

UNIVERSIDADE FEDERAL DE UBERLÂNDIA

INSTITUTO DE CIÊNCIAS BIOMÉDICAS

GRADUAÇÃO EM BIOMEDICINA

ANA LAURA COSTA OLIVEIRA

**COMPOSTOS COORDENADOS A OURO(I) COMO NOVOS COMPLEXOS
METÁLICOS CONTRA A REPLICAÇÃO DO VÍRUS MAYARO**

**“GOLD(I)-COORDINATED COMPOUNDS AS NEW METAL COMPLEXES
AGAINST MAYARO VIRUS REPLICATION”**

UBERLÂNDIA

2024

ANA LAURA COSTA OLIVEIRA

**COMPOSTOS COORDENADOS A OURO(I) COMO NOVOS COMPLEXOS
METÁLICOS CONTRA A REPLICAÇÃO DO VÍRUS MAYARO**

Trabalho de Conclusão de
Curso apresentado como requisito
para obtenção do título de Bacharel em
Biomedicina na Universidade Federal
de Uberlândia.

Orientadora: Prof^ª Dr^ª Ana Carolina
Gomes Jardim
Coorientador: Dr. Igor de Andrade
Santos

UBERLÂNDIA

2024

**COMPOSTOS COORDENADOS A OURO(I) COMO NOVOS COMPLEXOS
METÁLICOS CONTRA A REPLICAÇÃO DO VÍRUS MAYARO**

Trabalho de Conclusão de
Curso apresentado como requisito
para obtenção do título de Bacharel
em Biomedicina na Universidade
Federal de Uberlândia.

Banca examinadora formada por:

Profa. Dra. Ana Carolina Gomes Jardim

Dr. Guilherme Rodrigues Fernandes Campos

Me. Uriel Enrique Aquino Ruiz

GOLD(I) COORDINATED COMPOUNDS AS NEW METAL COMPLEXES AGAINST MAYARO VIRUS REPLICATION

Ana Laura Costa Oliveira¹, Igor Andrade Santos¹, Igor Santos Oliveira², Laiane dos Santos Oliveira², Pedro Henrique de Souza Guarda², Camilla Abbehausen², Ana Carolina Gomes Jardim^{1,3}.

¹ Institute of Biomedical Science, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.

² Institute of Chemistry, University of Campinas, Brazil

³ Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University, São José do Rio Preto, SP, Brazil.

***Corresponding author:** Professor Ana Carolina Gomes Jardim, Institute of Biomedical Science, ICBIM/UFU, Avenida Amazonas, 4C- Room 216, Umuarama, Uberlândia, Minas Gerais, Brazil, CEP: 38405-302.

Tel: +55 (34) 32258682

E-mail: jardim@ufu.br

Keywords: Antiviral, metallic compounds, gold compounds, Mayaro virus, Mayaro fever.

ABSTRACT

Mayaro virus (MAYV) is an arbovirus responsible for Mayaro fever, a neglected emerging disease that causes hyperthermia, muscle pain, and skin rashes. Aggravatingly, the infection can develop into a chronic arthralgia and polyarthralgia. Currently, there are no vaccines or antiviral drugs for the treatment of MAYV. In this context, Gold (Au) complexes, specially Au(I)-based ones, have previously shown antiviral activity against several viruses. Here we evaluated the potential of the novel Au(I) coordinated to 1,3-bis(2,4,6-trimethylphenyl)-imidazol-2-ylidene (IMes) complexes Au(IMes)Cl, Au(IMes)Sbtz, Au(IMes)Stzn, Au(IMes)Spym and Au(IMes)2tu as antiviral candidates against MAYV *in vitro*. To this, Vero E6 cells were infected with MAYV-*nanoluc*, an infectious clone carrying the *nanoluciferase* reporter gene, in the presence or absence of compounds for 24 h, when virus titres were evaluated. The screened compounds presented no cytotoxic and demonstrated strong anti-MAYV activity, reducing over 76% of MAYV infection. Dose-response assay were performed, and Au(IMes)Spym and Au(IMes)2tu showed the highest selective indexes (9 and 8.9, respectively) among the tested complexes, being selected for further analysis. Antiviral effects of Au(IMes)Spym and Au(IMes)2tu on different stages of the MAYV replicative cycle were investigated, demonstrating that the compounds were able to inhibited all stages of MAYV infection, suggesting a broad spectrum potential. Our findings show that these Au(I) coordinated compounds are an interesting source of molecules for the development of future antivirals candidates against Mayaro fever.

INTRODUCTION

The *Mayaro virus* (MAYV) is a zoonotic arbovirus responsible for Mayaro fever, a neglected emerging disease in regions of Central America and South America (Andreolla *et al.*, 2022). MAYV was first isolated from the blood of forest workers in Tobago and Trinidad islands in 1954 (Anderson *et al.*, 1957), and, since then, the virus has been causing sporadic outbreaks in countries of Latin America (Lorenz; Ribeiro; Neto, 2019; Caicedo *et al.*, 2021).

The MAYV particle consists in a positive single-stranded RNA genome, associated with a nucleocapsid, and involved by envelope membrane (Abbo *et al.*, 2023; Kim *et al.*, 2022). MAYV genome, of approximately 11.5 kb in size, contains two open reading frames (ORFs). The first ORF is translated in the non-structural viral proteins (nsP) nsP1, nsP2, nsP3, and nsP4; and the second ORF is translated into the structural proteins capsid (C), envelope 1 (E1), 2 (E2), and 3 (E3), 6K, and Transframe (TF), obtained after cleavage of non-structural and structural precursor polyproteins (Kim *et al.*, 2022; Pujhari *et al.*, 2022).

MAYV belongs to the *Togaviridae* family and the *Alphavirus* genus. It is classified into the Semliki complex subgroup with seven other viruses: *Chikungunya virus*, *Bebaru virus*, *Ross River virus*, *Getah virus*, *Onyong-nyong virus*, *Semliki Forest*, and *Una virus*, with serological similarity (Paschoalino *et al.*, 2023; Weber *et al.*, 2023). Therefore, cross-reactivity can be observed in traditional serological tests, making challenging the accuracy of MAYV infections diagnosis and the reporting of disease incidence (Cereghino *et al.*, 2023; Weber *et al.*, 2023).

MAYV is mainly transmitted through the bite of female mosquitoes of genus *Hemagogus spp.*, however, the virus has also been detected in *Aedes spp.*, *Culex spp.*, *Mansonia spp.*, *Psorophora spp.* and *Sabethes spp.* mosquitoes (Hernández-Sarmiento *et al.*, 2024; Krokovsky *et al.*, 2023). After transmission, MAYV infected patients can develop the Mayaro fever disease, with symptoms which include fever between 39 and 40.2 °C, chill, myalgia, retro-orbital pain, arthralgia, skin rashes, vomiting, and diarrhea (Rosa *et al.*, 2023; Valdés-Torres *et al.*, 2022). In some cases, serious complications such as myocarditis, haemorrhagic, and neurological manifestations may occur (Abbo *et al.*, 2023; Mutricy *et al.*, 2022). Similar to *Chikungunya virus* infection, MAYV causes arthralgia in 50 to 90% of infected patients during the acute period of infection. After this phase, about 54% of infected humans remain with chronic arthralgia symptoms for months or years, affecting the main joints and causing disabling conditions (Anestino *et al.*, 2024; Hernández-Sarmiento *et al.*, 2024; Krokovsky *et al.*, 2023).

Currently, there are no licensed antiviral treatment or vaccines against MAYV (Andreolla *et al.*, 2022; Paschoalino *et al.*, 2023). Therefore, the treatment for Mayaro fever consists of administering antipyretics, analgesics, and non-steroidal anti-inflammatory drugs to reduce symptoms (Andreolla *et al.*, 2022; Sun, J.; Wu, D., 2019). This fact associated with: i) underreported cases of MAYV infection; ii) antibody cross-reactivity and the similarity of clinical manifestations to other arboviruses such as CHIKV, *Orthoflavivirus denguei* (DENV) and *Orthoflavivirus zikaense* (ZIKV) (Cereghino *et al.*, 2023; Mutricy *et al.*, 2022; Saatkamp *et al.*, 2021; Weber *et al.*, 2023); iii) high potential for dissemination, due to susceptible vectors (Caicedo *et al.*, 2021; De Curcio *et al.*, 2022; Krovovsky *et al.*, 2023); and iv) the increasing cases of MAYV infections in non-endemic areas in the Americas (Celone *et al.*, 2024; Diagne *et al.*, 2020; Lorenz; Chiaravalloti-Neto, 2024; Sessions *et al.*, 2023), warrants for the need for the identification of new molecules capable of inhibiting MAYV, that can be applied into the development of future antiviral drugs.

In this context, Inorganic Medicinal Chemistry encompassing metal coordinated compounds arises as vast and innovative approach for drug antiviral development, especially those coordinated with Gold (Au). The Au coordinated compounds have been explored for their medical applications since 1890 (Benedek, 2004; Delgado *et al.*, 2020; Sadler; Sue, 1994). Their basic structure can be developed from Au(I), Au(II) or Au(III), associated to organic or inorganic ligands, the most common forms being Au(I) and Au(III) (Jürgens; Casini, 2017; Sadler; Sue, 1994). It is worth noting that Au(III) compounds can suffer the reduction process in living organisms resulting in Au(I) species (Berners-Price; Filipovska, 2011; Delgado *et al.*, 2020).

The first gold-based drug approved to be used in humans was Auranofin, employed to the treatment of rheumatoid arthritis patients since 1985 (Bombardier *et al.*, 1986; Kean; L. Patrick Hart; W. Watson Buchanan, 1997; Yamashita, 2021). Interestingly, Auranofin was further exploited towards its biological activity, demonstrating that it possesses antiparasitic activity against *S. mansoni* and *P. falciparum* (Kuntz, *et al.*, 2007; Sannella, *et al.*, 2008), anticancer properties (Babaei *et al.*, 2021; Khedr *et al.*, 2023), as well as antiviral activities against the *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2) (Laplantine *et al.*, 2022), *Human immunodeficiency virus 1* (HIV-1) (Lewis *et al.*, 2011), and CHIKV (Langsjoen *et al.*, 2017). Additionally, gold ions have been also employed as nanoparticles named as AuNPs (Li *et al.*, 2019), which have demonstrated to increase biological effect, as well as making the molecules active against viruses such as *Alfainfluenzavirus influenzae*

(H1N1) (Babaei *et al.*, 2021), *Simplexvirus humanalpha 1* (HSV) (Halder *et al.*, 2018), SARS-CoV-2 (Karges, 2021), and *Foot-and-Mouth Disease Virus* (FMDV) (Rafiei *et al.*, 2016), and HIV-1 (Di Gianvincenzo *et al.*, 2010).

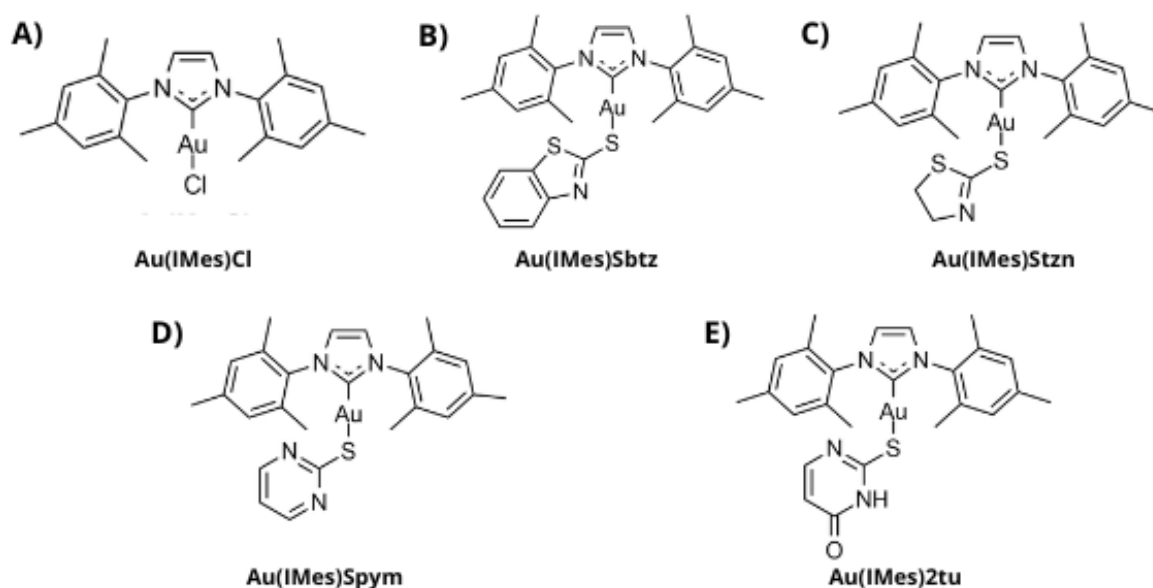
Considering the therapeutic potential encompassing Au coordinated compounds, herein, the metal complexes Au(IMes)Sbtz, Au(IMes)Stzn, Au(IMes)Spym, Au(IMes)2tu, and Au(IMes)Cl were screened towards their antiviral activity against MAYV. This series of organometallic compounds is based on Au(I) and has the ligand 1,3-bis(2,4,6-trimethylphenyl)-imidazol-2-ylidene (IMes), classified as an N-heterocyclic carbene (NHC). Au(IMes)Cl has the chloride ion as its second ligand. Additionally, these complexes ligands that contain molecules with donor sulphur atoms, which are 2-mercaptobenzthiazole (HSbtz), 2-mercaptothiazoline (HStzn), 2mercaptopyrimidine, (HSpym) and 2-thiouracil (2tuH) (Fereidoonenezhad *et al.*, 2019; Schuh *et al.*, 2012; Xiao, Q. *et al.*, 2022). Au(IMes)Spym and Au(IMes)2tu were further characterized into their mode of action and demonstrates the potential of Au(I) metal complexes as an innovative approach in antiviral drug development against MAYV.

METHODS

Compounds

The K[AuCl₄] and tetrahy- drothiophene (tht) were supplied by Sigma-Aldrich (Brazil) and used as received. Solvents, salts, and common lab reagents were used as received. The synthesis of [AuCl(tht)], 1,3-Bis (mesityl) imidazolium chloride (IMes · HCl), and Au(IMes)Cl was performed as previously described (Aires *et al.*, 2022). The complexes Au(IMes)Sbtz, Au(IMes)Stzn, Au(IMes)Spym, and Au(IMes)2tu were synthesized and characterized by Prof. Camilla Abbehausen from the State University of Campinas (UNICAMP), using Au(IMes)Cl as scaffold, in which the metal ion Au(I) is linked to a carbon atom and a sulphur atom with different substituents (**Figure 1**). The complexes were dissolved in dimethyl sulfoxide (DMSO, v/v), a coordinating solvent, and stored at –20°C. For all the assays performed, untreated control was performed by treating cells with media containing DMSO 0.1%.

Figure 1. Chemical Structures of Au(IMes)Cl, Au(IMes)Sbtz, Au(IMes)Stzn, Au(IMes)Spym, and Au(IMes)2tu.



Cell culture

Vero-E6 cells (kidney tissue derived from a normal adult African green monkey, ATCC E6) and BHK-21 cells (fibroblasts derived from Syrian golden hamster kidney; ATCC CCL-10) were cultivated in Dulbecco's modified Eagle's medium (DMEM, Sigma-aldrich), supplemented with 100 U/mL of penicillin (Hyclone laboratories, USA), 100 mg/mL of streptomycin (Hyclone laboratories, USA), 1% dilution of stock of non-essential amino acids (Hyclone laboratories, USA), 5% of fetal bovine serum (FBS, Hyclone laboratories, USA), in a humidified incubator at 37°C and 5% CO₂.

Rescue of MAYV-*nanoluc* reporter virus

The infectious MAYV expressing *nanoluciferase* reporter (MAYV-*nanoluc*) (Marinho *et.al.*, *under review*), was designed based on the MAYV isolate BeAr 20290 (Li, 2019). To virus production, BHK-21 cells were seeded at 8x10⁵ cells/well in a 6-well plate, and after 24 hours the cells were transfected with 5µg of CMV-MAYV-*nanoluc* plasmid, using lipofectamine 3000® and Opti-Mem medium to rescue MAYV-*nanoluc* reporter virus. 48 h post transfection, the supernatant containing MAYV-*nanoluc* particles was collected, centrifuged, and stored at -80°C.

The MAYV-*nanoluc* viral titre was determined by plaque assay, in which Vero E6 cells were seeded in 24 wells plates at 8x10⁴ cells/well 24 h prior to the infection. The cells were

infected with a ten-folds serial dilution of MAYV-*nanoluc* and incubated at 37°C. After 1 h, fresh medium supplemented with 1% Penicillin-Streptomycin (Cultilab, Campinas, SP, Brazil), 1% FBS (Gibco – Thermo Fisher Scientific, Waltham, MA, USA) and 1% carboxymethylcellulose (CMC, Sigma- Aldrich, St. Louis, MO, USA) was added to the cells and incubated for 48 h. Afterwards, cells were fixed with 10% formaldehyde (Merck, Darmstadt, Germany) and stained with 1% violet crystal (Merck, Darmstadt, Germany). The number of viral foci was counted to determine the viral titre, expressed as plaque-forming units per milliliter (PFU/mL).

Cell viability through MTT assay

To identify Vero-E6 cells viability in the presence or absence of compounds, cells were seeded in 96-well plates at 5×10^3 cells/well and treated with compounds at 50, 10, and 2 μ M for 72 h at 37°C with 5% of CO₂. After that, media-containing compound was removed and a MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma–Aldrich) at 1 mg/mL solution was added to each well, incubated for 30 minutes and replaced with 300 μ l of DMSO to solubilize the formazan crystals. The absorbance was measured at 560 nm on Glomax microplate reader (Promega). DMSO 0.1% was used as untreated control. Cell viability was calculated according to the equation $(T/C) \times 100\%$, in which T and C represent the optical density of the treated well and control groups, respectively. The results were calculated using GraphPad Prism 8.0.

Antiviral assay

Briefly, Vero-E6 cells were seeded at a density of 5×10^4 cells/well into 48 well plates. After 24h, cells were infected with MAYV- *nanoluc* at a multiplicity of infection (M.O.I) of 0.1 in the presence of Au(IMes)Cl at 10 μ M, and Au(IMes)Sbtz, Au(IMes)Stzn, Au(IMes)Spym, and Au(IMes)2tu at 2 μ M (the highest non-cytotoxic concentration determined by cell viability assay). After 24 h, samples were harvested using *Renilla-luciferase* lysis buffer (Promega) and virus replication levels were quantified by measuring *Renilla-luciferase* activity using the *Renilla luciferase* Assay System (Promega). The cell viability was performed in parallel in the same conditions. DMSO was used as untreated control. The antiviral activity was calculated according to the equation $(T/C) \times 100\%$, where T and C represent the mean of the treated well and control group, respectively. The results were calculated using GraphPad Prism 8.0.

Dose-response assay

Au(IMes)Spym and Au(IMes)2tu were submitted to a dose response assay to determine their effective concentration of 50% (EC_{50}) and cytotoxic concentration of 50% (CC_{50}). To this, Vero E6 cells (5×10^4 cells/well) were added of each compound in a two-folds serial dilution ranging from 0.39 μ M to 50 μ M in the presence or absence of MAYV-*nanoluc* at an MOI of 0.1. After 24h, viral replication and cell viability assays were performed as described above. The EC_{50} and CC_{50} were calculated using GraphPad Prism 8.0, and used to calculate the selectivity index ($SI = CC_{50}/EC_{50}$).

Time of drug-addition assays

To evaluate the effect of the compounds on different stages of MAYV replication, time of drug-addition assays were performed. In all assays, Vero-E6 cells were seeded in a concentration of 5×10^4 cells/well, treated with Au(IMes)Spym at 3 μ M and Au(IMes)2tu at 6 μ M, infected with MAYV-*nanoluc*, and replication levels were quantified 24 h post infection (h.p.i.) employing *Renilla Luciferase* assay described above. These selected concentrations were obtained from the dose response assay, where the highest noncytotoxic concentration that inhibits viral replication were used.

To identify if the compounds can protect the cells against MAYV-*nanoluc*, cells were pre-treated with metal compounds for 1h at 37 °C, followed by supernatant removal, PBS washing (three times), and added of medium containing the virus (MOI of 0.1).

To evaluate the effect of each molecule on MAYV-*nanoluc* entry steps, cells were treated with each complex and simultaneously infected with the virus (MOI of 1) for 1 h at 37 °C. Then, the supernatant was removed, washed three times with PBS, and DMEM 2% FBS was added.

The virucidal effect was investigated by incubating the compounds with the virus (MOI of 2) for 1h. This solution was then transferred to Vero E6 cells. After 1 h of incubation, cells were washed with PBS and DMEM 2% FBS was added.

On the other hand, to evaluate the activity of gold compounds on the post-entry stages of MAYV infection, cells were infected with the virus (MOI of 0.1) for 1 hour. Afterwards, supernatant was removed, washed three times with PBS, and incubated with the compounds up to 24 h.p.i..

Statistical analysis

Individual experiments were performed in triplicate and all assays were carried out a minimum of three times to confirm reproducibility. Statistical differences were considered significant when $p < 0.05$. GraphPad Prism 8.0 software was used to assess statistical differences of means of readings. The EC_{50} and CC_{50} were calculated using a non-linear regression considering $\log(\text{inhibitor})$ vs. response, with variable slope (four parameters).

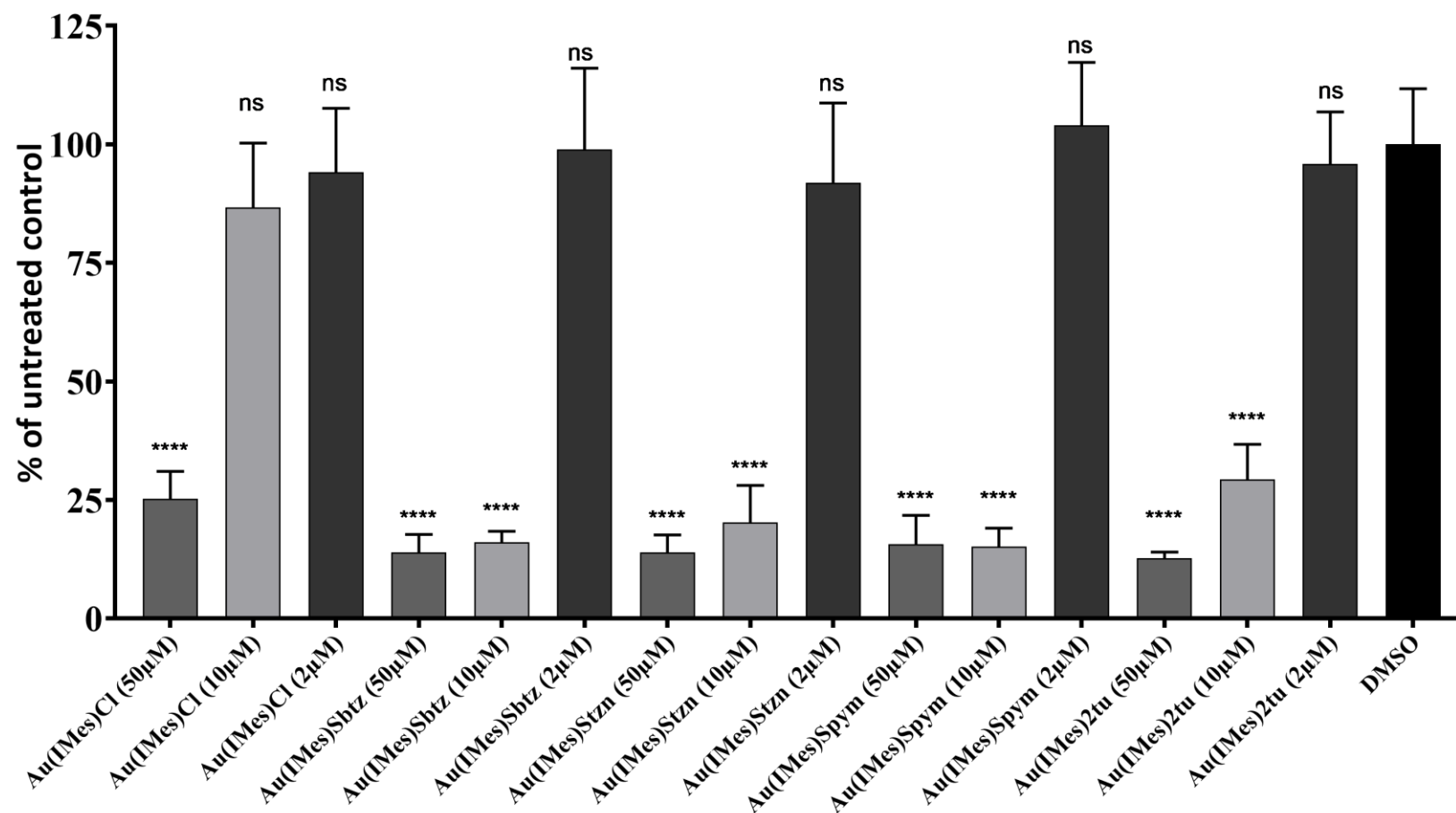
RESULTS

Au(I) complexes inhibit MAYV infectivity *in vitro*

In order to investigate the antiviral potential of Au(I) complexes, an initial cytotoxicity screen was first performed by treating Vero E6 cells with all metal complexes at the concentrations of 50, 10, and 2 μM for 72 h, when cell viability was measured by MTT assay. As a result, Au(IMes)Cl was viable at 10 μM (87%), while Au(IMes)Sbtz, Au(IMes)Stzn, Au(IMes)Spym, and Au(IMes)2tu were viable at 2 μM (99%, 92%, 104%, and 96%, respectively) (**Figure 2**).

.

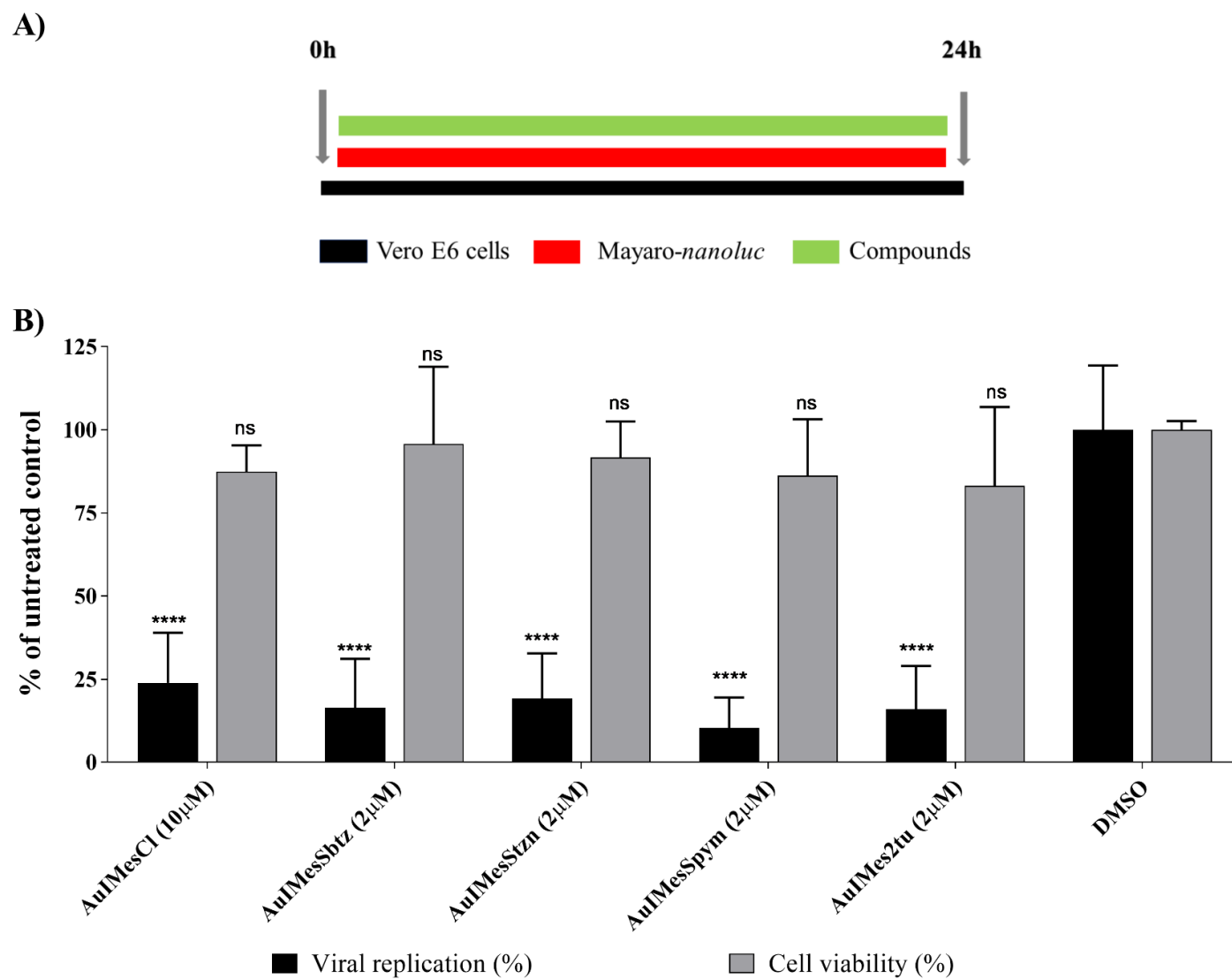
Figure 2. Cell viability of Au(IMes)Cl, Au(IMes)Sbtz, Au(IMes)Stzn, Au(IMes)Spym, and Au(IMes)2tu at tested concentrations.



Vero-E6 cells were treated with metal complexes at 50, 10, and 2 μ M for 72 h at 37°C with 5% of CO₂. After that, media-containing compound was removed and MTT at 1 mg/mL solution was added to each well, incubated for 30 minutes and replaced with 100 μ l of DMSO to solubilize the formazan crystals. The absorbance was measured at 560 nm. DMSO 0.1% was used as untreated control. Mean values $\pm$ SD of a minimum of three independent experiments each measured in triplicate. Values of $p < 0.5$ were considered statistically significant. (****) $p < 0.0001$, while (ns) were considered non-significant. The image was generated using GraphPad Prism 8.

Then, the highest non-cytotoxic concentration of each compound, selected from cell viability assay, was used to screen the complexes towards its anti-MAYV activity, using MAYV-*nanoluc*. To this, Vero E6 cells were infected by MAYV-*nanoluc* and treated simultaneously with Au(IMes)Cl at 10 μ M or Au(IMes)Sbtz, Au(IMes)Stzn, Au(IMes)Spym, and Au(IMes)2tu at 2 μ M for 24h, when cells were harvested and luminescence was quantified by the *Renilla luciferase* assay. Cell viability was measured by the MTT assay in parallel (**Figure 3A**). As an outcome, Au(IMes)Cl, Au(IMes)Sbtz, Au(IMes)Stzn, Au(IMes)Spym, and Au(IMes)2tu were able to significantly decrease MAYV viral replication by 76%, 84%, 81%, 90%, and 84%, respectively ($p < 0.0001$) (**Figure 2B**).

Figure 3. Antiviral activity of Au(I) coordinated compounds on MAYV-*nanoluc* replication



A) Schematic description of the assay. **B)** Vero-E6 cells were infected with MAYV-*nanoluc* (MOI of 0.1) and treated with the higher non-cytotoxic concentration of each compound. After 24 hours of infection the cells were lysed, and the replication were measured by Renilla-luciferase assay. Cell viability assay was performed in parallel. DMSO 0.1% was used as untreated control. Mean values $\pm$ SD of a minimum of three independent experiments each measured in triplicate. Values of $p < 0.5$ were considered statistically significant. (****) $P < 0.0001$, while (ns) were considered non- significant. The image was generated using GraphPad Prism 8.

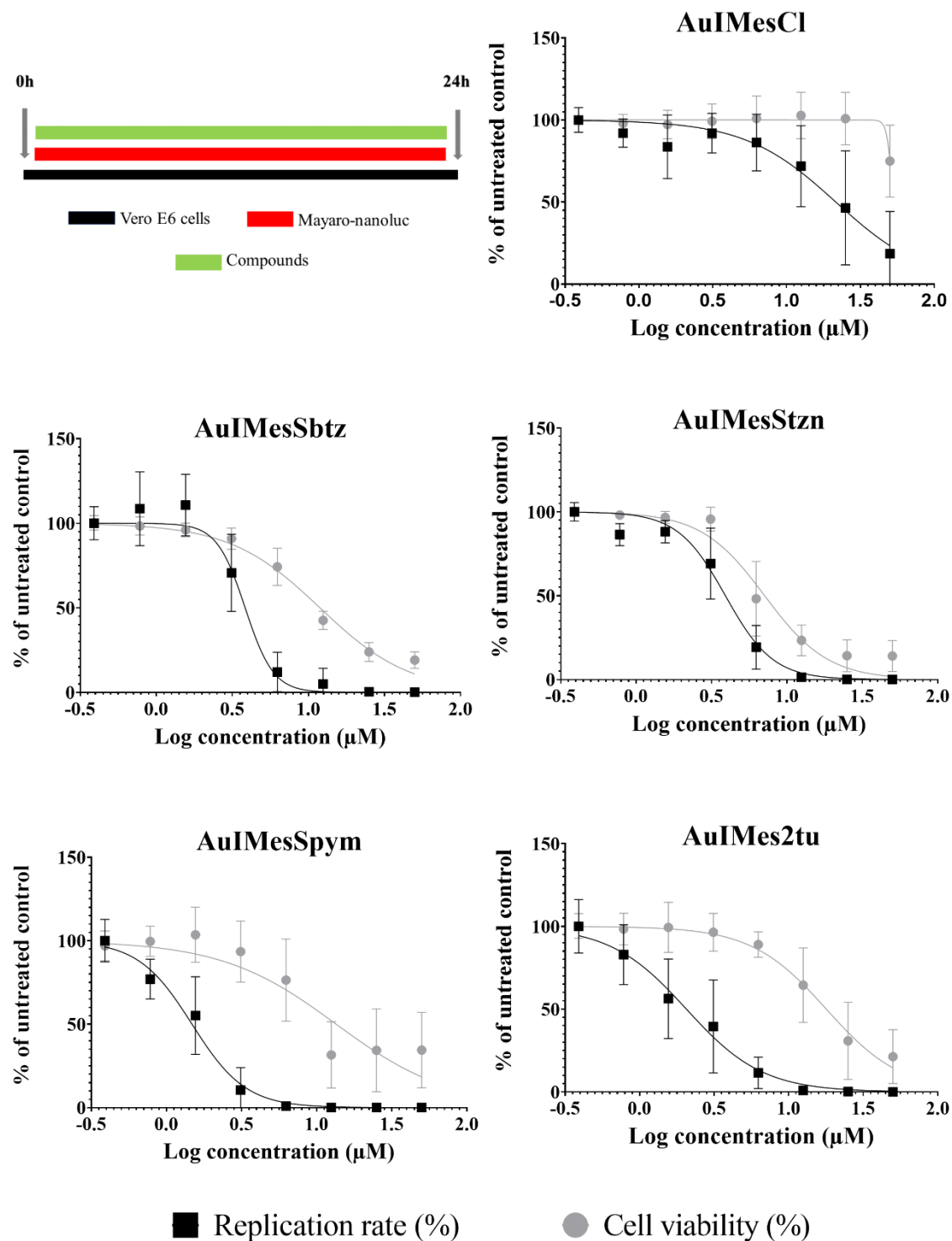
Au(I) complexes decrease viral replication with different selectivity indexes

The active compounds were further exploited towards their cytotoxicity and antiviral profiles employing a dose-response assay to determine the effective concentration of 50% (EC_{50}) and cytotoxic concentration of 50% (CC_{50}) values. For this, Vero E6 cells were infected by MAYV-*nanoluc* and simultaneously treated with Au(IMes)Cl, Au(IMes)Sbtz, Au(IMes)Stzn, Au(IMes)Spym, and Au(IMes)2tu at concentrations ranging from 0.39 to 50 μ M in a two-folds serial dilution. As a result, the compound Au(IMes)Cl exhibited an EC_{50} of 21.5 μ M, a CC_{50} of 52.5 μ M, and an selectivity index (SI) of 2.4, while the complexes: i) Au(IMes)Sbtz: exhibited an EC_{50} of 3.8 μ M, a CC_{50} of 11.9 μ M, and an SI of 3.1; ii) Au(IMes)Stzn: displayed an EC_{50} of 3.9 μ M, a CC_{50} of 7.1 μ M, and an SI of 1.8; iii) Au(IMes)Spym had an EC_{50} of 1.5 μ M, a CC_{50} of 13.5 μ M, and an SI of 9.0; and iv) Au(IMes)2tu showed an EC_{50} of 2.0 μ M, a CC_{50} of 17.9 μ M, and an SI of 8.9 (**Table 1; Figure 4**).

Table 1. Values of EC_{50} , CC_{50} and an SI of the tested Au(I) coordinated compounds.

Compounds	EC_{50} (μ M)	CC_{50} (μ M)	SI
Au(IMes)Cl	21.5	52.5	2.4
Au(IMes)Sbtz	3.8	11.9	3.1
Au(IMes)Stzn	3.9	7.1	1.8
Au(IMes)Spym	1.5	13.5	9.0
Au(IMes)2tu	2.0	17.9	8.9

Figure 4. Dose-response curve of Au(I) complexes against MAYV-*nanoluc* in Vero E6 cells.



(A) Representative scheme of the dose-response assay in Vero E6 cells (black bar) infected with MAYV-*nanoluc* (red bar) and treated with each compound (green bar) for 24 h at concentrations ranging from 0.39 to 50 μ M, in a two-folds serial dilution. The effects of Au(IMes)Cl (B), Au(IMes)Sbtz (C), Au(IMes)Stzn (D), Au(IMes)Spym (E) and Au(IMes)2tu (F) are demonstrated by CC_{50} indicated by ● and EC_{50} indicated by ■. Mean values of three independent experiments each measured in triplicate including the standard deviation are shown. The image was generated using GraphPad Prism 8.

Au(I) complexes impair different stages of the MAYV infection cycle

Due to the highest values of SI of Au(IMes)Spym and Au(IMes)2tu, these complexes were selected for further exploiting their antiviral effect on MAYV replication cycle. To this end, time of drug-addition assays were performed, in which the treatments were carried out with Au(IMes)Spym at 3 μ M or Au(IMes)2tu at 6 μ M.

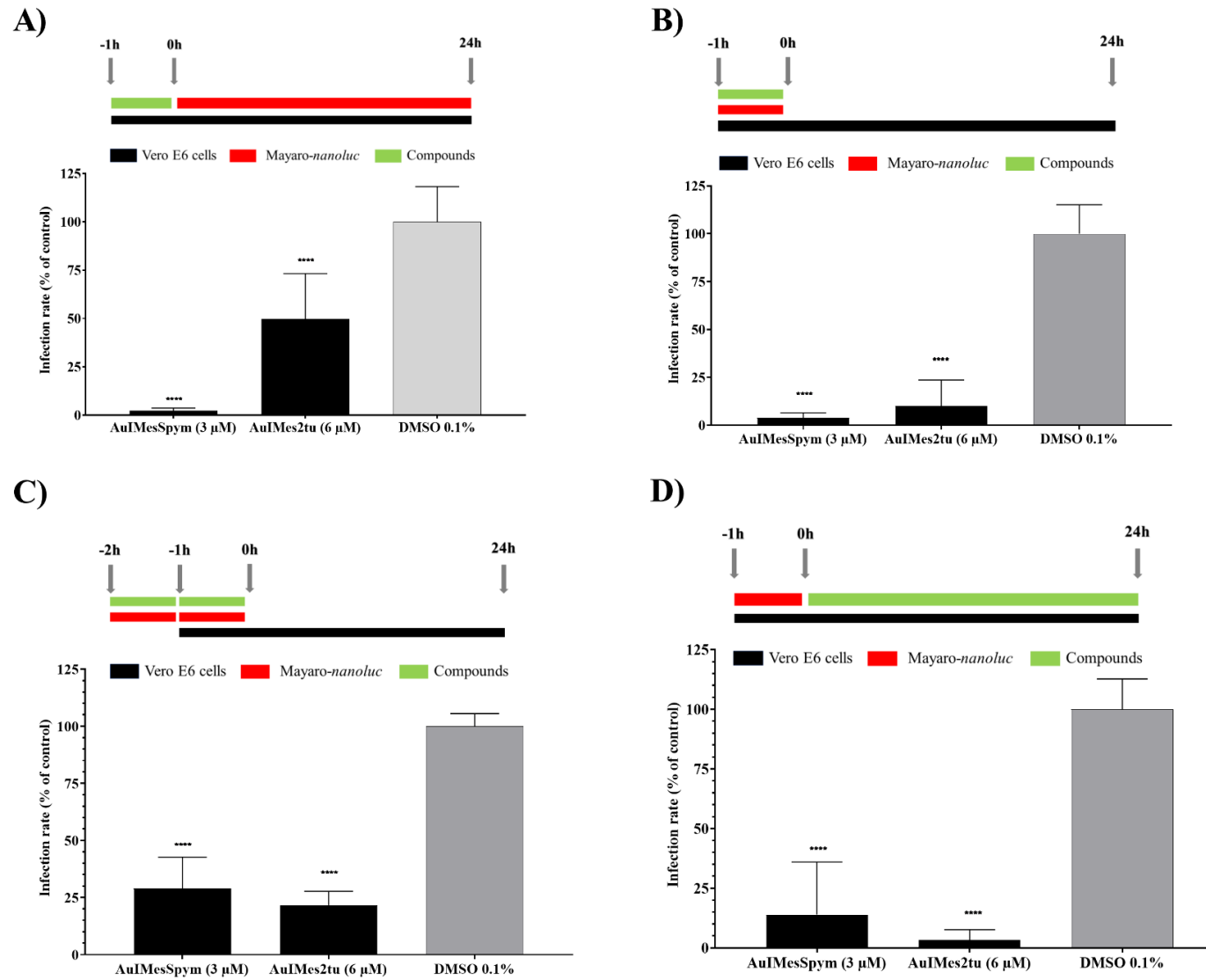
Firstly, the protective effect of Au(I) complexes was evaluated by pre-treating Vero-E6 cells with Au(IMes)Spym or Au(IMes)2tu for 1h, followed by infection with MAYV-*nanoluc* for the remaining 24h, when replication levels were assessed. As an outcome, MAYV-*nanoluc* replication significantly decreased in 98% and 50% in the presence of Au(IMes)Spym and Au(IMes)2tu, respectively ($p < 0.0001$) (**Table 2; Figure 5A**). These data indicate that these compounds could be acting on host cells and preventing MAYV infection.

Table 2. Antiviral effects of Au(I) compounds in the time of drug-addition assay.

Inhibition of MAYV replication (%)				
Compounds	Protective	Virucidal	Entry	Post-entry
Au(IMes)Spym	98	71	96	86
Au(IMes)2tu	50	78	90	97

Figure 5. Effect of Au(I) compounds on different stages of MAYV replicative cycle.

Figure 5. Effect of Au(I) compounds on different stages of MAYV replicative cycle



A) Pre-treatment. Vero E6 cells were treated with compounds for 1h at 37 °C. Then the supernatant was removed, and the cells were washed with PBS (three times) and added of medium containing the virus at an MOI of 0.1. **B) Entry.** Complexes and the virus (MOI of 1) were simultaneously added to cells for 1 h. The cells were washed with PBS (three times) and DMEM 2% FBS was added. **C) Virucidal.** The virus supernatant (MOI of 2) was incubated for 1h with compounds, then they were added to Vero E6 cells for an extra hour. Then, cells were washed three times with PBS and DMEM 2% FBS was added. **D) Post-entry.** Vero E6 cells were incubated with MAYV-*nanoluc* (MOI 0.1) for 1h. Afterwards, supernatant was removed, cells were washed three times with PBS, and incubated with compounds for the remaining 24h. For all protocols, *Renilla* luciferase assay were used to get the luminescence. Schematic representation of each time-based assay as shown by Vero E6 cells (black bars), compound (green bars), MAYV-*nanoluc* (red bars). Mean values standard deviation of three independent experiments each measured in triplicate. (****) $P < 0.0001$. The image was generated using GraphPad Prism 8.

Further, to identify if the compounds were inhibiting MAYV entry to the host cells, virus and compounds were simultaneously added to the cells and incubated for 1h, followed by media removal, washing with PBS, and replacement with fresh medium for 24h. As a result, Au(IMes)Spym and Au(IMes)2tu strongly inhibited viral replication by 96 and 90%, respectively ($p < 0.0001$) (**Table 2; Figure 5B**). Due to the strong entry activity of both compounds on MAYV entry to the host cells, a virucidal assay was performed by pre-incubating the supernatant containing MAYV-*nanoluc* at an MOI of 2 with Au(IMes)Spym or Au(IMes)2tu for 1h, and then adding the inoculum to the cells for further 1h. After the incubation, cells were washed with PBS and replaced by fresh media. Interestingly, Au(IMes)Spym and Au(IMes)2tu significantly reduced MAYV replication in 71% and 78%, respectively ($p < 0.0001$). Therefore, suggesting that both compounds could be interacting with MAYV virus particle and preventing its entry (**Table 2; Figure 5C**).

Finally, to investigate the effect of Au(IMes)Spym and Au(IMes)2tu on the post-entry stage of MAYV replication, the cells were first infected with MAYV-*nanoluc*, and after 1h, the supernatant was removed, cells were washed with PBS to remove unbound viral particles and were added of medium containing Au(IMes)Spym or Au(IMes)2tu. As an outcome, viral replication was reduced in 86% by Au(IMes)Spym and 97% by Au(IMes)2tu ($p < 0.0001$) (**Table 2; Figure 5D**). These results suggest that these Au(I) complexes are also affecting post-entry stages of virus replicative cycle.

DISCUSSION

The organometallic compounds encompassing gold ions have demonstrated various biological activities in recent decades, being active against bacteria, parasites, as well as demonstrating antitumor effects (Amini *et al.*, 2023; Babaei *et al.*, 2021; Jürgens; Casini, 2017; Khedr *et al.*, 2023; Roy *et al.*, 2018; Timoszyk; Grochowalska, 2022). Besides the seminal studies on the Auranofin anti-HIV effects, gold-NHC has been studied as anti-SARS-CoV-2 with excellent results (Gil-Moles *et al.*, 2020; Karges, 2021; Lewis *et al.*, 2011; Reiser, *et al.* 2012; Rothan *et al.*, 2020). Our research group has been studying the effects of Au(I) complexes, especially bearing phosphines and N-heterocyclic carbenes, in CHIKV and other arboviruses (Aires, 2022). Considering the potential of MAYV to cause outbreaks and the lack of antivirals to abrogate its viral replication, the investigation of new molecules as antiviral candidates is needed. Herein, the organometallic molecules Au(IMes)Cl, Au(IMes)Sbtz, Au(IMes)Stzn, Au(IMes)Spym and Au(IMes)2tu were explored as potential inhibitors of MAYV, as well as their activities on MAYV replicative cycle were evaluated.

Our results showed that all compounds were able to reduce MAYV viral replication at specific concentrations, however Au(IMes)Spym and Au(IMes)2tu presented the highest selective indexes, representing the most attractive molecules here investigated. Interestingly, considering the chemical structure of the compounds analysed here, the substitution of the chloride by a sulfur donor ligand Au(IMes)Cl for a sulfur donor ligands resulted in more cytotoxic compounds for VeroE6 cells, however, except by Au(IMes)Stzn, the compounds were able to inhibit MAYV replication in low concentrations, presenting a better SI when compared to Au(IMes)Cl. What is more, the presence of thiazole was related with enhancing cytotoxicity in mammalian cells when combined with chalcones (Ayati *et al.*, 2018; Pathania; Narang; Rawal, 2019). But in another case the thiazole when combined to thiosemicarbazones resulted in the inhibition of viral replication to lower EC₅₀ concentrations against other arboviruses, such as *Orthoflavivirus flavi* (YFV) and Saint Louis encephalitis virus (SLEV), when (Pacca *et al.*, 2017). In comparison, the compounds containing thiazolidines derivatives, such as Au(IMes)Sbtz and Au(IMes)Stzn have the lowest SI, and especially Au(IMes)Stzn presented a even lower SI than Au(IMes)Cl. On the other hand, compounds bearing the pyrimidine derivative ligands, have the best SI, despite their cytotoxicity to VeroE6.

Due to the highest SIs, Au(IMes)Spym and Au(IMes)2tu were selected for further assessing the effect of these molecules on the stages of MAYV replicative cycle. The Au(IMes)Spym were able to completely protect cells against infection while Au(IMes)2tu

decreased viral infection in 50%, which agrees with previous data showing that Au(I) complexes were able to protect cells against CHIKV (Aires *et al.*, 2022). This may be related to the fact that Au(I) compounds inhibit the enzyme Thioredoxin Reductase (TrxR), which is responsible for reducing the amount of reactive oxygen species (ROS) (Delgado *et al.*, 2020; Dong *et al.*, 2022), thus resulting in an increase in intracellular ROS levels and impairing viral replication (Benhar *et al.*, 2016; Meuren *et al.*, 2022; Zhang, Z.; Rong, L.; Li, Y.-P., 2019). This suggests that the presence of Au(I) might be inducing intracellular antiviral response that can decrease viral replication even if the virus enters the cells, a hypothesis to be further investigated.

In addition, Au(IMes)Spym and Au(IMes)2tu were also able to block viral entry in over 90%, and demonstrated virucidal activity, suggesting the interaction of the compounds to virus glycoproteins and/or envelope. Interestingly, Au nanoparticles were reported as SARS-CoV-2 inhibitors by disrupting viral membrane (Tang *et al.*, 2023), but also were able to interact with Ebola virus glycoproteins, as well as with HIV envelope glycoproteins gp41, gp120, and gp160, inhibiting the entry of the virus into T CD4⁺ cells (Budhadev *et al.*, 2020; Di Gianvincenzo *et al.*, 2010; Fonteh; Keter; Meyer, 2010; Okada *et al.*, 1993). Historically, auranofin affects glycoproteins in HIV-1 (gp120) by an oxidative pathway (Chirullo *et al.*, 2013; Reiser, *et al.*, 2012). Additionally, Auranofin and Au complexes containing gallic acid were capable to inhibit the endocytosis of SARS-CoV-2 and HSV-1 (Halder *et al.*, 2018; Laplantine *et al.*, 2022). Therefore, it is possible to suggest that the new Au(I) molecules described here are capable of blocking MAYV entry through interactions with virus glycoproteins or by inhibiting endocytosis and/or disrupting viral membrane. This could be expected, since the Au(I) ion can increase affinity for selenols and thiol-containing proteins, allowing different interactions with the viral envelope and cellular receptors (Berners-Price; Filipovska, 2011). However, more studies are needed to better understand the mode of action of these compounds on viral entry.

Although both Au(IMes)Spym and Au(IMes)2tu presented activity in protecting the cells and impairing viral entry, their outstanding activity was also seen in the post-entry stage, inhibiting viral replication up to 97%. Due to the lipophilicity of Au(I) complexes (Delgado *et al.*, 2020; THOMAS *et al.*, 2022), it can trespass cell membrane and act on viral post-entry steps as observed herein. The Au(I) drug Auranofin has been described as capable of reducing CHIKV viral RNA in Vero E6 cells (Langsjoen *et al.*, 2017), and other Au(I) triphenylphosphine complexes also interact with dsRNA, which significantly interferes in CHIKV replication (Aires *et al.*, 2022). In addition, Au(I) complexes were described as inhibitors of HIV reverse transcriptase and protease, as well as SARS-CoV-2 main protease (Mpro) (Fonteh; Keter;

Meyer, 2010; Massai et al., 2022; Tang *et al.*, 2023). Moreover, Au(NHC) complexes were able to affect the stacking of nitrogenous bases and interacting with two different enzymes, bovine pancreatic ribonuclease (RNase A) and hen egg white lysozyme (Annunziata *et al.*, 2022). In this sense, this post-entry activity could be related to: i) the interaction of the complexes with viral genome; ii) interaction with viral dsRNA, an intermediate of viral replication; iii) interaction with viral proteases; iv) and/or inducing cellular antiviral responses, such as reactive oxygen species (ROS).

Additional assays are needed to unveil the mode of action of Au(I) complexes against MAYV. So far, it is possible to suggest that the complexes here investigated are attractive compounds to be further exploited into the drug development against MAYV infection.

CONCLUSION

In summary, the data presented here demonstrates that Au(I) complexes of NHC and sulphur were able to strongly inhibit MAYV replication, demonstrating the potential of the metal complexes as antivirals against this arbovirus. From the data presented here, the compounds Au(IMes)Spym and Au(IMes)₂tu had a better ratio of cytotoxicity and antiviral activity, and were able to inhibit all stages of MAYV replicative cycle. More studies must be carried out to unveil the mechanism of action of these compounds, as well as to identify its potential *in vivo*. It is important to emphasize that the data described here could be useful for the development of future antivirals against MAYV, and provide a relevant advance to public health in the fight against Mayaro fever. Also, our results contribute to highlight the metal complexes as strong candidates for antiviral drug development against emergent and endemic viruses.

REFERENCES:

- ABBO, S. R. et al. Comparative Efficacy of Mayaro Virus-Like Particle Vaccines Produced in Insect or Mammalian Cells. **Journal of Virology**, v. 97, n. 3, 30 mar. 2023.
- AIRES, R. L. et al. Triphenylphosphine gold(I) derivatives promote antiviral effects against the Chikungunya virus. **Metallomics**, v. 14, n. 8, 1 jan. 2022.
- AMINI, S. M. et al. The Therapeutic Effects of Curcumin-coated Gold Nanoparticle Against Leishmania Major Causative Agent of Zoonotic Cutaneous Leishmaniasis (ZCL): An In Vitro and In Vivo Study. **Current Microbiology**, v. 80, n. 4, p. 104, 13 fev. 2023.
- ANDERSON, Charles R.; WATTLE, George H.; AHIN, Norman W.; *et al.* Mayaro Virus: A New Human Disease Agent. **The American Journal of Tropical Medicine and Hygiene**, v. 6, n. 6, p. 1012–1016, 1957.
- ANDREOLLA, Ana Paula; BORGES, Alessandra Abel; BORDIGNON, Juliano; *et al.* Mayaro Virus: The State-of-the-Art for Antiviral Drug Development. **Viruses**, v. 14, n. 8, p. 1787, 2022.
- ANESTINO, T. A. et al. The impact of arthritogenic viruses in oral tissues. **Journal of Applied Microbiology**, 6 fev. 2024.
- ANNUNZIATA, A. et al. Halo complexes of gold(I) containing glycoconjugate carbene ligands: synthesis, characterization, cytotoxicity and interaction with proteins and DNA model systems. **Dalton Transactions**, v. 51, n. 27, p. 10475–10485, 12 jul. 2022.
- AYATI, A. et al. Synthesis and biological evaluation of 4-amino-5-cinnamoylthiazoles as chalcone-like anticancer agents. **European journal of medicinal chemistry**, v. 145, p. 404–412, 1 fev. 2018.
- BABAEI, Abouzar; MOUSAVI, Seyed Mahmoud; GHASEMI, Marzie; *et al.* Gold nanoparticles show potential in vitro antiviral and anticancer activity. **Life Sciences**, v. 284, p. 119652, 2021.
- BENEDEK, T. G. The History of Gold Therapy for Tuberculosis. **Journal of the History of Medicine and Allied Sciences**, v. 59, n. 1, p. 50–89, 2004.
- BENHAR, M. et al. Dual targeting of the thioredoxin and glutathione systems in cancer and HIV. v. 126, n. 5, p. 1630–1639, 2 maio 2016.
- BERNERS-PRICE, S. J.; FILIPOVSKA, A. Gold compounds as therapeutic agents for human diseases. **Metallomics**, v. 3, n. 9, p. 863, 2011.
- BOMBARDIER, C. et al. Auranofin therapy and quality of life in patients with rheumatoid arthritis. Results of a multicenter trial. **The American Journal of Medicine**, v. 81, n. 4, p. 565–578, out. 1986.
- BUDHADEV, D. et al. Glycan-Gold Nanoparticles as Multifunctional Probes for Multivalent Lectin–Carbohydrate Binding: Implications for Blocking Virus Infection and Nanoparticle Assembly. *Journal of the American Chemical Society*, v. 142, n. 42, p. 18022–18034, 16 set. 2020.

CAICEDO, E.-Y. et al. The epidemiology of Mayaro virus in the Americas: A systematic review and key parameter estimates for outbreak modelling. **PLOS Neglected Tropical Diseases**, v. 15, n. 6, p. e0009418, 3 jun. 2021.

CELONE, M. et al. Understanding transmission risk and predicting environmental suitability for Mayaro Virus in Central and South America. **PLoS neglected tropical diseases**, v. 18, n. 1, p. e0011859, 2024.

CEREGHINO, C. *et al.* The E2 glycoprotein holds key residues for Mayaro virus adaptation to the urban *Aedes aegypti* mosquito. **PLOS Pathogens**, v. 19, n. 4, p. e1010491–e1010491, 2023.

CHIRULLO, B. et al. A candidate anti-HIV reservoir compound, auranofin, exerts a selective “anti-memory” effect by exploiting the baseline oxidative status of lymphocytes. v. 4, n. 12, p. e944–e944, 5 dez. 2013.

DE CURCIO, J. S. et al. Detection of Mayaro virus in *Aedes aegypti* mosquitoes circulating in Goiânia-Goiás-Brazil. **Microbes and Infection**, v. 24, n. 4, p. 104948, jun. 2022.

DELGADO, G. et al. Compostos quimioterápicos de ouro: uma visão geral dos complexos anticâncer de Au(I/III) em relação à estrutura do ligante. **Química Nova**, 2020.

DI GIANVINCENZO, Paolo; MARRADI, Marco; MARTÍNEZ-ÁVILA, Olga María; *et al.* Gold nanoparticles capped with sulfate-ended ligands as anti-HIV agents. **Bioorganic & Medicinal Chemistry Letters**, v. 20, n. 9, p. 2718–2721, 2010.

DIAGNE, Cheikh Tidiane; BENGUE, Michèle; CHOUMET, Valérie; *et al.* Mayaro Virus Pathogenesis and Transmission Mechanisms. **Pathogens**, v. 9, n. 9, p. 738, 2020.

DONG, G. et al. Au-24 as a potential thioredoxin reductase inhibitor in hepatocellular carcinoma cells. **Pharmacological Research**, v. 177, p. 106113, mar. 2022.

ESPOSITO, Danilo Lucas Alves; FONSECA, Benedito Antonio Lopes da; ESPOSITO, Danilo Lucas Alves; *et al.* Will Mayaro virus be responsible for the next outbreak of an arthropod-borne virus in Brazil? **Brazilian Journal of Infectious Diseases**, v. 21, n. 5, p. 540–544, 2017.

FEREIDONNEZHAD, M. et al. Synthesis and biological evaluation of thiolate gold(I) complexes as thioredoxin reductase (TrxR) and glutathione reductase (GR) inhibitors. *New journal of chemistry*, v. 43, n. 33, p. 13173–13182, 1 jan. 2019.

FONTEH, P. N.; KETER, F. K.; MEYER, D. HIV therapeutic possibilities of gold compounds. *BioMetals*, v. 23, n. 2, p. 185–196, 3 fev. 2010.

HALDER, Asim; DAS, Suvadra; OJHA, Durbadal; *et al.* Highly monodispersed gold nanoparticles synthesis and inhibition of herpes simplex virus infections. **Materials Science and Engineering: C**, v. 89, p. 413–421, 2018.

HERNÁNDEZ-SARMIENTO, L. J. et al. Mayaro virus infection elicits a robust pro-inflammatory and antiviral response in human macrophages. **Acta Tropica**, p. 107146–107146, 1 fev. 2024.

JÜRGENS, S.; CASINI, A. Mechanistic Insights into Gold Organometallic Compounds and their Biomedical Applications. *CHIMIA*, v. 71, n. 3, p. 92, 29 mar. 2017.

KARGES, Johannes; COHEN, Seth M. Metal Complexes as Antiviral Agents for SARS-CoV-2. **ChemBioChem**, 2021.

KEAN, W. F.; L. PATRICK HART; W. WATSON BUCHANAN. Auranofin. *Rheumatology*, v. 36, n. 5, p. 560–572, 1 maio 1997.

KHEDR, W. E. et al. Silver and gold nanoparticles: Eco-friendly synthesis, antibiofilm, antiviral, and anticancer bioactivities. **Preparative biochemistry & biotechnology**, p. 1–13, 23 ago. 2023.

KROKOVSKY, Larissa; RALPH, Carlos; RIBEIRO, Duschinka; *et al.* Dynamic of Mayaro Virus Transmission in *Aedes aegypti*, *Culex quinquefasciatus* Mosquitoes, and a Mice Model. **Viruses**, v. 15, n. 3, p. 799–799, 2023.

LANGSJOEN, Rose M; AUGUSTE, Albert J; ROSSI, Shannan L; *et al.* Host oxidative folding pathways offer novel anti-chikungunya virus drug targets with broad spectrum potential. **Antiviral Research**, v. 143, p. 246–251, 2017.

LAPLANTINE, Emmanuel; CHABLE-BESSIA, Christine; OUDIN, Anne; *et al.* The FDA-approved drug Auranofin has a dual inhibitory effect on SARS-CoV-2 entry and NF-κB signaling. **iScience**, v. 25, n. 10, p. 105066–105066, 2022.

LEWIS, Mark G.; DAFONSECA, Sandrina; CHOMONT, Nicolas; *et al.* Gold drug auranofin restricts the viral reservoir in the monkey AIDS model and induces containment of viral load following ART suspension. **AIDS**, v. 25, n. 11, p. 1347–1356, 2011.

LI, W. et al. AuNPs as an important inorganic nanoparticle applied in drug carrier systems. **Artificial Cells, Nanomedicine, and Biotechnology**, v. 47, n. 1, p. 4222–4233, 12 nov. 2019.

LI, Xiaodan; ZHANG, Hongqing; ZHANG, Yanan; *et al.* Development of a rapid antiviral screening assay based on eGFP reporter virus of Mayaro virus. **Antiviral Research**, v. 168, p. 82–90, 2019.

LORENZ, C.; CHIARAVALLOTI-NETO, F. Brazil reports an increased incidence of oropouche and mayaro fever in the amazon region. **Travel Medicine and Infectious Disease**, v. 58, p. 102692, 1 mar. 2024.

MASSAI, Lara; MESSORI, Luigi; MICALE, Nicola; *et al.* Gold compounds as cysteine protease inhibitors: perspectives for pharmaceutical application as antiparasitic agents. **BioMetals**, v. 30, n. 2, p. 313–320, 2017.

MASSAI, L. et al. Gold-Based Metal Drugs as Inhibitors of Coronavirus Proteins: The Inhibition of SARS-CoV-2 Main Protease by Auranofin and Its Analogs. v. 12, n. 11, p. 1675–1675, 11 nov. 2022.

MEUREN, L. M. et al. Infection of Endothelial Cells by Dengue Virus Induces ROS Production by Different Sources Affecting Virus Replication, Cellular Activation, Death and Vascular Permeability. **Frontiers in Immunology**, v. 13, 2 fev. 2022.

MUTRICY, R. et al. Mayaro virus infection in French Guiana, a cross sectional study 2003–2019. **Infection, Genetics and Evolution**, v. 99, p. 105243, abr. 2022.

PASCHOALINO, Marina; MARINHO, Mikaela dos Santos; SANTOS, Igor Andrade; *et al.* An update on the development of antiviral against Mayaro virus: from molecules to potential viral targets. **Archives of Microbiology**, v. 205, n. 4, 2023.

- PUJHARI, S. et al. Characterization of Mayaro virus (strain BeAn343102) biology in vertebrate and invertebrate cellular backgrounds. **Journal of General Virology**, v. 103, n. 10, 10 out. 2022.
- RAFIEI, Solmaz; REZATOFIGHI, Seyedeh Elham; ARDAKANI, Mohammad Roayaei; *et al.* Gold Nanoparticles Impair Foot-and-Mouth Disease Virus Replication. **IEEE Transactions on NanoBioscience**, v. 15, n. 1, p. 34–40, 2016.
- REISER, K. et al. Thioredoxin-1 and protein disulfide isomerase catalyze the reduction of similar disulfides in HIV gp120. **The International Journal of Biochemistry & Cell Biology**, v. 44, n. 3, p. 556–562, mar. 2012.
- ROSA, Rafael Borges; DE CASTRO, Emilene Ferreira; DE OLIVEIRA SANTOS, Débora; *et al.* Mouse Models of Mayaro Virus. **Viruses**, v. 15, n. 9, p. 1803, 2023.
- ROY, P. et al. Exploration of antifilarial activity of gold nanoparticle against human and bovine filarial parasites: A nanomedicinal mechanistic approach. **Colloids and Surfaces B: Biointerfaces**, v. 161, p. 236–243, 1 jan. 2018.
- SAATKAMP, C. J. et al. Mayaro virus detection in the western region of Pará state, Brazil. v. 54, 22 mar. 2021.
- SADLER, P. J.; SUE, R. E. The Chemistry of Gold Drugs. **Metal-Based Drugs**, v. 1, n. 2-3, p. 107–144, 1 jan. 1994.
- SANNELLA, A. R. et al. New uses for old drugs. Auranofin, a clinically established antiarthritic metallodrug, exhibits potent antimalarial effects in vitro: Mechanistic and pharmacological implications. **FEBS letters**, v. 582, n. 6, p. 844–847, 19 mar. 2008.
- SCHUH, E. et al. Gold(I) Carbene Complexes Causing Thioredoxin 1 and Thioredoxin 2 Oxidation as Potential Anticancer Agents. v. 55, n. 11, p. 5518–5528, 4 jun. 2012.
- SSEMAGANDA, A. et al. Gold(i) phosphine compounds as parasite attenuating agents for malaria vaccine and drug development. **Metallomics**, v. 10, n. 3, p. 444–454, 1 jan. 2018.
- SUN, J.; WU, D. Mayaro virus, a regional or global threat? **Travel Medicine and Infectious Disease**, v. 32, p. 101462, nov. 2019.
- TANG, H. et al. Anti-Coronaviral Nanocluster Restrains Infections of SARS-CoV-2 and Associated Mutants through Virucidal Inhibition and 3CL Protease Inactivation. *Advanced science*, v. 10, n. 13, 26 fev. 2023.
- THOMAS, S. et al. Bottom-up Synthesis of Water-Soluble Gold Nanoparticles Stabilized by N-Heterocyclic Carbenes: From Structural Characterization to Applications. *Chemistry - A European Journal*, v. 28, n. 56, 10 ago. 2022.
- TIMOSZYK, A.; GROCHOWALSKA, R. Mechanism and Antibacterial Activity of Gold Nanoparticles (AuNPs) Functionalized with Natural Compounds from Plants. **Pharmaceutics**, v. 14, n. 12, p. 2599–2599, 25 nov. 2022.
- VALDÉS-TORRES, P. et al. Honokiol and Alpha-Mangostin Inhibit Mayaro Virus Replication through Different Mechanisms. **Molecules**, v. 27, n. 21, p. 7362, 1 jan. 2022.
- WEBER, Whitney C; LABRIOLA, Caralyn S; KREKLYWICH, Craig N; *et al.* Mayaro virus pathogenesis and immunity in rhesus macaques. **PLOS Neglected Tropical Diseases**, v. 17, n. 11, p. e0011742–e0011742, 2023.

XIAO, Q. et al. Heteroleptic Gold(I)-bisNHC complex with excellent activity in vitro, ex vivo and in vivo against endometrial cancer. **European journal of medicinal chemistry**, v. 236, p. 114302–114302, 1 jun. 2022.

YAMASHITA, M. Auranofin: Past to Present, and repurposing. **International Immunopharmacology**, v. 101, p. 108272, dez. 2021.

ZHANG, Z.; RONG, L.; LI, Y.-P. Flaviviridae Viruses and Oxidative Stress: Implications for Viral Pathogeneses

is. **Oxidative Medicine and Cellular Longevity**, v. 2019, 19 ago. 2019.