Bioorganic & Medicinal Chemistry Letters 26 (2016) 3487–3490

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of 2-pyridinylpyrimidines as inhibitors of HIV-1 structural protein assembly

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ARTICLE INFO

Article history:
Received 24 May 2016
Revised 14 June 2016
Accepted 15 June 2016
Available online 16 June 2016

Keywords:
Assay
Assembly
Capsid
Inhibition
Pyrimidine

ABSTRACT

In an effort to identify an HIV-1 capsid assembly inhibitor with improved solubility and potency, we synthesized two series of pyrimidine analogues based on our earlier lead compound *N*-(4-(ethoxycarbonyl) phenyl)-2-(pyridine-4-yl)quinazoline-4-amine. In vitro binding experiments showed that our series of 2-pyridine-4-ylpyrimidines had IC_{50} values higher than 28 μM. Our series of 2-pyridine-3-ylpyrimidines exhibited IC_{50} values ranging from 3 to 60 μM. The congeners with a fluoro substituent introduced at the 4-*N*-phenyl moiety, along with a methyl at C-6, represent potent HIV capsid assembly inhibitors binding to the C-terminal domain of the capsid protein.

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of acquired immunodeficiency syndrome (AIDS). Since the introduction of highly active antiretroviral therapy (HAART), the mortality of AIDS patients has declined, and the life expectancy of people with HIV infection has increased markedly. However, treatment success can be compromised by the unwanted side-effects of current medications and by selection of drug-resistant viruses. Therefore, there remains a demand for novel approaches and targets for therapeutic intervention in the viral life cycle.[1,2]

The HIV-1 virion is built up by assembly of approximately 2500 Gag polyprotein molecules that are organized inside the budding virions.[3] For the virus to mature, these polyproteins have to be specifically processed by the viral protease into final viral proteins, including matrix, nucleocapsid, small spacer peptides, and capsid (CA).[4–6] The latter possesses N- and C-terminal domains that are tethered by a flexible linker. The conical shape of the mature HIV capsid enclosing the viral genome stems from its architecture—the CA subunits form hexamers and pentamers, contributing to the overall fullerene-type structure.

At present, there is no HIV-1 assembly inhibitor on the market. There are few reports of non-peptidic compounds targeting CTD of CA.[7–11] The other reported compounds bind to the NTD of CA and some of them affect capsid assembly. Three independent binding sites have been defined in the NTD. Acylhydrazones, thioureas, benzodiazepines, benzimidazoles and the urea-based derivative CAP-1, bind to the hydrophobic pocket at the base of the helical bundle.[12–16] A second binding site interacts with the ligand PF-3450074.[17] A third ligand-binding site lies near the flexible cyclophilin A binding loop.[18] In general, the reported CA NTD-binding compounds exhibit mostly micromolar affinity towards CA in vitro and weak antiviral activity. However, several effective nanomolar inhibitors have been reported.[15,17]

The discovery by Sticht et al.[19] of a 12-mer peptide (CAI) that is capable of binding to the CA C-terminal domain (CTD) and inhibits assembly of immature and mature-like particles in vitro enabled the development of a competitive binding assay designed to select CTD ligands. This new screening assay for assembly inhibitors

Abbreviations: AIDS, acquired immunodeficiency syndrome; CA, capsid; CAI, capsid assembly inhibitor; CTD, C-terminal domain; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; ITC, isothermal titration calorimetry; NMR, nuclear magnetic resonance; TLC, thin layer chromatography; TBTU, *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate.

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http://dx.doi.org/10.1016/j.bmcl.2016.06.039
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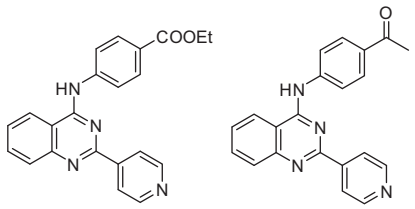
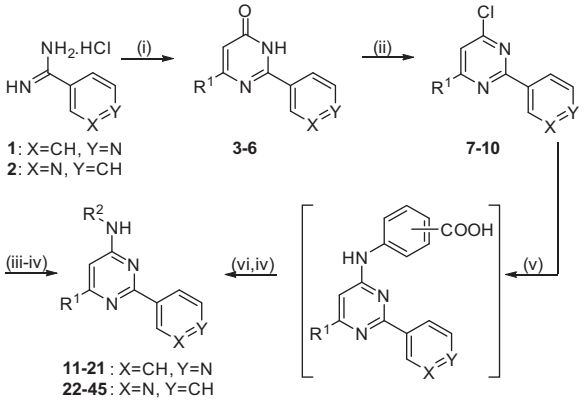


Figure 1. 4-Aminophenylquinazoline derivatives that bind to the C-terminal domain of the HIV-1 capsid protein.[7]



Scheme 1. Synthesis of pyrimidines **11**–**45**. R^1 = H, CH_3. The following reagents and conditions were used: (i) ethyl formylacetate sodium salt, ethanol, reflux or methyl acetoacetate, $NaHCO_3$, ethanol, reflux; (ii) neat $POCl_3$, 100 °C; (iii) aniline, HCl, dioxane, reflux or HCl, ethanol, water, reflux; (iv) HCl in dioxane; (v) aminobenzoic acid, HCl, ethanol, water, reflux; (vi) amine, TBTU, Et_3N, DMF.

utilizes an amplified luminescent proximity assay system (AlphaScreen). Briefly, interaction of CAI linked to donor beads with CA CTD on acceptor beads results in a strong luminescent signal originating from energy transfer from excited donor to acceptor beads. The presence of an effective assembly inhibitor causes the beads to separate, resulting in decreased luminescence.

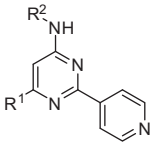
We previously identified a set of unique capsid assembly inhibitors binding to CA CTD from a high throughput screen.[7] Because the inhibitors compete with CAI, we proposed that they bind into a conserved hydrophobic groove in CA CTD.[20] We further optimized the identified hits to improve the inhibitory activity, resulting in the compounds shown in Figure 1. These compounds exhibit moderate affinity to the binding site in vitro, as determined by AlphaScreen assay and ITC.[7]

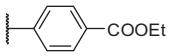
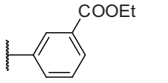
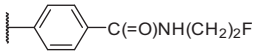
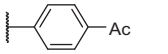
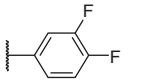
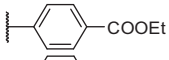
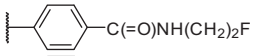
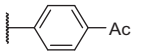
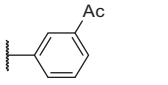
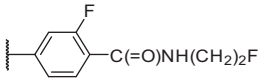
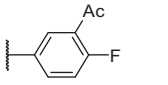
To improve the pharmacokinetics and binding of those quinazoline compounds, we searched for derivatives with superior solubility. From previous work,[7] it was obvious that the 4-*N*-phenyl and 2-pyridinyl moieties are crucial for binding. Therefore, we decided to replace the parent quinazoline scaffold with a smaller, and less hydrophobic, heterocyclic pyrimidine.

Here, we report evaluation of two series of *N*-4-phenyl-2-pyridinylpyrimidines that are substituted at the *N*-phenyl moiety. Pyridine-4-ylpyrimidines (compounds **11**–**21**) and pyridine-3-ylpyrimidines (compounds **22**–**45**), shown in Scheme 1, are bioisosteres that act as new classes of capsid assembly inhibitors binding to CA CTD.

We explored the structure–activity relationship (SAR) of these compounds with regard to substitution of the *N*-phenyl moieties. From previous work,[7] we concluded that the attachment of electron-withdrawing groups lead to the decrease of in vitro IC_{50} values determined by AlphaScreen assay. In particular, carbonyl and carboxyl functionalities were superior to sulfonamides and carboxamides, except for *N*-2-fluoroethylcarboxamide.

Table 1
Structure–activity relationship of the pyridine-4-yl derivatives



Compound	R^2	IC_{50} (μM)
11*		28 ± 3
12*		>100
13*		>100
14*		>100
15*		81 ± 8
16**		36 ± 3
17**		>100
18**		54 ± 5
19**		>100
20**		>100
21**		83 ± 7

The IC_{50} value was determined as the compound concentration sufficient for decreasing the AlphaScreen signal by 50%. For experimental details, see Supplementary materials.

* 2,4-Disubstituted pyrimidine derivative (R^1 = H).

** 2,4,6-Trisubstituted pyrimidine derivative (R^1 = Me).

The compounds reported here were synthesized according to the general route outlined in Scheme 1. The commercially available 3-/4-cyanopyridines were first converted to corresponding amidines **1** and **2** by treatment with sodium methoxide and later with ammonium chloride.[21] The resulting amidines were either combined with the sodium salt of ethyl formylacetate, which was prepared by aldol condensation of ethyl acetate with ethyl formate, or with methyl acetoacetate to yield pyrimidinones **3**–**6**.[21,22] Subsequent treatment[23] with phosphorus oxychloride yielded 4-chloropyrimidines **7**–**10**, which were subjected to nucleophilic displacement of the chlorine atom by variously substituted anilines.[7,24] To prepare the carboxamide series (**13**, **17**, **20**, **28**–**31**, **39**–**44**), a different synthetic approach was used. An appropriate chloropyrimidine was first treated with aminobenzoic acid, and when TLC indicated its disappearance, the entire reaction mixture was evaporated to dryness and subjected to standard amidation using TBTU as a coupling agent. We found that this stepwise approach was superior to the nucleophilic aromatic substitution carried out with corresponding *N*-alkyl aminobenzamides in terms of yield and versatility. Finally, all prepared compounds were converted into the corresponding hydrochloride salts.

We started with preparation of pyridine-4-ylpyrimidines, which are congeners of the compounds shown in Figure 1. The first round of SAR studies revealed that ablation of the annulated benzene ring from the parent quinazoline led to a significant decrease