′), 5.23 (m, 1H, H-2′), 1.95 (d, *J*<1 Hz, 3 H, H-4′a), 1.81 (d, *J*=1.3 Hz, 3H, H-4′b); ^{13}C NMR δ: 127.7 (C-1), 122.6 (C-1a), 121.7 (C-2), 134.0 (C-3), 114.9 (C-4), 142.0 (C-4a), 114.7 (C-5), 140.1 (C-5a), 142.7 (C-6), 121.8 (C-7), 129.4 (C-8), 120.8 (C-8a), 177.3 (C-9), 45.6 (C-1′), 118.5 (C-2′), 137.1 (C-3′), 25.6 (C-4′a), 18.4 (C-4′b). Anal. calcd. for $C_{18}H_{16}ClNO$: C, 72.60; H, 5.42. Found: C, 72.38; H, 5.74.

Virology

Vero (African green monkey kidney) cells were grown in Eagle's minimum essential medium (MEM; GIBCO, New York, USA) supplemented with 5% inactivated calf serum and 50 µg/ml gentamycin. The C6/36 HT mosquito cell line from *Aedes albopictus* was cultured at 33°C in L-15 Leibovitz medium (GIBCO, USA) supplemented with 0.3% tryptose phosphate broth, 0.02% glutamine, 1% MEM non-essential amino acids solution and 5% fetal calf serum. For the maintenance medium (MM), the serum concentration was reduced to 1.5%. The following virus strains were used: JUNV strain IV4454, JUNV strain XJCl3, Tacaribe virus (TCRV) strain TRLV 11573, lymphocytic choriomeningitis virus (LCMV) strain Armstrong, DENV-1 strain Hawaii, DENV-2 strain NGC, DENV-3 strain H87 and DENV-4 strain 8124. Stocks of the arenaviruses JUNV, TCRV and LCMV were propagated and titrated by plaque formation in Vero cells, whereas stocks of DENV were prepared in C6/36 HT cells and titrated by plaque assay in Vero cells.

Antiviral activity was determined by a virus yield inhibition assay. Vero cells grown in 24-well plates were infected at a multiplicity of infection (MOI) of

0.1 plaque-forming units/cell. After 1 h adsorption at 37°C, cells were washed and incubated with MM with or without serial two-fold dilutions of each compound. Ribavirin (Sigma-Aldrich, St. Louis, USA) was used as a reference anti-arenavirus substance. After 48 h of incubation at 37°C, supernatant cultures were harvested and extracellular arenavirus and DENV yields were determined by a plaque assay [26,27]. The 50% effective concentration (EC_{50}) was calculated as the concentration required to reduce virus yield by 50% in the compound-treated cultures compared with untreated ones. Cytotoxicity was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma-Aldrich, USA) method in Vero cells using conditions equivalent to those used in previously described antiviral assays [27]. The 50% cytotoxic concentration (CC_{50}) was calculated as the compound concentration required to reduce the MTT signal by 50% compared with untreated controls. All determinations were performed twice and each in duplicate.

Results

This series of *N*-substituted acridones was initially evaluated for cytotoxicity and antiviral activity against JUNV (strain IV4454) in Vero cells. As shown in Table 1, the group of 10-allyl-9(10*H*)-acridones (**3a–3g**) included the more effective and selective anti-JUNV compounds. Five of the seven derivatives did not exert any cytotoxic effect on Vero cells up to the maximum tested concentration of 1,000 μM. Compounds **3f** and **3c** exhibited the highest inhibitory effect against JUNV with EC_{50} values of 4.7 and 5.5 μM, respectively, and, because both acridones lacked toxicity for uninfected cells, the SI (ratio CC_{50}/EC_{50}) were >212.8 and >181.8, respectively. Furthermore, both **3f** and **3c** possessed greater activity and selectivity than ribavirin (the only compound in clinical use for arenavirus treatment), which was evaluated in parallel as a reference substance. Compound **3e** also elicited an interesting level of activity against JUNV, having an EC_{50} of 7.3 μM; however, due to its highest toxicity, the SI of **3e** was only 14.9. The other 10-allyl-9(10*H*)-acridones were inactive or showed very weak inhibitory effect against JUNV. The acridones with other *N*-substitutions (compounds **4**, **5** and **6**) exhibited a great level of toxicity for Vero cells coupled with a weak or lack of anti-JUNV activity.

Experiments were then conducted to examine the same series of acridone derivatives for activity against DENV-2 (strain NGC) in Vero cells. The profile of susceptibility for this virus was comparable to JUNV response (Table 1). Maximum potency and selectivity were found among 10-allyl-9(10*H*)-acridones, and, again, **3c** and **3f** were the most effective derivatives with potent antiviral activity at concentrations well below the CC_{50}. In fact, the SI of **3c** and **3f** against DENV-2 were >400 and >322.6, respectively, the highest values observed for this series of acridones. The remaining *N*-substituted acridones, including **4**, **5** and **6** derivatives, lacked selective anti-DENV-2 activity, which is similar as the results observed for the JUNV experiment. The weak anti-DENV activity exhibited by ribavirin (Table 1) is in accordance with data reported by other investigators [28,29].

Given the very selective inhibitory action exhibited by acridones **3c** and **3f** against both JUNV and DENV-2, these compounds were chosen to further evaluate their

Table 1. Cytotoxicity and antiviral activity of acridone derivatives against JUNV and DENV-2

		JUNV		DENV-2	
Compound	CC_{50}, μM*	EC_{50}, μM†	SI¶	EC_{50}, μM†	SI¶
3a	112.4 ±2.2	34.2 ±1.9	3.3	25.0 ±4.5	4.5
3b	>1,000	>100	Inactive	13.5 ±3.5	>74.1
3c	>1,000	5.5 ±0.5	>181.8	2.5 ±0.3	>400
3d	>1,000	44.2 ±0.9	>22.7	>50	Inactive
3e	109.0 ±5.3	7.3 ±0.2	14.9	7.4 ±0.2	14.7
3f	>1,000	4.7 ±0.2	>212.8	3.1 ±0.1	>322.6
3g	>1,000	>100	Inactive	81.2 ±4.0	>12.3
4a	87.6 ±0.8	33.4 ±2.9	2.6	>50	Inactive
5c	541.6 ±7.0	>100	Inactive	>100	Inactive
6a	165.5 ±2.7	43.1 ±3.5	3.8	>50	Inactive
6c	70.2 ±4.8	>50	Inactive	17.7 ±1.7	3.9
6e	276.2 ±6.8	53.3 ±0.6	5.2	>100	Inactive
6f	74.0 ±0.9	>50	Inactive	>50	Inactive
Ribavirin	>400	18.5 ±1.7	>21.6	62.0 ±1.9	>6.5

*Fifty percent cytotoxic concentration (CC_{50}): compound concentration required to reduce cell viability by 50%, determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. Values are the mean from duplicate independent tests ±SD. †Fifty percent effective concentration (EC_{50}): compound concentration required to reduce virus yields by 50%. Values are the mean from duplicate independent tests ±SD. ¶Selectivity index (SI): CC_{50}/EC_{50}. DENV, dengue virus; JUNV, junin virus.

Table 2. Spectrum of antiviral activity of compounds **3c** and **3f**

Virus	3c		3f	
	EC_{50}, μM*	SI†	EC_{50}, μM*	SI†
Arenavirus				
JUNV strain IV4454	5.5 ±0.5	>181.8	4.7 ±0.5	>212.8
JUNV strain XJCl3	20.5 ±0.8	>48.8	4.5 ±0.2	>223.2
TCRV	27.2 ±0.9	>36.8	13.4 ±0.9	>74.6
LCMV	29.4 ±1.7	>34.0	7.3 ±1.2	>136.3
Flavivirus				
DENV-2	2.5 ±0.3	>400	3.1 ±0.1	>322.6
DENV-1	8.3 ±0.9	>120.5	15.9 ±0.3	>62.9
DENV-3	10.0 ±0.4	>100	5.8 ±0.1	>170.9
DENV-4	98.2 ±2.1	>10.2	19.9 ±0.3	>50.3

*Fifty percent effective concentration (EC_{50}): compound concentration required to reduce virus yields by 50%. Values are the mean from duplicate independent tests ±SD. †Selectivity index (SI): 50% cytotoxic concentration/EC_{50}. The 50% cytotoxic concentration for both **3c** and **3f** is >1,000 μM (Table 1). DENV, dengue virus; JUNV, junin virus; LCMV, lymphocytic choriomeningitis virus; TCRV, Tacaribe virus.

spectrum of antiviral activity against arenaviruses and flaviviruses. Both compounds were assayed against another strain of JUNV (the attenuated XJCl3 strain, which was obtained by serial passage from the prototype XJ strain), TCRV (another New World arenavirus) and against the Old World arenavirus and prototype of the family LCMV. As seen in Table 2, the profile of susceptibility of the three viruses to these compounds was similar to that observed for the IV4454 strain of JUNV. The EC_{50} of **3c** against each virus strain varied between 5.5 and 29.4 μM, whereas the EC_{50} of **3f** was lower than 10 μM, except for **3f** against TCRV with an EC_{50} of 13.4 μM. With respect to the flaviviruses, both **3c** and **3f** were evaluated against the other three serotypes of DENV and were found to be antivirally active against all DENV serotypes, with significant selectivity (Table 2).

As a first approach to characterize the action of these compounds on HFV, the possibility that they acted directly either on the virus particles or on the cells to be infected was investigated. When cell monolayers were pre-incubated with compound for 2 h at 37°C and removed before JUNV infection, both acridones were not effective in reducing virus yield at 48 h post infection (Figure 2). Similarly, following incubation of virus with compound for 2 h at 37°C before cell infection reduction in remaining JUNV, infectivity was only around 30% at the highest concentration tested (100 μM). By contrast, when the compounds were added to cells simultaneously with virus inoculum (as in the screening assay shown in Table 1), virus titre in cell supernatants at 48 h post infection was significantly reduced at concentrations 15–20-fold lower and in a dose-dependent manner (Figure 2). Similar results were obtained for both compounds **3c** and **3f** and DENV-2 (data not shown). Thus, the inhibitory effect of acridones against HFV was entirely exerted through a blockade in virus multiplication during the infectious process.

Discussion

The major finding of this study is the first demonstration that acridone derivatives have a potent antiviral activity that block *in vitro* multiplication of HFV belonging to *Arenaviridae* and *Flaviviridae*, such as JUNV and DENV. Both viruses were included in the category A priority viral pathogen list of the Centers for Disease Control and Prevention [30]. Category A pathogens are those agents that would produce the greatest impact if used in a bioterror attack because of their potential for widespread dissemination, high virulence, capacity for inducing fear and anxiety in the population, and lack of an effective chemotherapy.

Here, the infection of Vero cells with these viruses was selectively inhibited by synthesized *N*-allyl acridones without effects on cell viability. Additionally, the most active compounds **3c** and **3f** exhibited a wide spectrum of antiviral activity against New World and Old World arenaviruses as well as the four serotypes of DENV. This last property is of remarkable significance because the four serotypes cocirculate in epidemic regions of America and Asia, and it is well known that the reinfection with a different serotype represents an important risk factor for the development of severe forms of dengue haemorrhagic fever [21]. For this reason, the ability to protect against all DENV serotypes, as reported here for acridones, should be mandatory for any antiviral substance to be considered as a promising dengue chemotherapeutic agent.

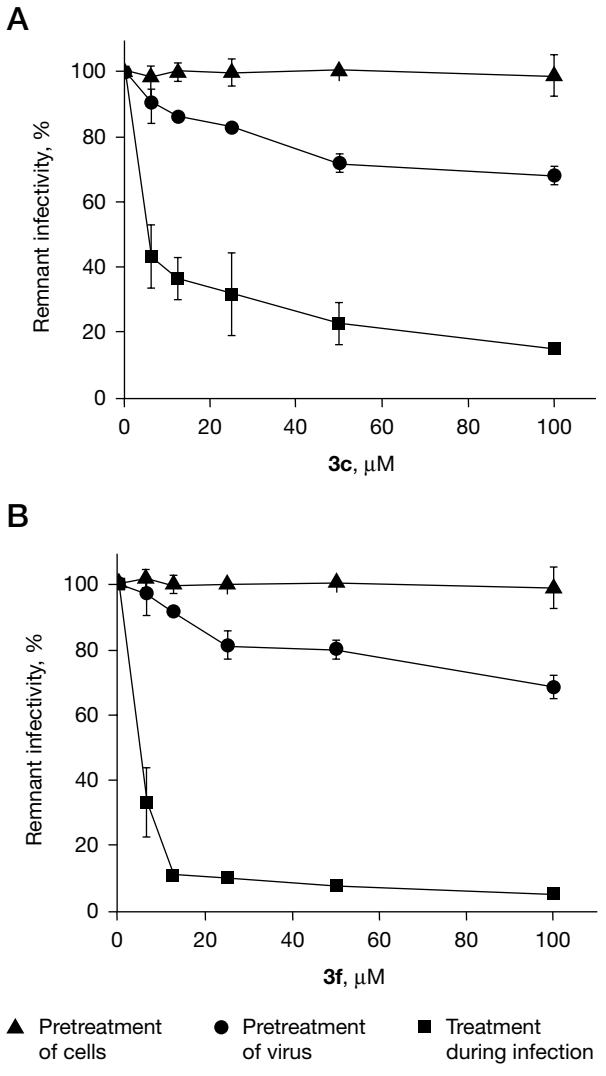
Although the structural diversity of tested compounds prevents a more substantive comparison of structure-activity at this time, the analysis of this group of acridones highlights some initial trends on functional substituents with their impact on the antiviral activity. The *N*-substitution with carbohydrate groups by cycloaddition generated very cytotoxic derivatives, whereas *N*-allyl

derivatives were by far the more selective group of virus inhibitors. The presence of halogen substituents in *N*-allyl acridones, in general, reduced toxicity and increased antiviral effect. In particular, the 6-chloro substitution seemed to be an important element, whereas the halogen substitution in other positions of ring A or B lead to a minor improvement of antiviral activity. In fact, the selectivity indices of the very active **3c** and **3f** compounds was significantly higher than values reported for other classes of acridone derivatives in the literature.

The identification of the target in the virus multiplication cycle for the inhibition of HFV by *N*-substituted acridones will allow further investigations with regards to the structure-activity relationships in this system. Acridone-based substances have been recently reported as potent inosine monophosphate dehydrogenase inhibitors [19], an enzyme that is the main target of ribavirin, mycophenolic acid, tiazofurin, selenazofurin and other compounds studied as potential agents against highly pathogenic RNA viruses [20]. The acridones **3c** and **3f** exerted their antiviral effect during the intracellular multiplication cycle of arenaviruses and DENV, but it remains to be determined if blockade is located at viral RNA synthesis.

According to results presented here and the considerations mentioned above, it will be interesting to analyse the influence of diverse halogen substitutions in the series of *N*-propanedienyl and *N*-butenyl acridones, as well as to elucidate the precise target of the potent 6-chloro-*N*-allyl derivatives in JUNV and DENV infection.

Figure 2. Treatment of cells or virus with **3c** or **3f** prior to and during infection



Pretreatment of cells or virus with **3c** (**A**) or **3f** (**B**). Pretreatment of cells: Vero cells were pre-incubated with maintenance medium containing different compound concentrations for 2 h at 37°C; then, supernatants were removed, cells were washed with phosphate-buffered solution and infected with Junin virus (JUNV) at a multiplicity of infection of 0.1 in absence of compound. Virus yields were determined at 48 h post infection. Pretreatment of virus: JUNV suspensions containing 6×10^5 plaque-forming units were incubated with an equal volume of maintenance medium with or without different compound concentrations for 1 h at 37°C. Then, mixtures were diluted and remaining infectivity was determined by plaque assay. Treatment during virus infection: compound was added to Vero cells simultaneously with JUNV at a multiplicity of infection of 0.1 and maintained during 1 h at 37°C. Virus yields were determined at 48 h post-infection. For the three treatments, results are expressed as percent of inhibition in compound-treated cultures compared with untreated ones. Each value is the mean of duplicate assays ±SD.

Disclosure statement

The authors declare no competing interests.

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