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Research paper

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

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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

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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).

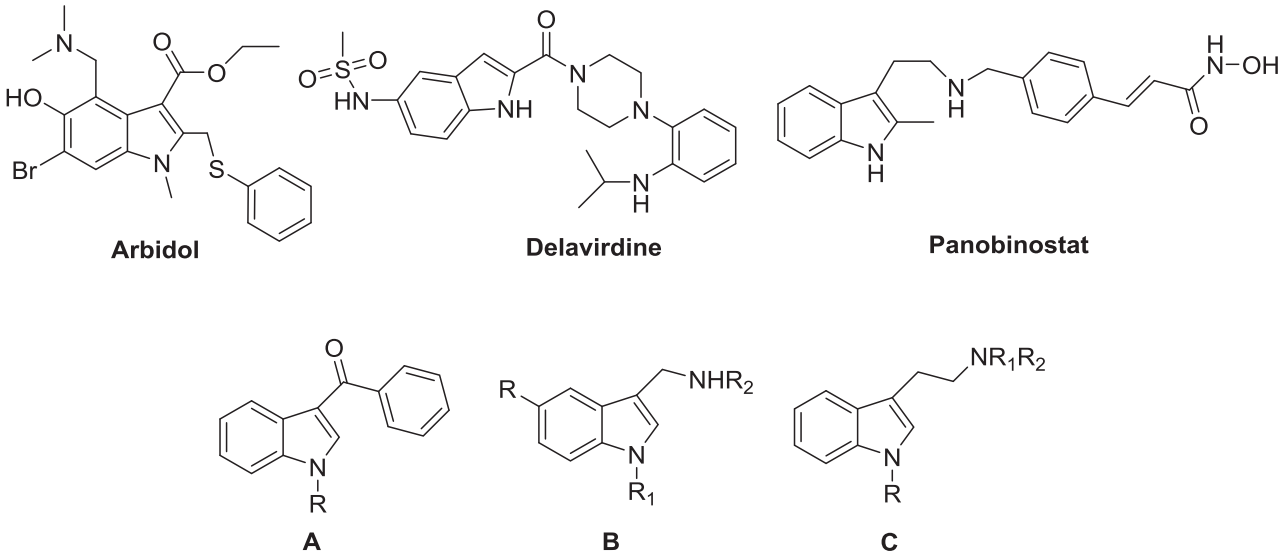
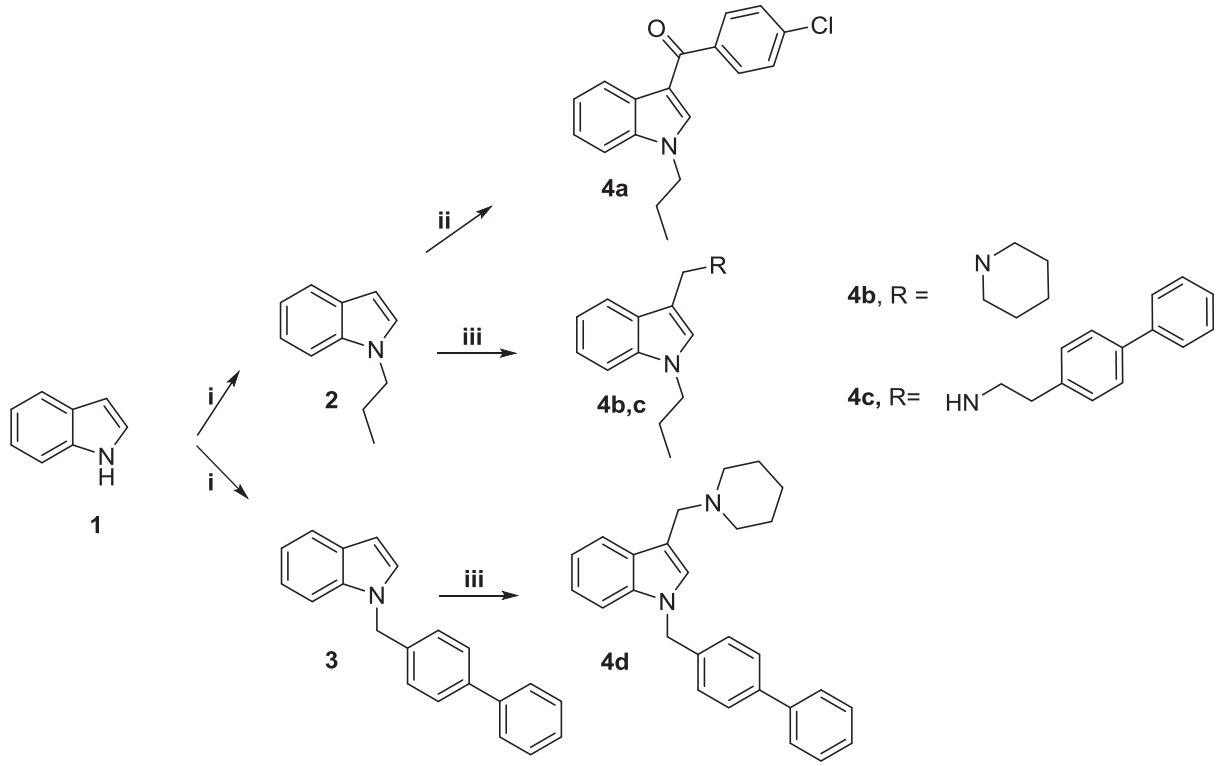
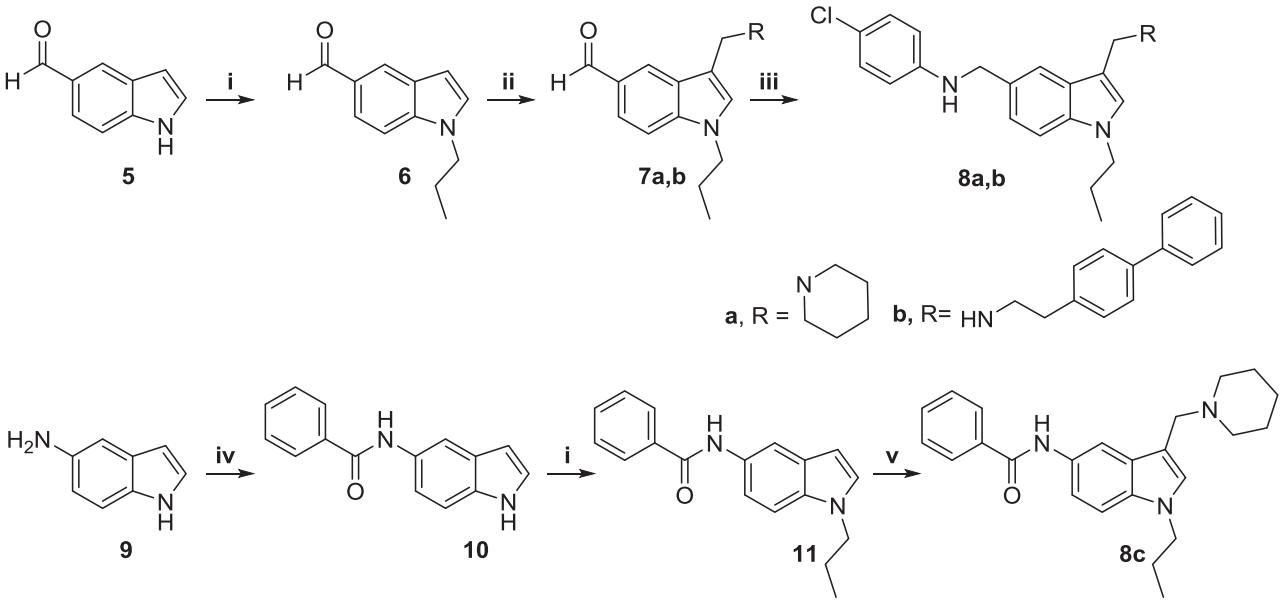


Fig. 1. Indole-containing antiviral compounds. Structure of indole (A, B) and tryptamine (C) derivatives described in this work.



Scheme 1. Synthesis of N- substituted-3- acyl or 3-methylamine indole derivatives **4a-d**. [a] Reagents and conditions, i: NaH, n-propyl iodide or 4-phenylbenzyl iodide, DCM/DMF, r.t, ii: $AlCl_3$, 4-chlorobenzoyl chloride, CH_3CN. iii: CH_2O, TFA, RNH_2, DCM, r.t.

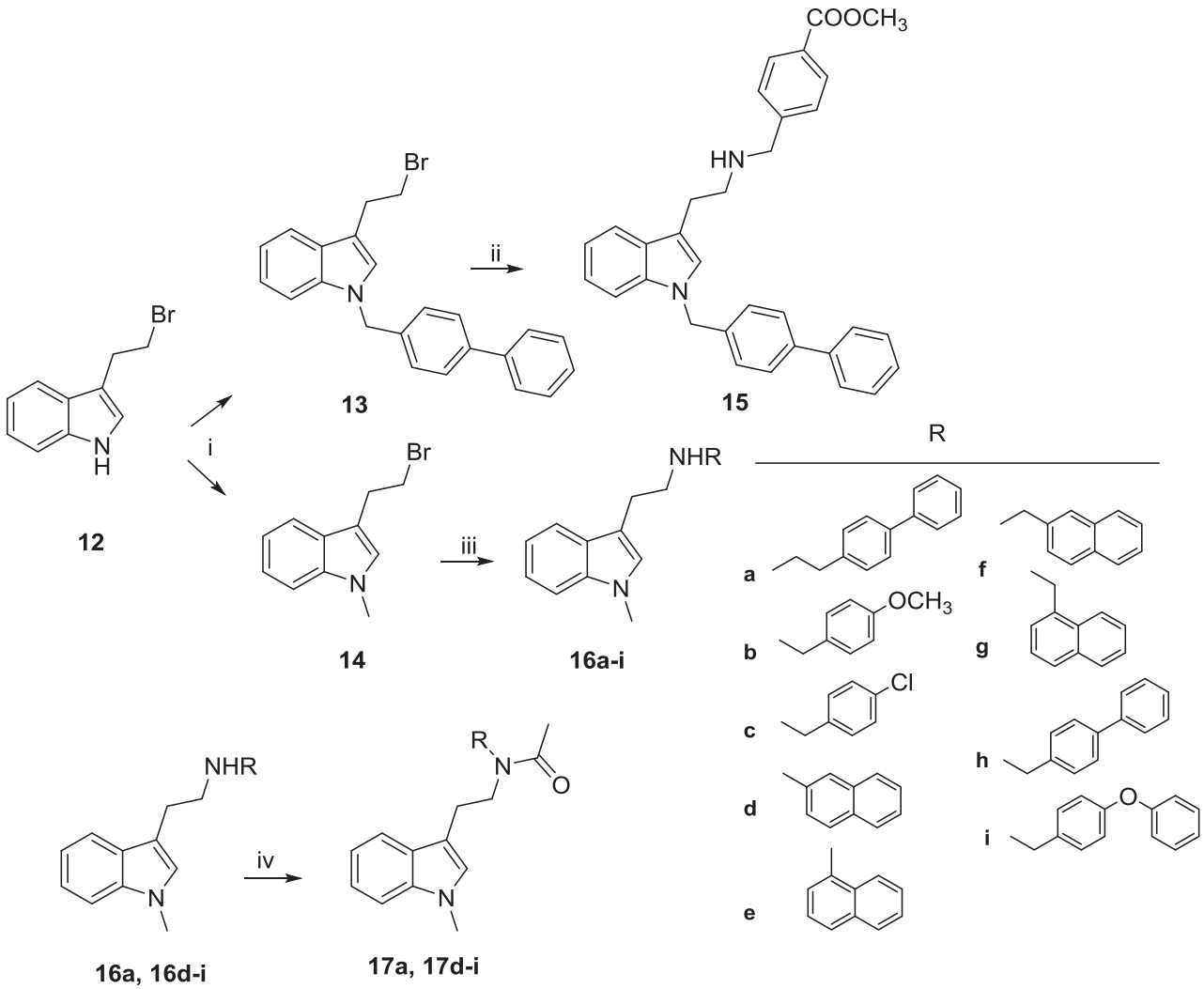


Scheme 2. Synthesis of 5-Substituted-3-methylamine indole derivatives **8a-c.** [a] Reagents and conditions, i: NaH, n-propyl iodide, DCM/DMF, r.t; ii: CH2O, TFA, piperidine or biphenylethylamine, DCM, r.t; iii: 4-Cl aniline, DCM/CH3COOH, 1,5 h reflux, then (CH3COO)3BH4, 3–5 h, r.t iv: HOBt, HBTU, DIPEA, benzoic acid, DCM/DMF, v: CH2O, TFA, piperidine, DCM, r.t.

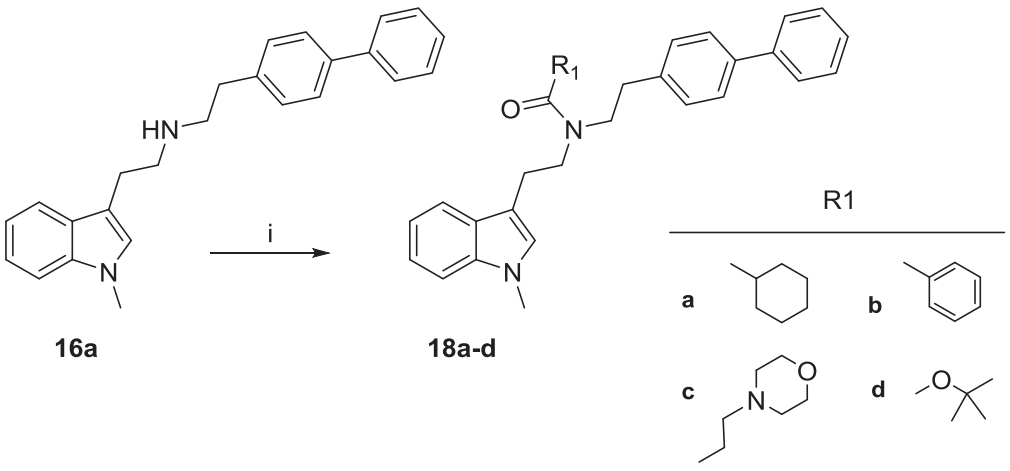
The acyl derivatives **17**, except **17d** and **17e**, and **18** were obtained as a mixture of rotamers due to the atropisomerism of the amide function, as confirmed by ROESY experiments, which showed exchange cross-peaks between the hydrogen signals of the two conformers (as examples see NMR spectra of compounds **17f** and **17 d** in Supporting Information) [38,39].

2.2. Antiviral activity

First, compounds **4**, **8**, and **15, 16** and **17a** were evaluated for their ability to inhibit the replication of VZV in human embryonic lung (HEL) cells, and the results were compared to those obtained for the reference compounds acyclovir and brivudin (Table 1). The



Scheme 3. Synthesis of tryptamine derivatives **15, 16** and **17**. a Reagents and conditions, i: NaH, n-propyl iodide or 4-phenylbenzyl iodide, DCM/DMF, r.t; ii: methyl 4-(aminomethyl) benzoate, (CH3COO)2Pd, TEA, THF, μW 100 °C, 20′ iii: R'NH2 CH3COO)2Pd, TEA, THF, μW 100 °C, 20′, iv ClCOCH3, TEA, DCM, 30′, r.t.

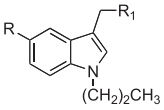
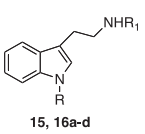
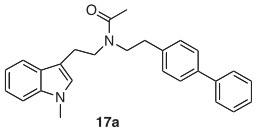


Scheme 4. Synthesis of acyl derivatives **18a-c**. [a] Reagents and conditions, i: ClCOR or Boc2O, TEA, DCM, 30′-24 h, r.t.

antiviral activity was expressed as EC50, being the compound concentration required to reduce virus-plaque formation (VZV) by 50%.

As shown in Table 1, the N-propyl-3-acyl (**4a**) and N-propyl-3-piperidine methyl (**4b**) derivatives do not displayed antiviral or cytotoxic activities at 100 μM, while the substitution of piperidine

Table 1
Activity of compounds **4**, **8, 15, 16** and **17a** against varicella-zoster virus (VZV) in human embryonic lung (HEL) cells.

Comp.	R	R_1	EC_{50} (μM)[a]			CC_{50} (μM)[b]
			TK^+	TK^-		
			OKA	07–1	YS-R	
						
4a	Propyl	Phenylcarbonyl	>100	>100	nd	>100
4b	Propyl	Piperidinemethyl	>100	>100	nd	>100
4c	Propyl	Biphenylethylaminemethyl	>0.8	>0.8	nd	1.7
4d	Biphenylmethyl	Piperidinemethyl	>4	>4	nd	6.9
						
8a	4-Ph-$NHCH_2$	Piperidine	>4	>4	nd	15
8b	4-Ph-$NHCH_2$	Biphenylethylamine	>4	>4	nd	9.7
8c	Ph-CO-NH	Piperidine	>20	>20	nd	31
 15, 16a-d  17a						
15	Biphenyl methyl	Biphenyl ethyl	nd	>4	>4	7.3
16a	Methyl	Biphenyl ethyl	>0.8	>4	nd	20
16b	Methyl	4-(OCH_3)benzyl	>20	>20	nd	28
16c	Methyl	4-(Cl) benzyl	>4	>4	nd	22
16d	Methyl	2-naphtyl	>100	>100	nd	41
17a (1)[c]			3.6	3.1	nd	31
17a (2)			2.5	1.7	2.1	39
Acyclovir (1)			2.1	26		224
Acyclovir (2)			1.9	165	nd	191
Brivudin (1)			0.019	33		300
Brivudin (2)			0.035	103	nd	160

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU).
[b] Cytotoxic concentration required to reduced cell growth by 50%.
[c] Experiment number.

at the 3-position by a biphenylethylamine group and of the propyl group at the N-1 indole by a biphenylmethyl moiety dramatically increased the cytotoxic activity of resultant compounds **4c** and **4d**, with CC50 values of 1.7 and 6.9 μM, respectively. Compounds **8 a-c**, derivatized at the C-5 indole position, proved less cytotoxic, CC50 ≈ 10–30 μM, but were also ineffective as inhibitors of VZV-induced plaque formation at similar concentrations.

The most interesting results were obtained for the tryptamine derivatives. In this series, the presence of a methyl group at the N-indole position lead to weaker cytotoxic derivatives (**16a-d** *versus* **15**, CC50 20–41 *versus* 7.3 μM), while the acylation of the amine group (**17a**) also reduces cytotoxicity (CC50 ≈ 31–39 μM) but with an inhibitory effect on the anti-VZV activity. Tryptamine derivative **17a** was indeed able to inhibit replication of TK+ and TK- VZV strains with EC50 values in the range of 1.7–3.6 μM (Table 1). The potency of **17a** against OKA strain (EC50 = 2.5–3.6 μM) was found comparable to that of the reference drug acyclovir (EC50 = 1.9–2.1 μM) but two orders of magnitude lower than that of brivudin (EC50 = 0.02–0.03 μM). However, the activity of this compound against thymidine kinase-deficient VZV strains, both 07-1 and YS-R, was maintained in the micromolar range with EC50 = 1.7–3.1 μM being, at least, one order of magnitude more active than acyclovir (EC50 = 33–103 μM) and brivudin (EC50 = 26–165 μM).

The activity of these compounds against other members of the *Herpesviridae family* was tested in HEL cell cultures. As shown in Table 2 none of these compounds was found active either towards cytomegalovirus (HCMV, AD-169 strain and Davis strain) or against herpes simplex virus-1 (HSV-1; KOS and thymidine kinase-deficient acyclovir-resistant) (HSV-1/TK- KOS ACVr strains) or against herpes simplex virus-2 (HSV-2; G strain).

The compounds were not active against other DNA viruses such as vaccinia virus, adenovirus-2, and feline herpesvirus. All these compounds also lacked inhibitory activity against a broad variety of RNA viruses, including HIV-1 and HIV-2, feline coronavirus, vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus, para-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, influenza A virus (H1N1 and H3N2 subtypes) and influenza B virus (data not shown).

To further explore the SAR within the tryptamine series, we next prepared a second set of **17a** analogs featuring a) a modified aromatic moiety linking the acetamide group (compounds **17 d-i**) and b) a modified acyl group (compounds **18a-d**).

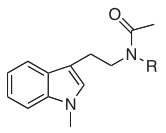
As shown in Table 3, substitution of biphenyl ethyl moiety by the more rigid naphthyl (compounds **17d**, **17e**) or their superior analog naphthylmethyl groups (**17f**, **17g**), as well as, the introduction of a biphenylmethyl or phenyloxybenzyl moieties (**17h**, **17i**) led to a complete loss of the antiviral activity. However, compounds containing 1-naphtyl (**17e**) or 1-naphtyl methyl (**17g**) groups or the inferior analog biphenylmethyl group (**17h**) were less cytotoxic. On the other hand, substitution of the acylmethyl group by bulkier alkyl groups such as cyclohexyl, morpholinethyl and *tert*-butyloxy

Table 2
Activity of compounds **4**, **8**, **15**, **16**, and **17a** against cytomegalovirus (HCMV), herpes simplex virus-1 (HSV-1) and herpes simplex virus-1 (HSV-2) in human embryonic lung (HEL) cell cultures.

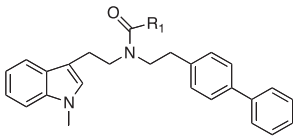
Comp.	Antiviral activity EC50 (μM)[a]					Cytotoxicity (μM)
	HMCV		HSV-1		HSV-2	MMC[b]
	AD-169	Davis	(KOS)	TK^- KOS ACV^r	(G)	
4a	nd	>100	>100	>100	>100	>100
4b	>4	>4	>100	>100	>100	>100
4c	nd	>0.16	>100	>100	>100	4
4d	nd	>4	>20	>20	>20	20
8a	nd	>4	>20	>20	>20	20
8b	nd	>4	>4	>4	>4	20
8c	nd	>20	>100	>100	>100	100
15	>4	>4	>100	>100	>100	20
16a	>4	>4	>100	>100	>100	>4
16b	>20	>20	>20	>20	>20	100
16c	>20	>20	>20	>20	>20	20
16d	76	100	>100	>100	>100	>100
17a	>4	>4	>100	>100	>100	20
Ganciclovir	3.1	2.1	0.09	100	0.03	>100
Cidofovir	0.38	0.51	0.4	1.2	0.7	>250
Acyclovir			0.08	112	0.2	>250
Brivudin			0.007	50	96	>250

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque-forming units (PFU).
[b] Maximum cytotoxic concentration that cause a microscopically detectable alteration of morphology.

Table 3
Activity of acyl analogs **17** and **18** against varicella-zoster virus (VZV) in human embryonic lung (HEL) cells..



17a, 17d-i



18a-d

Compounds	R	R_1	EC_{50} (μM)[a]		MCC (μM)[b]
			TK+	TK-	
			OKA	07–1	
17a	Biphenylethyl	Methyl	2.6	2.0	35
17d	2-naphtyl		>4	>4	20
17e	1-naphtyl		>20	>20	100
17f	2-naphtylmethyl		>4	>4	20
17g	1-naphtylmethyl		>20	>20	100
17h	Biphenylmethyl		>20	>20	100
17i	Phenyloxybenzyl		>4	>4	20
18a		Cyclohexyl	>4	>4	20
18b		Phenyl	20	20	>100
18c		Morpholinethyl	>4	>4	20
18d		t-butyloxy	>4	>4	20
Acyclovir			3.8	145	>440
Brivudin			0.026	143	>300

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU).
[b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.

groups (compound **18a, 18c, 18d**) led to an annihilation of antiviral activity. In this series of compounds, only the benzoyl derivative **18b** somewhat maintains the antiviral activity at an EC_{50} of 20 μM, displaying also a reduced cytotoxic activity (MMC >100 μM).

3. Conclusions

The identification of a new family of non-nucleoside anti-VZV agents based upon the indole template has been reported. We have shown that the substitution on the tryptamine moiety strongly influences the activity/toxicity of these compounds. In particular, the concomitant presence of a biphenylethyl and a methyl(phenyl) carboxy group at the amino group of tryptamine is a requisite for antiviral activity. In fact, the N-biphenylethyl acetamide derivative **17a** displayed an interesting inhibition against VZV and was endowed with a selectivity of 10–20 (ratio CC50/EC50). It must be also considered that **17a** showed similar activity against TK+ and TK- VZV strains, thus indicating a mechanism of action independent from the virus-encoded thymidine kinase. Additionally, this compound is able to exert antiviral activity exclusively on VZV, being totally inactive against other members of *Herpesviridae family* as well as against a wide variety of RNA virus strains suggesting a selective action on a specific antiviral target untargeted by the currently existing compounds. The possible mechanism of action of this novel class of compounds will be further investigated.

4. Experimental section

4.1. Chemistry

Reagents, starting materials, and solvents were purchased from Sigma-Aldrich (Milan, Italy) and used as received. Reactions were carried out with magnetic stirring in round-bottomed flasks unless otherwise noted. Moisture-sensitive reactions were conducted in oven-dried glassware under a positive pressure of dry nitrogen, using pre-dried, freshly distilled solvents. Microwave assisted reactions were performed in a Biotage Initiator + reactor. Analytical thin layer chromatography (TLC) was performed on pre-coated glass silica gel plates 60 (F254, 0.25 mm, VWR International). Purifications were performed by flash column chromatography on silica gel (230–400 mesh, Merck Millipore). NMR spectra were recorded on Varian Mercury-400 apparatus. 1HNMR and 13C NMR spectra were recorded with a Varian-400 spectrometer, operating at 400 and 100 MHz, respectively. Chemical shifts are reported in δ values (ppm) relative to internal Me4Si, and J values are reported in hertz (Hz). The following abbreviations are used to describe peaks: s (singlet), d (doublet), dd (double double), t (triplet), q (quadruplet) and m (multiplet). ESI-MS experiments were performed on an Applied Biosystem API 2000 triple-quadrupole

spectrometer. Combustion microanalyses were performed on a Carlo Erba CNH 1106 analyzer, and were within 0.4% of calculated values and confirmed >95% purity for the final products.

4.1.1. General procedure for the synthesis of N-alkylated indole intermediates (**2, 3, 6, 11, 13** and **14**)

Indole (**1**, 1.0 eq) or 1H-indole-5-carbaldehyde (**5**, 1.0 eq), or N-(1H-indol-5-yl)benzamide (**10**, 1.0 eq), or 3-(2-Bromoethyl)indole (**13**, 1.0 eq.) were dissolved in a mixture of anhydrous DCM/DMF (2/1 v/v) under magnetic stirring and the temperature was set to 0 °C. To this solution, 1.5 equivalents of NaH were added portionwise and the mixture was allowed to react for 30 min. Then, 1.5 equivalents of alkyl iodide [methyl iodide, or n-propyl iodide, or 4-(phenyl)iodomethylbenzene] in DCM were added dropwise and the reaction was warmed to room temperature and maintained under stirring for further 12 h. The reaction was then quenched by a 10% aqueous solution of citric acid and washed with brine. The organic layer was separated, dried over anhydrous Na2SO4, filtered and evaporated *in vacuo*. Crude products were purified by column chromatography using *n*-hexane/ethyl acetate (4:1 v:v) as mobile phase. N-alkylated compounds were obtained in 75% yield (derivative **2**); 80% yield (derivatives **3** and **13**); 67% yield (derivative **6**); 55% yield (derivative **11**) and 50% yield (derivative **14**).

4.1.2. Synthesis of derivative **4a**

To a solution of 1-propyl-1H-indole (**2**) in dry CH3CN was added aluminium trichloride (15 eq) and benzoyl chloride (10 eq), the reaction was stirred at room temperature overnight. The resulting mixture was dried *in vacuo* and reconstituted in DCM. The organic phase was washed with water (3 × 50 mL), dried over anhydrous Na2SO4, filtered, concentrated and purified by column chromatography using *n*-hexane/ethyl acetate (3/2) as mobile phase. Compound **4a** was obtained in 32% yield.

4.1.3. Synthesis of derivatives **4b-d**

A solution of formaldehyde (2 eq.), trifluoroacetic acid (2 eq.) and 2 equivalents of the proper amine (piperidine or bifenylethilamine) was added portionwise to a solution of the intermediate **2** or **3** (1 eq.) in DCM. The mixture was stirred for 3 h at room temperature, then was washed with water (3 × 50 mL), dried over anhydrous Na2SO4, filtered, concentrated and purified by column chromatography using DCM/MeOH (9:1 v/v) as mobile phase. Compounds **4b-d** were obtained in 25%–38% yield.

4.1.4. Synthesis of derivatives **8a-b**

The intermediate **7a** or **7b** (1.0 eq) was dissolved in a solution of DCM:CH3COOH (5:1 v/v) at room temperature. To this solution 2.0 equivalents of 4-chloroaniline was added and the mixture was warmed to reflux for 1.5 h. Then, 1.8 equivalents of sodium triacetoxyborohydride were added portionwise and the mixture was allowed to reflux for further 3–5 h. After cooling to room temperature, NaOH 1 N was added. The organic phase was separated and extracted one more time with the alkaline solution. Then it was dried over Na2SO4, filtered and concentrated *in vacuo*. The crude products were purified by column chromatography using mixtures of DCM/MeOH as eluent giving desired compounds **8a** and **8b** in 24% and 30% yield respectively.

4.1.5. Synthesis of derivative **8c**

To a solution of 5-amino indole (**9**, 1.0 eq) in dichloromethane/DMF (20 mL/5 mL) were successively added benzoic acid (1.1 eq), HBTU (1.2 eq), HOBt (1.2 eq), and DIEA (2.4 eq), and stirring was continued at room temperature for 24 h. Afterward, the reaction mixture was diluted with dichloromethane (20 mL), and the resulting solution was washed successively with 10% citric acid (2 × 25 mL), 10% NaHCO3 (2 × 25 mL), and water (2 × 25 mL), dried over Na2SO4, and evaporated to dryness. Intermediate **10** was obtained after flash chromatography using *n*-hexane/ethyl acetate 2/1 as ratio with 63% yield. Substitution with propyl group of indolic N1 was carried out using the same conditions described elsewhere, to give compound **11** with 44% yield. Mannich reaction performed on position 3 of intermediate **11** follows the procedure previously described yielded final compound **12** in 24% yield.

4.1.6. General procedure for the synthesis of derivatives **15,** and **16a-i**

One equivalent of intermediate **14a** or **14b** was dissolved in THF and 1.5 eq of the proper amine, 1.5 eq of TEA, 1.5 eq of NaI and 0.3 eq of (CH3COO)2Pd were added to this solution. The reaction was conducted under μW, at 100 °C, for 20 min. The resulting mixture was filtered through Celite, dried *in vacuo* and reconstituted in DCM. The organic phase was washed with water (3 × 50 mL), dried over anhydrous Na2SO4, filtered, concentrated and purified by column chromatography using DCM/MeOH (9:1 v/v) as mobile phase giving derivative **15** in 38% yield and intermediates **16a-i** in 55–75% yield.

4.1.7. General procedure for the synthesis of derivatives **17a-i** and **18a-c**

One equivalent of intermediate **16a-i** was dissolved in DCM and 1.5 eq of the proper acyl chloride and 1.5 eq of TEA, were added to the solution. The reaction was conducted for 20 min at room temperature. The organic phase was washed with water (3 × 50 mL), dried over anhydrous Na2SO4, filtered, concentrated and purified by column chromatography using *n*-hexane/ethyl acetate (2:1 v/v) as mobile phase. Final derivatives **17a-i** and **18a-d** were obtained as an atropisomer mixtures in 42–58% yield.

4.1.8. Synthesis of derivative **18d**

To a mixture of 16a (1.0 eq.) in DCM, (Boc)2O (3.0 eq.) was added followed by TEA (1.5 eq.). The mixture was allowed to stir at room temperature for 12 h. Afterward, the reaction mixture was diluted with dichloromethane (20 mL), and the resulting solution was washed with 10% citric acid (2 × 25 mL). The organic phase was dried over Na2SO4, and evaporated to dryness. Compound 17 k was obtained as atropisomer mixture after flash chromatography using *n*-hexane/ethyl acetate 2/1 in 58% yield.

4.2. Antiviral assays

The compounds were evaluated against different herpes viruses, including varicella-zoster virus (VZV) strains Oka and YS, TK^- VZV strains 07-1 and YS-R, herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strain G, human cytomegalovirus (HCMV) strains AD-169 and Davis as well as feline herpes virus (FHV), the poxvirus vaccinia virus (Lederle strain), adenovirus-2, parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, respiratory syncytial virus (RSV), feline coronavirus (FIPV) and influenza A virus subtypes H1N1 (A/PR/8), H3N2 (A/HK/7/87) and influenza B virus (B/HK/5/72) and human immune deficiency virus (HIV-1 (III_B) and HIV-2 (ROD)). The antiviral assays were based on inhibition of virus-induced cytopathicity (CPE) or (VZV) plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey kidney cells (Vero), human epithelial cervix carcinoma cells (HeLa), human CD_4^+ T-lymphocyte cells (CEM), Crandell-Rees feline kidney cells (CRFK), or Madin Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100

$CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 VZV plaque forming units (PFU) and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral CPE or plaque formation (VZV) was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50}, the concentration required to reduce virus-induced cytopathicity or viral plaque formation by 50%.

4.3. Cytotoxicity assays

Cytotoxicity measurements were based on the inhibition of HEL cell growth. HEL cells were seeded at a rate of 5 × 103 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter. The 50% cytostatic concentration (CC50) was calculated as the compound concentration required to reduce cell growth by 50% relative to the number of cells in the untreated controls. CC50 values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Cytotoxicity was expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that causes a microscopically detectable alteration of cell morphology.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2016.09.014. These data include MOL files and InChiKeys of the most important compounds described in this article.

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