European Journal of Medicinal Chemistry 82 (2014) 600–611

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design and synthesis of a new series of modified *CH*-diarylpyrimidines as drug-resistant HIV non-nucleoside reverse transcriptase inhibitors

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ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form
11 May 2014
Accepted 25 May 2014
Available online 5 June 2014

Keywords:
Non-nucleoside HIV RT inhibitors
Diarylpyrimidines
Cyanide group
Double mutant HIV-1
Docking analysis
SAR

ABSTRACT

This article reports the design, synthesis and antiviral evaluation of a new series of non-nucleoside reverse transcriptase inhibitors (NNRTIs). The basic skeleton of these target 18 molecules is diarylpyrimidine featuring a substituted amino group between the pyrimidine scaffold and the aryl wing. All of the new compounds have been characterized by spectra analysis. The entire target molecules were evaluated for their *in vitro* anti-HIV activity with controlling group of FDA approved drugs. Most of them showed good to potent activities against wild-type (WT) HIV-1 with IC_{50} values in the range of 0.0175–69.21 μM. 2-(4-Cyanophenylamino)-4-(2-cyanovinylphenylhydrazonomethyl)pyrimidine (**1d**) displayed potent anti-HIV-1 activity against WT HIV-1 with a selectivity index (SI) of 106367 and an IC_{50} value of 1.75 nM, which was 47 fold lower than that of AZT. Compound **1d** also showed a broad-spectrum inhibitory activity, with an IC_{50} value of 5.33 μM and 5.05 μM against both HIV-1 double-mutated (K103N/Y181C) strain and HIV-2 strain, respectively. The preliminary structure–activity relationship (SAR) was also investigated. The binding modes with HIV-1 RT for both the wild type and mutant type have also been discussed.

1. Introduction

Human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT) is one of the key enzymes in the HIV life cycle [1], which represents one of the main targets for designing selective agents for the treatment of HIV/AIDS [2]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) against HIV-1 are the main and important drugs to combating with the plague of AIDs [3]. Among the classes of compounds to which the US FDA-approved RT inhibitors belong [4], the three most important main types of NNRTIs are aromatic heterocyclic compounds, including HEPTs [5,6], DABOs [7,8], diarylpyrimidines (DAPYs) [9–11], *etc* [12]. All these therapeutic agents shared the pyrimidine ring as the main structural skeleton (Fig. 1) [6], which could directly bind with the allosteric site, located 10–15 Å away from the catalytic site of RT [13–15]. While the emergence of RT mutations (such as Y181C, K103N, *etc.*) rapidly confers resistance to the first-generation NNRTIs, highly potent second-generation NNRTIs have also been identified [1,16,17], such as the Y181C mutation-associated diarylpyrimidine (DAPY) analogs [18], represented by TMC125 [19–21] and TMC278 [22–24], which have been corroborated by many crystallographic data [25–27].

The crystal structures of DAPY/HIV-1 RT complexes and the relative molecular modeling results have revealed some important features of enzyme–ligand interactions, which are crucial for maintaining the antiviral activity against a wide range of resistance mutations [27,28]. DAPYs shared the similar pharmacophore with

Abbreviations: NNRTIs, non-nucleoside reverse transcriptase inhibitors; HEPTs, 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio) thymines; DABOs, 3,4-dihydro-2-alkyl-6-benzyl-pyrimidin-4(3*H*)-ones; DAPYs, diarylpyrimidine derivatives; NVP, nevirapine; DLV, delavirdine; HIV-1 RT, Human immunodeficiency virus type 1 reverse transcriptase; US FDA, United States Food and Drug Administration; NNRTIs, non-nucleoside RT inhibitors; EFV, efavirenz; TMC125, etravirine; TMC278, rilpivirine; NNIBP, nonnucleoside 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-diphenyltetrazolium bromide; $CCID_{50}$, 50% cell culture infectious dose; CC_{50}, 50% cytotoxic concentration; EC_{50}, 50% effective concentration; Kd, dissociation constant; IC_{50}, 50% inhibitory concentration.

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http://dx.doi.org/10.1016/j.ejmech.2014.05.059
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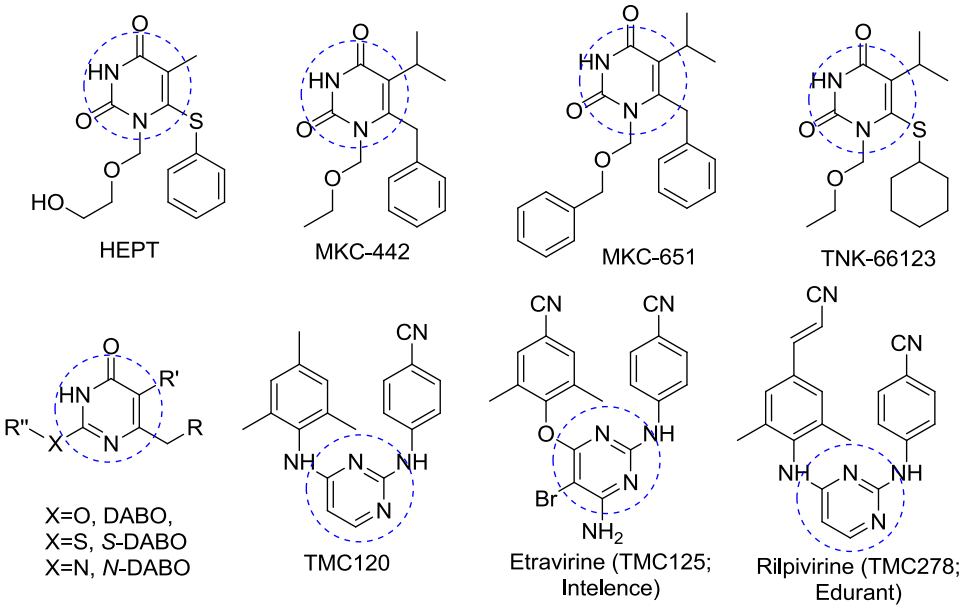

Fig. 1. Structure of some important NNRTIs including HEPT, DABCO and DAPYs.

the first-generation NNRTIs, including the hydrophobic center, the hydrogen bond donor and acceptors, the active binding conformation of DAPYs resembled a horseshoe [29] or "U" shape when bound in the non-nucleoside inhibitor binding pocket (NNIBP) [25,27]. For example, TMC125 could be taken as an example to delineate this interaction mechanism in details as following: the ether and amino linkages of the two cyanophenyl groups of the TMC125 could provide sufficient flexibility to allow strong π–π stacking interactions with the aromatic ring of Y181, Y188, F227 and W229 [27,30], respectively. It was also suggested that DAPY NNRTIs could bind to RT through torsional flexibility ("Wiggling") and repositioning flexibility ("Jiggling") [25]. Based on the above concepts, a general strategy for designing flexible drugs against a variety of drug-resistant mutants HIV-1 strains has been proposed.

Considerable efforts made on the structural modifications of the linker group of *CH_2*-DAPYs might end up with various effects on the biological activity *via* the assistance of the different substituted groups on both sides of the aromatic rings [31,32]. It has already been shown that there are still some possibilities to achieve the anti-drug resistance effects by modifying the linker group between the left benzene ring and the central pyrimidine ring [33]. In order to identify more potent DAPYs as possible NNRTIs, we have modified DAPY derivatives with cyclopropylamino groups attached to the CH_2-linker between the left benzene ring and the central pyrimidine ring [34]. The results have shown that both the cyano and the cyanovinyl groups are beneficial for enhancement of the anti-HIV activity. We therefore proposed that it might be a short cut to start from fixing the cyano or cyanovinyl group while changing the attached group on the CH_2 linker. On the other hand, either a hydrazine [35] (**2**, Fig. 2) or hydroxyl [36,37] (**3**, Fig. 2) groups has also been proven to be very beneficial for improving the antiviral activity, especially increasing the drug resistance activities, which have been also verified by many biological assays [37].

Guided by the mechanism of interaction between DAPY and HIV-1 RT as well as the related SAR analysis of these analogues, we wanted to find more drug candidates against the drug-resistant HIV-1 strains. In this paper, we therefore have designed and synthesized another series of new 4-(substituted-amino)arylmethyl DAPYs derivatives (**1**, Fig. 2) accordingly. The target molecules shared the pyrimidine skeleton and the hydrazine or the hydroxyl-CH_2 linker, as well as the cyano and the cyanovinyl groups on both of the aromatic rings. The chemical structures of all the new target molecules have been characterized by spectra analysis data. The anti-HIV screening tests show that almost all of the compounds show moderate to excellent activity against HIV, of which compound **1d** is the best one. The relative SAR discussions were also investigated together with the comparative docking analysis.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds **1a**–**1r** was shown in Scheme 1. Starting from commercially available material 2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (**4**) and iodomethane, 2-(methylthio)-2,3-dihydropyrimidin-4(1*H*)-one (**5**) were obtained *via* methylation. Methylthio ether **5** was transformed into 4-((4-oxo-1, 2, 3, 4-tetrahydropyrimidin-2-yl)amino) benzonitrile (**6**) through the reaction with excess amount of 4-cyanoniline at high temperature of 180 °C. The purification of **6** was achieved by washing with organic solvent. The important intermediates 4-(4-chloropyrimidin-2-yl-amino) benzonitriles (**7**) was easily prepared from intermediate **6** *via* refluxing with $POC1_3$. The key intermediates, oxo-*CH_2*-DAPYs (**9a**–**9d**) were conveniently synthesized from benzonitriles **7** in two steps through our previously reported modified methods [31,38]. The cyanation of **9b** with CuCN at 150 °C for 10 h in anhydrous DMF gave the corresponding 4-cyano intermediate **9c** [34]. The acrylonitrile derivative **9d** was prepared *via* the coupling reaction, which was conducted on **9b** with classical Heck conditions using palladium (II) diacetate as a catalyst in the presence of sodium acetate in DMA [34]. Imino compounds **10a**–**10r** were synthesized by refluxing **9a**–**9d** with various substituted amines in ethanol with the assistance of small amount of acetic acid and anhydrous sodium sulfate, respectively. Reductions of **10a**–**10r** with sodium cyanoborohydride in ethanol provided the target compounds **1a**–**1r** (Scheme 1).

2.2. Biological activity

The activity and cytotoxicity of these newly synthesized DAPY analogues were evaluated in MT-4 cells for their ability to