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Research paper

Identification of an indol-based derivative as potent and selective varicella zoster virus (VZV) inhibitor

Simona Musella [a, 1], Veronica di Sarno [a, 1], Tania Ciaglia [a], Marina Sala [a], Antonia Spensiero [a], Maria Carmina Scala [a], Carmine Ostacolo [b], Graciela Andrei [c], Jan Balzarini [c], Robert Snoeck [c], Ettore Novellino [b], Pietro Campiglia [a], Alessia Bertamino [a, **], Isabel M. Gomez-Monterrey [b, *]

[a] Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, 84084, Fisciano, SA, Italy
[b] Department of Pharmacy, University of Naples "Federico II", Via D. Montesano 49, 80131, Napoli, Italy
[c] Department of Microbiology and Immunology, Rega Institute for Medical Research, Leuven, Belgium

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ABSTRACT

We report the synthesis and antiviral activity of a new family of non-nucleoside antivirals, derived from the indole nucleus. Modifications of this template through Mannich and Friedel-Crafts reactions, coupled with nucleophilic displacement and reductive aminations led to 23 final derivatives, which were pharmacologically tested. Tryptamine derivative **17a** was found to have a selective inhibitory activity against human varicella zoster virus (VZV) replication in vitro, being inactive against a variety of other DNA and RNA viruses. A structure-activity relationship (SAR) study showed that the presence of a biphenyl ethyl moiety and the acetylation at the amino group of tryptamine are a prerequisite for anti-VZV activity. The novel compound shows the same activity against thymidine kinase (TK)-competent (TK^+) and TK-deficient (TK^-) VZV strains, pointing to a novel mechanism of antiviral action.

1. Introduction

Varicella zoster virus (VZV) is a ubiquitous and highly infectious human virus that belongs to the *herpesviridae* family. It is classified within the group of α-herpesviruses which also includes herpes simplex virus (HSV) [1]. A VZV primary infection leads to acute varicella or "chickenpox", while reactivation of latent virus, established in cranial nerve and dorsal root ganglia, causes herpes zoster (shingles). The course of varicella is generally benign in immune-competent children, but can cause severe morbidity and mortality in adults and in immune-compromised individuals [2]. Complications of herpes zoster in immune-competent hosts include post-herpetic neuralgia (PHN), a persistent pain syndrome, which is the most challenging complication particularly in older individuals [2,3]. Central nervous system (CNS) complications can follow both primary infection and reactivation of VZV [4,5]. The most serious manifestations arise when VZV invades the spinal cord or cerebral arteries after reactivation of the virus, causing diseases such as myelitis and focal vasculopathies [2,4,5]. Other neurological complications of herpes zoster include motor neuropathy, particularly in patients with zoster ophthalmicus [6,7]. In patients with the acquired immune deficiency syndrome (AIDS), transplant recipients, and cancer patients, VZV infection can be associated with severe acute retinal necrosis (ARN), a disease with poor prognosis [8,9]. The outcomes of varicella and herpes zoster have been dramatically improved by the development of safe and effective antiviral drugs with potent activity against VZV [10]. Three oral guanine-based antivirals are approved worldwide for the treatment of VZV-associated diseases: acyclovir, valacyclovir, and famciclovir [11]. The thymidine analog brivudin has been licensed for the therapy of herpes zoster in some European and Central American countries [12]. These drugs are (a) nucleoside analogs that after predominant phosphorylation by the virus-encoded thymidine kinases (TKs), act as competitive inhibitors of the viral DNA polymerase or alternate substrates to

* Corresponding author.
** Corresponding author.
E-mail addresses: abertamino@unisa.it (A. Bertamino), imgomez@unina.it (I.M. Gomez-Monterrey).
[1] These authors contributed equally to this work.

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the natural triphosphates, inhibiting DNA replication [13]. Other anti-VZV nucleoside inhibitors such as the stearyl/valyl diester valomaciclovir and the valyl-ester prodrug of the bicyclic nucleoside analog (BCNA) FV100 are under clinical investigation [14–17].

One of the limitations of the use of nucleoside derivatives is the emergence of single and multiple drug resistance which could be partially avoided with the use of non nucleoside compounds [18,19]. A drug of choice for treatment of acyclovir-resistant VZV disease is foscarnet, a direct inhibitor of viral DNA polymerase that is not dependent on viral TK for activation [20–22]. A number of small molecules have been identified and reported as potent and selective VZV inhibitors with different mechanisms of action. Some examples are the 4-oxo-dihydroquinoline [23] and 4-oxo-dihydrothieno [2,3-b]pyridine derivatives [24] as inhibitors of the viral DNA polymerases, the oxadiazolephenyl derivative (ASP2151) as a helicase-primase inhibitor [25], and N-α-methylbenzyl-N′-arylthiourea derivatives that interfere with the function of the viral ORF54 protein, impairing morphogenesis of the capsid [26,27]. Finally, a series of 4-benzyloxy-γ-sultone derivatives has been also reported as non-selective VZV inhibitors with unknown mechanism of action [28]. Given the difficulty of identifying initial hit compounds in this field, where the synthesized compounds are *in primis* subject to a cellular screening, we considered of interest to use a privileged scaffold as effective starting point in the search for anti-VZV ligands [29]. Indole and its bioisosteres, as privileged scaffolds, represent one of the most important structural motifs in drug discovery [30–32], and it is widely used in antiviral research [32,33]. Arbidol [34] and delavirdine [35], are examples of marketed indole-containing antiviral drugs, whereas Panobinostat (LBH589) [36], being a HDAC (histone deacetylase) inhibitor, is actively undergoing clinical evaluation against human immunodeficiency virus (HIV) type 1 (See Fig. 1).

However the use of the indole-based structures in the research of anti-herpes virus agents is rather unusual. Hence we explored the minimum structural requirements for anti-VZV activity starting from this easily derivatizable scaffold. Two small libraries were synthesized based on substituted indoles (A, B) and tryptamines (C). Their cytotoxic and antiviral activity was then evaluated using cellular assays. Some interesting structure-activity relationships were evidenced regarding the N-1 and C-3 substituents.

2. Results and discussion

2.1. Chemistry

Compounds **4a-d** were prepared starting from indole **1** according to Scheme 1. N-1 alkylation of **1** with propyl iodide or 4-phenylbenzyl iodide in DCM/DMF using NaH as base, gave the corresponding intermediates **2** and **3**, respectively. The 3-acyl derivative **4a** was obtained from **2** by Friedel-Crafts acylation, using 4-chlorobenzoyl chloride and AlCl3 in acetonitrile (32% yield). Functionalization of **2** and **3** through a Mannich reaction, using formaldehyde and piperidine/or biphenyl ethyl amine, and TFA as catalyst, led to 3-methylamine derivatives **4b-d** (25–38% yield).

The 5-substituted indole derivatives (**8a-c**) were synthesized using the indole-5-carboxyaldehyde (**5**) and the 5-aminoindole (**9**) as starting material and following the two-synthetic strategy indicated in Scheme 2. **5** was first N-alkylated (**6**) and then subjected to a Mannich reaction to obtain the corresponding aldehydes **7a** and **7b**, as described above. Treatment of these intermediates with 4-chloro aniline and sodium triacetoxyborohydride in reductive amination conditions, led to final products **8a** and **8b** in 24% and 30% yield, respectively. On the other hand, reaction of **9** with benzoic acid using HOBt/HBTU as coupling agents gave N-(1H-indol-5-yl)benzamide **10** in 63% yield. N-alkylation of **10** with n-propyl iodide followed by Mannich reaction with formaldehyde and piperidine afforded final compound **8c**.

The tryptamine-based derivatives **15, 16a-i** were prepared following Scheme 3. N-alkylation of 3-(2-bromoethyl)-1H-indole (**12**) with methyl iodide or 4-phenylbenzyl iodide led to derivatives **13** and **14** in 67% and 61% yield, respectively. Nucleophilic displacement of the bromine atom in these intermediates by different commercially available amines was performed under microwave conditions, using palladium acetate as catalyst, obtaining the compounds **15**, **16a-i** in 38–75% yield [37].

Treatment of compounds **16a** and **16d-i** with acetyl chloride gave the corresponding acetyl derivatives **17a** and **17d-i** (42–58% yield).

Analogously, treatment of **16a** with different aromatic and aliphatic acyl chlorides gave the corresponding acylated compounds **18a-c** (48–57% yield). The carbamoyl derivative **18d** was obtained in 58% yield by reaction of **16a** with di-*tert*-butyl dicarbonate in DCM/TEA (See Scheme 4).

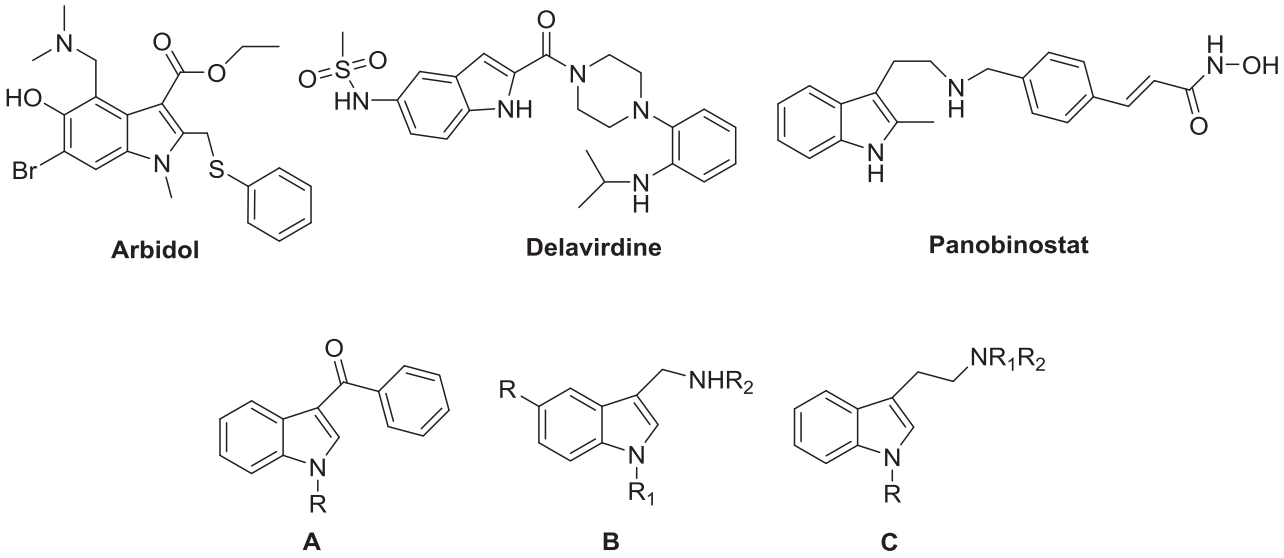

Fig. 1. Indole-containing antiviral compounds. Structure of indole (A, B) and tryptamine (C) derivatives described in this work.