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Original article

Molecular design, synthesis and biological evaluation of BP-*O*-DAPY and *O*-DAPY derivatives as non-nucleoside HIV-1 reverse transcriptase inhibitors

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ABSTRACT

This paper reports the synthesis and antiviral evaluation of a series of non-nucleoside reverse transcriptase inhibitors (NNRTIs) that combine the peculiar structural features of diarylpyrimidine derivatives (DAPYs) and benzophenone derivatives (BPs). The DAPY derivatives bearing benzoyl or alkoxyl substitutes on the A-ring showed the inhibitory activity against wild-type HIV-1 at the cellular level within the range of EC_{50} values from micromolar to nanomolar. Among these compounds, **1u** exhibited the most potent anti-HIV-1 activity ($EC_{50} = 0.06 \pm 0.01$ μM, SI > 6260), which were about 1.8-fold more active than nevirapine (NVP) and delavirdine (DLV). In addition, the binding modes with HIV-1 RT and the preliminary SAR studies of these derivatives were also considered for further investigation.

1. Introduction

Human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT) is a key enzyme in the HIV replicative cycle. It represents one of the main targets for the treatment of HIV/AIDS. Nowadays, thirteen RT inhibitors have been approved by the United States Food and Drug Administration (USFDA) for clinical use as anti-HIV drugs, among which the non-nucleoside RT inhibitors (NNRTIs, Fig. 1) are nevirapine (NVP), delavirdine (DLV), efavirenz (EFV), etravirine (TMC125) and rilpivirine (TMC278) [1–5]. The NNRTIs can directly inhibit HIV reverse transcriptase enzyme by binding to an allosteric site, located around 10–15 Å away from the catalytic site [6]. With the group of the NNRTIs, the emergence of RT mutations rapidly confers resistance to the first-generation NNRTIs, such as the Y181C mutation associated with NVP resistance and the K103N mutation associated with EFV resistance [7]. However, the diarylpyrimidine (DAPY) analogs, represented by TMC125 and TMC278, have been identified as highly potent second-generation NNRTIs [8]. It was found that NVP and DLV inhibited K103N/Y181C RT mutants at a range of $EC_{50} > 10$ μM to $EC_{50} > 100$ μM in cell-based assays, while TMC125 and TMC278 could inhibit K103N/Y181C RT mutants at an EC_{50} value of 5 nM and 0.8 nM, respectively [9].

The crystal structures of HIV-1 RT/DAPY complexes and/or molecular modeling studies have revealed some important features of enzyme–ligand interaction, helping to maintain the antiviral activity against a wide range of resistance mutations [10,11]. Although DAPYs shared a similar pharmacophore including hydrophobic center, hydrogen bond donor and acceptor as mentioned for the first-generation NNRTIs (NEV, DLV and EFV), the binding conformation of DAPYs resembled a horseshoe or "U" shape when

Abbreviations: NNRTIs, non-nucleoside reverse transcriptase inhibitors; DAPYs, diarylpyrimidine derivatives; BPs, benzophenone derivatives; NVP, nevirapine; DLV, delavirdine; HIV-1 RT, Human immunodeficiency virus type 1 reverse transcriptase; USFDA, United States Food and Drug Administration; NNRTIs, non-nucleoside RT inhibitors; EFV, efavirenz; TMC125, etravirine; TMC278, rilpivirine; NNIBP, non-nucleoside inhibitor binding pocket; wt, wild-type; BPs, benzophenone derivatives; SI, selectivity index; AZT, zidovudine; ESI, electrospray ionization; TMS, tetramethylsilane; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide; $CCID_{50}$, 50% cell culture infectious dose; OD, optical density; CC_{50}, 50% cytotoxic concentration; EC_{50}, 50% effective concentration; K_d, dissociation constant; IC_{50}, 50% inhibitory concentration.

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bound in the non-nucleoside inhibitor binding pocket (NNIBP) [9,10]. For instance, in the molecule of TMC125, the ether and amino linkages of the two cyanophenyl substituents provide sufficient flexibility to allow strong π–π stacking interactions with Y181, Y188, F227 and W229 [10]. It was suggested that DAPY NNRTIs bind to RT through torsional flexibility ("wiggling") and repositioning flexibility ("jiggling") [9]. This concept proposes a general strategy for designing flexible drugs to target a variety of resistant mutants. Recently, some novel DAPY analogs [8,12] and triazine derivatives [13] have also been investigated as NNRTIs, whereas these analogs did not show highly potent antiviral activity against clinically relevant mutant strains.

In addition, numerous structurally different non-nucleoside compounds have also been investigated for inhibitory effects against HIV replication. A class of novel NNRTIs, benzophenone derivatives (BPs) was developed starting from a lead with moderate activity against wild-type (wt) HIV and little or no activity against clinically relevant mutants in a high-throughput screening [14,15]. For example, GW678248 (Fig. 1) exhibited very low EC_{50} values in antiviral assays: 0.5 nM against wild-type, 1 nM against K103N and 0.7 nM against Y181C, respectively [14]. Upon binding of GW678248 to RT, the B-ring is placed at the top hydrophobic pocket that is lined with the aromatic residues Y181, Y188, and W229, while the A-ring placed a "down" position and interacting with V179 via the *p*-chloro substituent on the A-ring [15]. Additionally, the interaction of the chloro substituent with the main-chain carbonyl of Y188 is also maintained [15]. As shown by the structure modifications of BPs, the benzophenone group was essential for maintaining high anti-HIV activity [15]. The RT-bound structure of BPs pointed to a number of features that aided in the design flexible drugs to target a variety of resistant mutants.

In our present study, we tried to combine the special structural features of diarylpyrimidines (DAPYs) and benzophenones (BPs) to develop new NNRTIs, which originated from the concept of molecular hybridization [16,17]. Twenty-one analogs (Fig. 2) were selected for synthesis and evaluation of *in vitro* antiviral activity against wt HIV-1, double mutant HIV-1 (K103N/Y181C) and HIV-2. The preliminary SAR studies and molecular docking analysis for further investigation are also discussed.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds (**1a**–**u**) is shown in Scheme 1. The key intermediates 4-(4-chloro-pyrimidin-2-ylamino)benzonitriles (**2a**–**c**) were synthesized according to our previous reported method in 3 or 5 steps [18]. The 4-chloro-hydroxybenzophenone derivatives (**3**) were prepared from 4-chloroanisole via Friedel–Crafts acylation [14], and the others are commercial available. Treatment of **2a**–**c** with appropriate hydroxybenzophenone or phenol derivatives (**3**) in the presence of K_2CO_3 in anhydrous DMF at 80 °C under nitrogen atmosphere afforded the desired compounds **1a**–**u**. The yields varied from 52.2% to 98.4%.

In the reaction with hydroxybenzophenone derivatives (**3**), the use of **2b**–**c** led to lower yields than that of **2a**, which could be due to the steric hindrance from the methyl group substituted at the pyrimidine ring. Besides the hydroxybenzophenone derivatives (**3**) listed in Scheme 1, the reaction was also tried by using 4-chloro-2-(2-chlorobenzoyl)phenol and 4-chloro-2-(2, 6-dichlorobenzoyl)phenol as reagents, whereas the reaction failed at 80 °C and gave only degradation unknown compounds. When carried out at lower temperature (25–50 °C), the reaction still did not work. Thus, we also introduced some phenol derivatives for comparison.

2.2. Biological activity

The antiviral activity and cytotoxicity of the synthesized compounds (**1a**–**u**) were tested in MT-4 cells. Their capacity to inhibit the replication of wild-type (wt) HIV-1 strain III_B, the double mutant HIV-1 strain RES056 (K103N + Y181C) and the HIV-2 strain ROD was evaluated [19,20]. The results, expressed as EC_{50}, CC_{50}, and SI (selectivity index) values are given in Table 1. FDA-approved drugs, including the NNRTIs NEV, EFV, DLV, and the NRTI zidovudine (AZT) were used as reference according to the same procedure. Additionally, the biological data of TMC125 evaluated by the same laboratory [12], and the previously reported antiviral data of

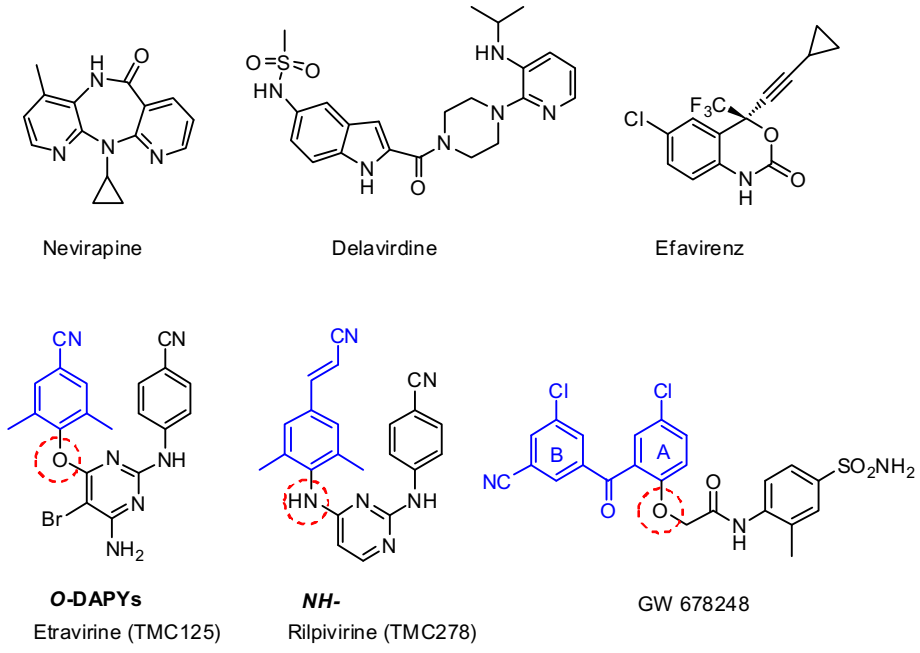

Fig. 1. Structures of currently FDA-approved clinical NNRTIs and novel benzophenone NNRTI derivatives (GW678248 as the example).