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Research paper

Perylenyltriazoles inhibit reproduction of enveloped viruses

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ABSTRACT

1-Substituted 4-perylen-2(3)-yl-1,2,3-triazoles, easily accessible by 'click' reaction and combining in one molecule a polyaromatic unit and a nitrogen heterocycle, were found to strongly inhibit the reproduction of enveloped viruses. 5-[4-(Perylen-3-yl)-1,2,3-triazol-1-yl]-uridine and 2-[1-(2-hydroxyethyl)-1,2,3-triazol-4-yl]perylene show EC_{50} of 0.031 and 0.023 μM, respectively, against tick-borne encephalitis virus (TBEV). Remarkably, the nucleoside unit appears to be not essential for antiviral activity. These results provide deeper understanding of structural basis of activity for this new class of antivirals.

1. Introduction

Enveloped viruses, bearing a lipid membrane bilayer around the nucleocapsid, cause many severe diseases in humans (hemorrhagic fevers, hepatitides, encephalitides, pneumonias, etc.). Small molecules targeting viral membrane and membrane fusion process present a modern paradigm of broad spectrum antivirals with decreased possibility for resistance development, since viral membrane originates from host membranes [1,2]. Earlier, we discovered rigid amphipathic fusion inhibitors (RAFIs), 5-arylethynyl uracil nucleosides displaying high inhibitory activity against a number of enveloped viruses and acting by arrest of fusion between viral and cellular membranes [3,4]. Perylen-3-yl as aryl is the prerequisite for high antiviral activity of RAFIs, e.g. **dUY11** and **aUY11** (Fig. 1) showed the most potent inhibition of viral reproduction in cells infected by herpes simplex virus types 1 and 2, hepatitis C virus, influenza A virus, etc. [3,4]. Later, antiviral action of **dUY11** was also found to be light-dependent and its association with singlet oxygen production has been suggested [5]. Indeed, naturally occurring polyketides hypericin [6–17] and hypocrellin [18–22] containing perylenequinone motif (Fig. 1) have light-dependent activity against enveloped viruses. Hypericin produces singlet oxygen [23,24], including when bound to lipid vesicles [25]. On the other hand, the antiviral effect of hypericin was initially reported to be oxygen-independent [13,19], but later the statement was revised [15]. Nevertheless, the actual mechanism of antiviral action of hypericin is still not determined unequivocally [16,17]. Apparently, multiple modes of action are possible for perylene compounds, though broad spectrum activity against a number of enveloped viruses suggests involvement of some membrane-associated events. We synthesized derivatives and analogues of **dUY11** to elucidate structural basis for broad spectrum antiviral action of perylene RAFIs.

Recently, we found that RAFIs had a high activity against tick-borne encephalitis virus (TBEV) *in vitro* [26]. TBEV is an

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enveloped virus, a typical member of *Flavivirus* genus with clear epidemiological relevance [27]. Perylene RAFIs **dUY11** and **aUY11** with EC_{50} values ca. 0.02 μM [26] outperform other reported nucleoside [28–32] and non-nucleoside [33–35] TBEV inhibitors. Thus, we used TBEV as a model virus for testing new perylene compounds.

Our aim was to study further the impact of the rigid linker (Fig. 1) in perylene RAFIs on their antiviral activity. Insertion of a flexible CH_2OCH_2 unit between the ethynyl group and perylene moiety dramatically reduces the antiviral effect [3,26,36]. Hence, the idea was to replace ethynyl with another rigid linker. 1,2,3-Triazol-1,4-diyl unit emerging from stereospecific Cu(I)-catalyzed Huisgen–Meldal–Sharpless azide alkyne cycloaddition is widely used as an amide isostere [37,38]. We substituted a triple bond in RAFI-type perylene compounds with this heterocycle to break the linear geometry of perylene-to-uracil linker while keeping the molecule considerably rigid. Here we report the synthesis and antiviral activity of triazolylperylene compounds.

2. Results and discussion

2.1. Chemistry

Whereas 5-azidouracil nucleoside derivatives had been sporadically 'clicked' with terminal alkynes [39–41], it was never done with ethynylperylenes. The synthesis is shown in Scheme 1.

5-Bromo nucleosides **1** were aminated with benzylamine and 5′-O silyl protected to yield compounds **2**. The latter were hydrogenated to 5-amino compounds followed by diazotization/azidation [42] into azides **3**. Along with 3-ethynylperylene, we used in the 'click' reaction recently obtained [43] 2-ethynylperylene. Phenyl derivatives **4** were prepared to confirm the vital necessity of perylene residue for antiviral activity. 5′-O TBDMS protection makes 'click' products **4**, **5**, and **6** suitable for the work-up and chromatographic purification. The final deprotection produced the desired triazolyl-linked nucleosides **7**–**9**. We prepared model compounds **10a,b** by 'clicking' ethynylperylenes with 2-azidoethanol to figure out the role of the uracil heterocycle. In perylenyltriazoles **10a,b** the nucleoside part is completely eliminated by replacement with 2-hydroxyethyl group. The latter has no aromatic or rigid parts, but retains a polar hydroxyl group for keeping the amphipathic character of the whole molecule. The reaction conditions for transformations shown in Scheme 1 were not optimized for maximum yields.

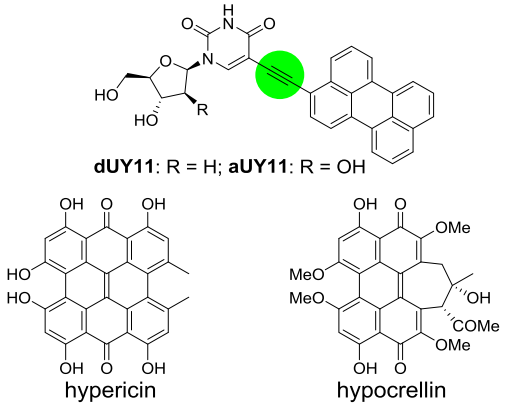


Fig. 1. Structures of broad spectrum antivirals. The marked ethynyl group is replaced with 1,2,3-triazol-1,4-diyl in this study.

2.2. Antiviral activity

Antiviral activity of triazolyl compounds was studied on porcine embryo kidney (PEK) cell line against TBEV strain Absettarov (GenBank ID KU885457) (Table 1). All the compounds were non-toxic for PEK cells in the concentrations used.

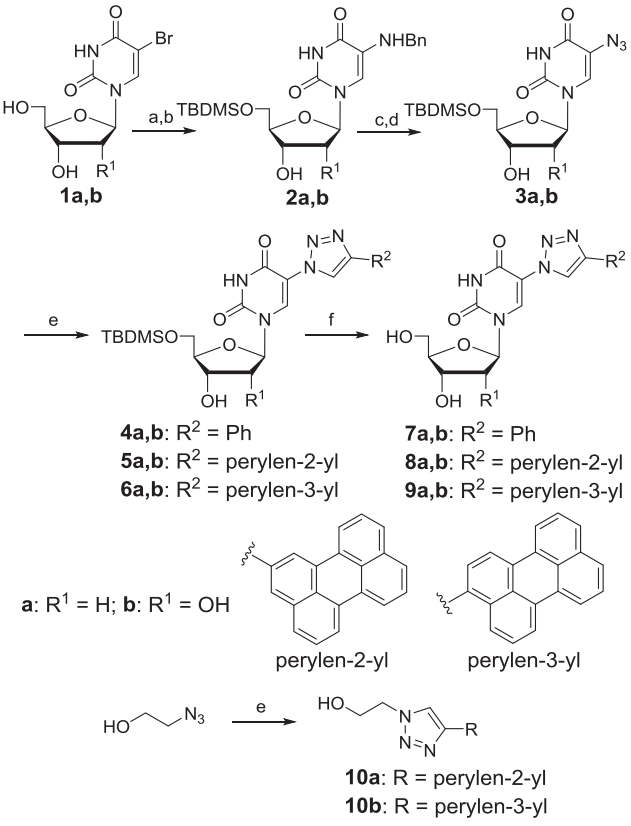
As expected, compounds **7a,b** lacking perylene moiety showed no antiviral activity. In contrast, all perylene compounds appeared to have high (submicromolar) inhibitory activity (Table 1), greatly exceeding recently reported anti-TBEV nucleosides [28–32] or other [33–35] TBEV inhibitors. The best results showed **9b** and **10a,** whose activity values were close to that of **dUY11**, which had been the most potent TBEV reproduction inhibitor reported to date [26].

Remarkably, there was no apparent relation between antiviral activity and perylene substitution position; one of the most active molecules (**9b**) contained a nucleoside residue (sugar + nucleobase) together with 3-substituted perylene, and another one (**10a**) did not contain a nucleoside residue at all and was a perylen-2-yl derivative.

Our preliminary observations showed that compounds **8**–**10** also expressed potent activity against hantaviruses, also enveloped viruses, thus confirming the broad spectrum of compounds' antiviral action (the data will be reported elsewhere).

2.3. Spectral properties

Photochemical destruction of viral membrane by reactive oxygen species generated by RAFIs requires light absorption [5].



Scheme 1. Synthesis of nucleoside and non-nucleoside perylenyltriazolyl compounds. Reagents and conditions: (a) $BnNH_2$, 90 °C, 3 h; (b) TBDMS-Cl, Py, 81% (**2a**), 64% (**2b**); (c) H_2, Pd/C, MeOH; (d) 1. $NaNO_2$, 80% AcOH; 2. NaN_3, 63% (**3a**), 46% (**3b**); (e) alkyne, CuI, TBTA, THF, 69% (**4a**), 84% (**4b**), 77% (**5a**), 88% (**5b**), 50% (**6a**), 58% (**6b**), 86% (**10a**), 81% (**10b**); (f) TBAF, DMSO, THF, 45% (**7a**), 48% (**7b**), 60% (**8a**), 65% (**8b**), 78% (**9a**), 84% (**9b**).