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Research paper

2-hydroxyisoquinoline-1,3(2*H*,4*H*)-diones (HIDs) as human immunodeficiency virus type 1 integrase inhibitors: Influence of the alkylcarboxamide substitution of position 4

Muriel Billamboz [a, b], Virginie Suchaud [a, b], Fabrice Bailly [a, b, *], Cedric Lion [a, c], Marie-Line Andréola [d], Frauke Christ [e], Zeger Debyser [e], Philippe Cotelle [a, b]

[a] Université Lille Nord de France, F-59000 Lille, France
[b] Centre de Recherches Jean-Pierre Aubert UMR-S1172, Faculté des Sciences Pharmaceutiques et Biologiques, 3 rue du Professeur Laguesse, BP83, F-59006 Lille, France
[c] UMR CNRS 8576, Unité de Glycobiologie Structurale et Fonctionnelle, F-59655 Villeneuve d'Ascq, France
[d] Laboratoire MFP, UMR 5234 CNRS, Université Bordeaux Segalen, FR Transbiomed, 146 Rue Léo Saignat, F-33076 Bordeaux, France
[e] Molecular Medicine, K.U. Leuven and IRC KULAK, Kapucijnenvoer 33, B-3000 Leuven, Flanders, Belgium

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ABSTRACT

Herein, we report further insight into the biological activities displayed by the 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (HID) scaffold. Previous studies have evidenced the marked fruitful effect of substitution of this two-metal binding pharmacophore at position 4 by phenyl and benzyl carboxamido chains. Strong human immunodeficiency virus type 1 integrase (HIV-1 IN) inhibitors in the low nanomolar range with micromolar (even down to low nanomolar) anti-HIV activities were obtained. Keeping this essential 4-carboxamido function, we investigated the influence of the replacement of phenyl and benzyl groups by various alkyl chains. This study shows that the recurrent halogenobenzyl pharmacophore found in the INSTIs can be efficiently replaced by an n-alkyl group. With an optimal length of six carbons, we observed a biological profile and a high barrier to resistance equivalent to those of a previously reported hit compound bearing a 4-fluorobenzyl group.

1. Introduction

Over the last two decades, intense efforts have been devoted to the development of HIV-1 integrase (IN) inhibitors and the pyrimidone scaffold was intensively investigated [1], leading to the release of Raltegravir (Fig. 1), the first HIV-1 IN inhibitor approved by the Food and Drug Administration (FDA) in 2007 [2], which opened up a new class of antiretrovirals agents [3,4]. It has become a preferred first line agent as part of the highly active antiretroviral therapy (HAART) treatment guidelines since 2009 [5]. Indeed it has a favorable long-term efficacy and safety profile in integrase-inhibitor-naïve patients with triple-class resistant HIV in whom antiretroviral therapy is failing. However, after several years of clinical use, resistance to this integrase strand transfer inhibitor (INSTI) involving mutations at IN amino acids Y143, Q148, and N155 has quickly emerged [6–9]. Elvitegravir, the second FDA-approved INSTI, needs to be boosted by the pharmacoenhancer termed cobicistat and is prescribed as a potent once-daily single tablet also including two potent nucleos(t)ide reverse transcriptase inhibitors (NRTIs), emtricitabine and tenofovir [10–12]. Although this novel anti-HIV tablet could offer new perspective for patients failing existing antiviral regimes, cross resistance with raltegravir rules out any treatment option for patients failing on raltegravir therapy [13,14]. Dolutegravir, is a second generation integrase inhibitor that provides distinct advantages compared with first generation integrase inhibitors. Unlike raltegravir, dolutegravir in coformulation with abacavir and lamivudine can be given once daily for patients

Abbreviations: AZT, azidothymidine; CPE, cytopathic effect; CYP, cytochrome P450; FC, fold-change in EC_{50} relative to wild-type strain of HIV-1; FDA, food and drug administration; LEDGF, lens epithelium-derived growth factor; HAART, highly active antiretroviral therapy; HID, 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione; HIV-1, human immunodeficiency virus type 1; IN, integrase; INSTI, integrase strand transfer inhibitor; 3′-P, 3′-processing; PFV, prototype foamy virus; RT, reverse transcriptase; ST, strand transfer; TI, therapeutic index; TOA, time of addition.

* Corresponding author. Centre de Recherches Jean-Pierre Aubert UMR-S1172, Faculté des Sciences Pharmaceutiques et Biologiques, 3 rue du Professeur Laguesse, BP83, F-59006 Lille, France.
E-mail address: fabrice.bailly@univ-lille1.fr (F. Bailly).

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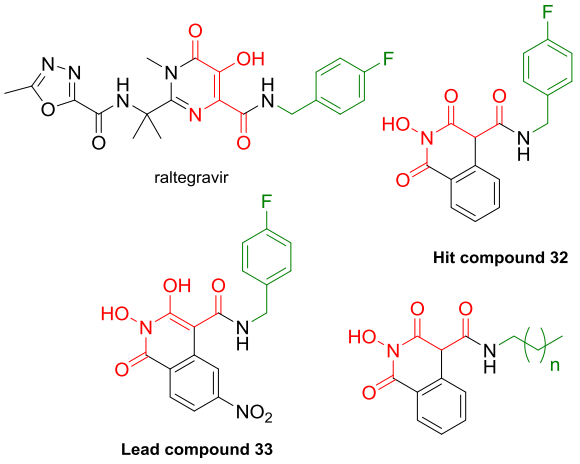


Fig. 1. Structures of the INSTI raltegravir, the hit compound **32**, the lead compound **33** and of herein studied *N*-alkyl carboxamides pointing out the key components of the HIV-1 IN inhibitory pharmacophore: the magnesium chelating moiety (red) and the hydrophobic halogenobenzyl or alkyl group (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

who are antiretroviral treatment naïve without the requirement of a pharmacokinetic booster which minimizes the drug–drug interaction potential of dolutegravir [15–17]. Moreover, dolutegravir showed a more robust resistance profile than raltegravir and elvitegravir although some viruses containing E138K, G140S, or R148H mutations had lower susceptibility and may also diminish the likehood of long-term clinical success [18,19]. All these INSTIs were shown to inhibit HIV-1 IN by chelating the two magnesium ions of the catalytic core through a triad of oxygen atoms [20,21]. Thus, in the current clinical context, integrase inhibitors should have a high genetic barrier to existing INSTIs to be novel serious candidates. For this purpose, alternative targets like the cellular cofactor lens epithelium-derived growth factor (LEDGF/p75) are promising targets [22,23] or continuous modulation of valuable scaffolds like naphthyridine is still relevant [24].

Following early pioneer studies on the 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (HID) scaffold [25,26], we recently reported the interesting biological properties of some derivatives substituted at position 4 by carboxamide chains [27–29]. These compounds displayed strong IN inhibitory potencies comparable to that of the clinically used raltegravir. One hit compound **32** (Fig. 1) potently inhibited ST and 3′ P IN catalytic activities while it kept activity against a panel of raltegravir-resistant HIV-1 variants and did not induce any resistance selection in cell culture [27]. A crystal structure of this compound bound to the wild-type prototype foamy virus (PFV) intasome revealed that the compact heterocyclic scaffold displaying all three Mg^{2+} chelating oxygen atoms from a single ring showed an overall binding mode similar to previous INSTIs [27]. Further substitution of this hit compound at position 7 by electron-withdrawing groups and particularly the nitro function led to the discovery of the lead compound **33** (Fig. 1) with low nanomolar anti-HIV potency [29].

Herein we elaborated and studied a novel series of 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-diones, in which we replaced the *p*-fluorobenzyl side chain by various alkyl chains. These synthetic modulations, aimed at generating more hydrophobic molecules, were performed in order to assess the influence of the nature of the side chain on the enzymatic inhibitory and antiviral properties within this scaffold. Owing to the nature of the hydrophobic pocket occupied by this substituent at the catalytic site (not only due to aromatic DNA bases pairs but also containing non-aromatic hydrophobic aminoacid residues), we investigated whether the N-benzylated chain at position 4 of the HID scaffold could be replaced by alkyl chains that would occupy this cavity through pure van der Waals interactions.

2. Results and discussion

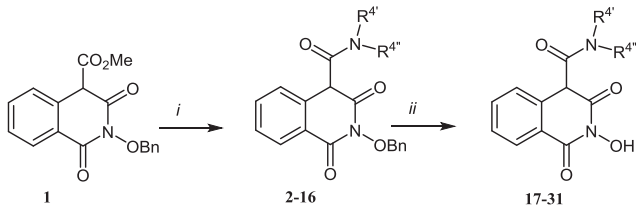
2.1. Chemistry

The target 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-diones were synthesized according to our previously reported method [28]. The key ester precursor **1**, methyl 2-(benzyloxy)-1,3-dioxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylate was converted to carboxamides **2**–**16** by addition-elimination of various amines. Finally, the *O*-benzyl protecting group was removed by action of boron trichloride or hydrogenation at room temperature over 5% Pd/C (Scheme 1). A series of primary carboxamides with increasing linear alkyl chains (3–9 carbons, **17**, **19**, **23**–**27**) were synthesized (Table 1), together with representative secondary amides (compounds **20**, **21** and **31**). The influence of the ramification (compound **18**, R^4 = isopropyl; compound **22**, R^4 = *tert*butyl) of the alkyl chain and of the bulkiness (compound **28**–**30**, R^4 = cyclopropyl, cyclopentyl, cyclohexyl) of the cycloalkyl chain was also briefly investigated.

2.2. Integrase and ribonuclease H inhibitory properties

Table 1 shows the biological properties of this series of *N*-alkyl-2-hydroxy-1,3-dioxoisoquinoline-4-carboxamides. As far as the compounds (**17**, **19**, **23**–**27**) with increasing linear chains are concerned, compounds **17** (R^4 = propyl), **26** (R^4 = octyl) and **27** (R^4 = nonyl) displayed weakened HIV-1 IN inhibition when compared with our reference hit and lead compounds **32** and **33**, with IC_{50} values of 1.82, 9.05 and 6.65 μM, respectively. Compounds **19**, **23**, and **25** (R^4 = butyl, pentyl, and heptyl) inhibit HIV-1 IN in the submicromolar IC_{50} range, with values from 0.65 μM to 0.86 μM. Compound **24** bearing an hexyl side-chain (IC_{50} 0.085 μM) is the only representative in this series that displayed excellent integrase inhibitory activity in the same nanomolar range as lead **33** (IC_{50} 0.010 μM). Fig. 2 shows the variation of the integrase inhibitory activity according to the length of the linear alkyl chain, clearly evidencing an optimal length of six carbons since compound **24** (R^4 = hexyl, IC_{50} = 0.085 μM) is 20- to 105-fold more active than compounds **17** (R^4 = propyl, IC_{50} = 1.82 μM), **26** (R^4 = octyl, IC_{50} = 9.05 μM) and **27** (R^4 = nonyl, IC_{50} = 6.65 μM) whereas there is only one-log difference with the close compounds **19** (R^4 = butyl, IC_{50} = 0.66 μM), **23** (R^4 = pentyl, IC_{50} = 0.65 μM) and **25** (R^4 = heptyl, IC_{50} = 0.86 μM).

The ramification of the propyl chain did not have a great impact on the integrase inhibition with close IC_{50} values of 1.82 and 4.43 μM for the linear and ramified counterparts **17** and **18**,



Scheme 1. Synthesis of target compounds **17**–**31**. Reagents and conditions: (i) 2.0 eq. R_4NH_2, toluene, Dean Stark apparatus, reflux, 15 h (45–83%); (ii) BCl_3, 5.0 or 6.0 eq, CH_2Cl_2, 15 min, rt then H_2O, 5 min, rt (49–88%) or H_2, 5% Pd/C, EtOAc/MeOH, rt, 4 h (52–90%).