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Original article

Design, synthesis and anti-HIV evaluation of novel diarylnicotinamide derivatives (DANAs) targeting the entrance channel of the NNRTI binding pocket through structure-guided molecular hybridization

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ABSTRACT

Through a structure-based molecular hybridization approach, a novel series of diarylnicotinamide derivatives (DANAs) targeting the entrance channel of HIV-1 NNRTIs binding pocket (NNIBP) were rationally designed, synthesized and evaluated for their anti-HIV activities in MT-4 cells together with the inhibition against the reverse transcriptase (RT) in an enzymatic assay. Encouragingly, most of the new DANAs were found to be active against wild-type HIV-1 with an EC_{50} in the range of 0.027–4.54 μM. Among them, compound **6b11** (EC_{50} = 0.027 μM, SI > 12518) and **6b5** (EC_{50} = 0.029 μM, SI = 2471) were identified as the most potent inhibitors, which were more potent than the reference drugs nevirapine (EC_{50} = 0.31 μM) and delavirdine (EC_{50} = 0.66 μM). Some DANAs were also active at micromolar concentrations against the K103N + Y181C resistant mutant. Compound **6b11** exhibited the highest enzymatic inhibition activity (IC_{50} = 20 nM), which is equal to that of efavirenz (EC_{50} = 20 nM) and 31 times higher than that of nevirapine (EC_{50} = 0.62 μM). Preliminary structure-activity relationships (SARs) and molecular modeling of these new DANAs have been discussed.

1. Introduction

Acquired immune deficiency syndrome (AIDS), mainly caused by human immunodeficiency virus type-1 (HIV-1) is still a prevalent disease worldwide. The most common and effective treatment of AIDS is the highly active antiretroviral therapy (HAART). Non-nucleoside reverse transcriptase (RT) inhibitors (NNRTIs) are important components of HAART with high antiviral potency, high specificity and low cytotoxicity [1]. Even so, a major problem is that the long-term efficacy of NNRTIs is limited by the rapid emergence of drug-resistant variants of HIV-1. Hence, the development of novel chemical entities with high affinity for the mutated RT has been a very active research field in recent years [2–4].

Among the more than 50 different series of NNRTIs that have been reported so far, diarylpyrimidine (DAPY) derivatives with superior activity profiles have attracted considerable attention over the past few years [5]. Up to now, two DAPY derivatives, etravirine (**TMC125**, ETV) and rilpivirine (**TMC278**, RPV), have been launched in the marketplace by FDA for the treatment of HIV infection in 2008 and 2011 respectively (Fig. 1). Besides, indolylarylsulfone (IAS) derivatives (lead compound **L-737,126** and **7e**) represent another series of new generation NNRTIs with broad spectrum of activity against drug-resistant HIV strains [4,6,7]. With an attempt to improve their anti-HIV-1 activity, most work on the modification of IASs was focused on introducing different substituents into the 2-carboxamide nitrogen [8–12].

Although DAPYs and IASs belong to two structurally different scaffolds, these two types of NNRTIs share a similar pharmacophoric feature and binding conformation. As shown in Fig. 2, a crystallographic overlay of **TMC125** [13] and **7e** [7] within their NNRTIs binding site (NNIBS) revealed that most fragments in their structures can overlap well with each other. The pyrrolidine ring of **7e** and the 2,6-dimethyl-4-cyanophenoxyl group of **TMC125** both participate in hydrophobic interactions with residues Tyr188 and

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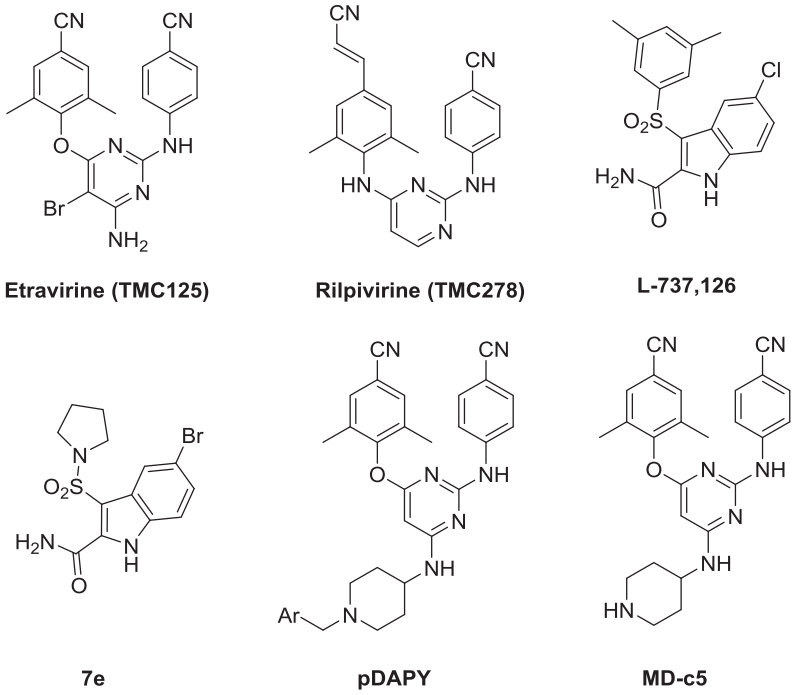

Fig. 1. The structures of representative DAPYs (**TMC125** and **TMC278**), IASs (**L-737,126** and **7e**), **pDAPY** and **MD–c5**.

Tyr188. The phenyl ring of the indolyl group of **7e** can overlap with the right aryl ring of **TMC125**, pointing toward the solvent exposed region. The 2-carbamoyl-pyrrole moiety functioning as the pyrimidine ring of **TMC125** developed critical hydrogen bonding with the backbone of Lys101. In addition, the NH_2 and Br groups on the central pyrimidine ring of **TMC125** pointed to the entrance channel which was surrounded by amino acids Leu100, Lys101, Glu138 and Val179 [13], the substituents at the 2-carboxamide nitrogen may also fit into the entrance channel in the docking study of IAS derivatives [10,14,15].

The entrance channel was first discovered in the structural optimization and molecular modeling study of IAS series [14,15]. More studies suggested that the introduction of nitrogen-containing aromatic heterocycles, amino acids, oligopeptides and so on at the 2-position of the indole ring of IAS will form the double hydrogen bonding with Lys101 and Glu138 of the channel, thus enhancing the potency against the wild-type or mutant virus strains [8–12]. According to the SAR studies of the DAPY derivatives, the substituents on the 5,6-position of the central pyrimidine ring was changeable [16]. The piperidinylamino-diarylpyrimidine (**pDAPY**) derivatives were designed through a molecular hybridization strategy in our previous studies [17]. Among the pDAPY derivatives, compound **MD–c5** was identified as the most potent inhibitor against wild-type and K103N + Y181C drug-resistant mutant HIV-1 strain with an EC_{50} value of 0.038 μM and 0.95 μM respectively (Fig. 1), which confirmed the design rationality of new multi-sites binding NNRTIs especially with the entrance channel targeting property.

Prompted by the analyses of the crystallographic overlay of **TMC125** and **7e**, the novel diarylnicotinamide scaffold was created using crystallographic overlays based molecular hybridization. As shown in Fig. 3, the preferred combination of the trisubstituted phenoxy ring (A-ring), the para-cyanoaniline moiety (B-ring), and the NH group linking the B ring and pyrimidine core (C-ring) of DAPYs were preserved. Substituents at the 2-carboxamide nitrogen in the IASs series were introduced to further improve the interaction with the entrance channel. These newly designed diarylnicotinamide derivatives (DANAs) were synthesized, and their anti-HIV activities against wild-type HIV-1 and HIV-2, as well as their activities against the double mutant HIV-1 strain (K103N + Y181C) were evaluated. Preliminary structure-activity relationships (SARs) and molecular modeling results of these new compounds were also discussed.

2. Chemistry

The expeditious and straightforward synthetic route is depicted in Scheme 1. The synthetic work started with the commercially available diethyl 1,3-acetonedicarboxylate (**1**). Reaction of **1** with triethyl orthoformate in acetic anhydride, followed by cyclisation with ammonia, resulted in formation of ethyl 4,6-dihydroxynicotinate (**2**). Treatment of **2** with phosphorus oxychloride gave ethyl 4,6-dichloronicotinate (**3**), which underwent nucleophilic substitution reaction with different substituted phenols under conditions of DMF/K_2CO_3, generating intermediate ethyl 6-chloro-4-(4-cyano-2,6-dimethylphenoxy)nicotinate (**4a**) and ethyl 6-chloro-4-(mesityloxy)nicotinate (**4b**). Then ethyl 4-(4-cyano-2,6-dimethylphenoxy)-6-(4-cyanophenylamino)nicotinate (**5a**) and ethyl 6-(4-cyanophenylamino)-4-(mesityloxy)nicotinate (**5b**) were obtained from **4a** and **4b** by Buchwald–Hartwig reaction with 4-aminobenzonitrile. The final derivatives **6a1-11** and **6b1-11** were obtained by ammonolysis of **5a** and **5b** using trimethylaluminium [18]. The synthesized compounds were characterized by physicochemical and spectral means. The MS and ^{1}H NMR, ^{13}C NMR spectral data were found in agreement with the assigned molecular structures.

3. Results and discussion

3.1. Anti-HIV activity evaluation

The newly synthesized DANAs were evaluated for their activity against wild-type HIV-1 strain (III_B), K103N + Y181C double mutant HIV-1 strain (RES056) and HIV-2 ROD strain in MT-4 cells using the