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Original article

Synthesis and antiviral activity of new phenylimidazopyridines and *N*-benzylidenequinolinamines derived by molecular simplification of phenylimidazo[4,5-*g*]quinolines

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ABSTRACT

Continuing our program of research concerning the antiviral activity of a wide series of new angular and linear azolo bicyclic and tricyclic derivatives, now we have simplified and modified the 4-chloro-2-(4-nitrophenyl)-3*H*-imidazo[4,5-*g*]quinoline **1**, which previously resulted the most active derivative, through either the elimination of the central ring or the opening of the imidazole ring, obtaining various imidazopyridines and *N*-benzylidenequinolinamines respectively.

Title compounds were tested in cell-based assays for cytotoxicity and antiviral activity against representatives of two DNA virus families as wells as against representatives of RNA virus families containing single-stranded, either positive-sense ($ssRNA^+$) or negative-sense ($ssRNA^-$), and double-stranded genomes (dsRNA). Some imidazo[4,5-*b*]pyridines emerged as new derivatives endowed with antiviral activity against Vaccinia Virus (VV) at concentrations ranging from 2 to 16 μM. In particular, compound **2b** demonstrate to be about 10 times more potent than Cidofovir, used as reference drug. Similarly, the imidazo[4,5-*c*]pyridines and *N*-benzylidenequinolinamines derivatives resulted active against Bovine Viral Diarrhoea virus (BVDV), at concentrations ranging from 1.2 to 28 μM. Above all compounds **1**, **3a** and **3f** showed an EC_{50} of the same order of magnitude of the reference drug, the 2′-C-methyl-guanosine. Moreover, several *N*-benzylidenequinolinamines showed an interesting activity against Respiratory Syncytial Virus (RSV) at concentrations between 12 and 26 μM.

1. Introduction

Over 200 species of DNA and RNA viruses are known to be able to infect and cause significant diseases in humans [1,2]. Examples of DNA pathogen viruses are Herpesvirus and Poxvirus [3]. Herpes viruses belongs to the *Herpesviridae* family, subdivided into numerous genera, comprehending Herpes simplex virus type 1 (HSV-1), that causes facial cold-sores, Herpes simplex virus type 2 (HSV-2), also called genital herpes, and Varicella-Zoster virus (VZV), which causes chickenpox in children and shingles in adults.

Particularly we focused our attention on HSV-1, the most common etiologic agent of sporadic fatal encephalitis worldwide [4]. The clinical syndrome is often characterized by the rapid inception of fever, headache and nausea, followed by acute or subacute onset of an encephalopathy whose symptoms include lethargy, confusion, and delirium [5]. It is estimated to affect at least 1 in 500,000 per year [6]. Nucleoside analogues acyclovir (ACV) and its derivatives with better bioavailability such as famciclovir, valacyclovir and penciclovir have become the first line drugs for prophylaxis and treatment of HSV infections. However, their wide use to treat Herpes Simplex infections and their long-term administration for the treatment of chronic infections in the immunocompromised host (such as the organ transplant patient or patient with AIDS) can lead to the development of ACV-resistant mutant strain [7]. As result, the efficacy of these nucleoside analogues has been compromised.

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Poxviruses (members of the *Poxviridae* family) are viruses that can infect both vertebrate and invertebrate animals. They are subdivided in four genera, among which the Orthopoxvirus genus include Variola virus, Vaccinia virus (VV), Cowpox virus and Monkeypox virus [8]. Before the introduction of vaccination and eradication of Variola virus's infection (smallpox) in 1970s, it caused significant morbidity in human populations with anywhere from 1 to 25 percent of mortality [9]. In recent years, however, the potential of the use of Variola virus or another Orthopoxvirus, as a bioterrorism weapon, has heightened our awareness as to our vulnerability to this disease since vaccination for smallpox was discontinued in 1980 [10–14]. This has stimulated efforts to develop new drugs for treatment of Poxvirus infections. This potential threat has resulted in a resurgent effect to identify and develop more agents that can be used in an emergency situation to treat these viral diseases.

RNA viruses include more than 350 different human pathogens and most of the etiological agents of emerging diseases. According to their type of genetic material, RNA viruses are classified as single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA). Further classification accounts for the ssRNA viruses polarity into negative-sense and positive-sense, or ambisense. Unfortunately, most of these human pathogens have no available vaccine, and only a few selective antiviral drugs are utilizable in the clinic to prevent and/or treat their infections. Between the ssRNA, viruses belonging to the *Flaviviridae* family cause clinically significant diseases in humans and animals. This virus family includes three genera: Pestiviruses (i.e., Bovine Viral Diarrhoea virus [BVDV]), Flaviviruses (i.e., Yellow Fever [YFV], Dengue [DENV], and West Nile [WNV]), and Hepaciviruses (Hepatitis C virus [HCV]). Actually, with the exception of YFV, there is no vaccine and some new drugs are actually in study [15–23].

Other important ssRNA viruses pathogens are those belonging to the *Picornaviridae* (Coxsackievirus, Poliovirus) and *Paramixoviridae* (Respiratory syncytial virus) and Rhabdoviridae (Vesicular Stomatitis Virus, VSV) families. These viruses cause a variety of illnesses, and at present, no specific antiviral therapy is available for the treatment [24,25].

Hence the need to identify new lead compounds targeting a distinct stages of replication cycle in a virus-specific way. In this context, according to an antiviral research multiannual program, our group described the series of 2-[4-substituted naphtyl] [26], 2-[4-substituted biphenyl] [27], substituted-2-styryl [28] and 5-acetyl-2-[2,3,4-substituted aryl] benzimidazoles [29]. After biological evaluation, most of these compounds emerged for their selective activity against Respiratory Syncytial virus (RSV), Yellow Fever virus (YFV) and Coxsackie virus type B5 (CVB-5). Afterwards, with the aim to increase the antiviral behaviour, we operated a bioisosteric modification replacing the benzimidazole ring with a benzotriazole nucleus to obtain the series of *N*-[4-(1*H*(2*H*)-benzotriazol-1(2)-yl)phenyl]alkylcarboxamides [30] and *N*,*N*′-bis[4-(1*H*(2*H*)-benzotriazol-1(2)-yl)phenyl]alkyldicarboxamides [31]. Meantime, to provide molecular diversity of benzotriazole and benzimidazole nucleus for further structure activity relationship (SAR) analysis, we synthesized and tested for antiviral activity three new classes of angular [32] and linear N-tricyclic compounds [33–35].

Recently we have reported new linear azolo tricyclic derivatives as selective inhibitors of the RNA-dependent RNA polymerases (RdRps) of various RNA virus [36]. Between them, imidazo[4,5-*g*]quinoline derivatives showed an interesting activity against some ssRNA+ (BVDV, HCV and CVB), and dsRNA (Reo) viruses. Notably the 4-chloro-2-(4-nitrophenyl)-3*H*-imidazo[4,5-*g*]quinoline (**1**), resulted endowed with high and selective activity against BVDV and HCV. Now, with the purpose to extend the SAR analysis we have took in account the simplification of imidazo[4,5-*g*]quinoline nucleus through the elimination of the central ring or molecular modification by the opening of the imidazole ring, obtaining imidazopyridines (**2**) and *N*-benzylidenequinolinamines (**3**) derivatives respectively (Fig. 1).

Here we report the chemical synthesis and the biological assessment of their antiviral activity.

2. Chemistry

All chemical structures of the synthesized compounds are depicted in Figs. 2 and 3. The nitrogen atom positions in imidazopyridines and the substituents of all compounds were chosen with the aim to evaluate the influence of electron-withdrawing groups, lipophilicity and/or steric hindrance, on the antiviral activities.

The synthetic route to obtain the designed imidazopyridines **2a**–**l** (Fig. 2) and their intermediates is described in Scheme 1. The diamines **4a**–**c** underwent nucleophilic attack by the corresponding bisulphite compounds **5a**–**e**, to give the imidazo[4,5-*b*] (**2a**–**j**) and [4,5-*c*]pyridine (**2k**,**l**).

In turn *N*-benzylidenequinolinamines **3a**,**b**,**f**–**h** were obtained by condensation of diaminoquinolines **6a**,**b** with bisulphite compounds **5a**,**b**,**f**,**g**, operating by soft condition in refluxed ethanol (Scheme 2). Whereas more extreme conditions, DMF at 130 °C, in past principally afforded the corresponding imidazo[4,5-*g*]quinolines [36].

Bisulphites **5a**–**g** were obtained in high yields from the commercially available corresponding aldehydes (Aldrich) with $Na_2S_2O_5$ in ethanol, according to the procedure used by Shriner and Land [37].

3. Results, discussion and conclusion

Title compounds **2a**–**l** and **3a**,**b**,**f**–**h** were evaluated in cell based assays for their cytotoxicity and antiviral activity against a panel of RNA and DNA viruses. Among single-stranded, positive RNA viruses (ssRNA+), we considered a Retrovirus (Human Immunodeficiency Virus type 1, HIV-1), two Picornaviruses (Coxsackie Virus type-5, CVB-5, and Poliovirus type-1, Sabin strain, Sb-1), and viruses representative of two of the three genera of the Flaviviridae family, i.e., a Flavivirus (Yellow Fever Virus, YFV), and a Pestivirus (Bovine Viral Diarrhoea Virus, BVDV). Among single-stranded, negative RNA viruses (ssRNA-) a Paramyxoviridae (Respiratory Syncytial Virus, RSV) and a Rhabdoviridae (Vesicular Stomatitis Virus, VSV) were selected as representatives. Among double-stranded RNA (dsRNA) viruses, a Reoviridae family member (Reo-1) was included. Finally,

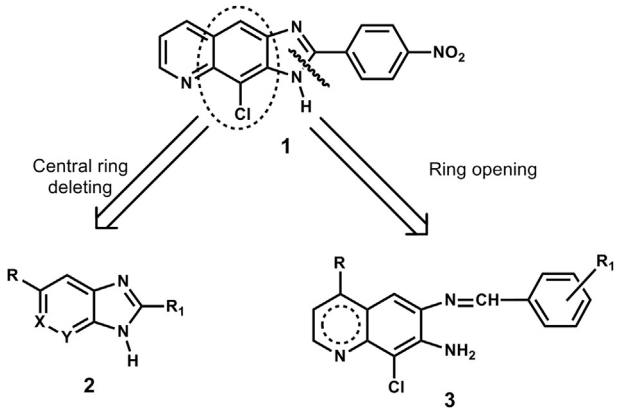

Fig. 1. Molecular modification of the lead compound **1**.