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Bioorganic & Medicinal Chemistry Letters 17 (2007) 260–265
Bioorganic & Medicinal Chemistry Letters

4-Aminopyrimidines as novel HIV-1 inhibitors

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Received 1 August 2006; revised 15 September 2006; accepted 18 September 2006
Available online 10 October 2006

Abstract—A series of 4-aminopyrimidines (**1**) was identified as novel HIV inhibitors of unknown molecular target. Structural modifications were carried out to establish its SAR and identify the linking site for target identification. A number of analogs were found to possess single digit inhibitory activity for HIV replication. Several analogs with various potential linkers, including a biotinated analog, also exhibited excellent potency, and could serve as tools for the identification of novel anti-HIV targets.

The introduction of highly active antiretroviral therapy (HAART) has significantly decreased morbidity and mortality among patients infected with human immunodeficiency virus type 1 (HIV-1).[1,2] However, HIV-1 can acquire resistance against all currently available antiretroviral drugs. Significant resistance has been generated against the currently marketed viral reverse transcriptase inhibitors (RTI) and protease inhibitors (PI).[3,4] Resistance has also been observed for the newly approved entry inhibitor Enfuvirtide (T-20).[5] Moreover, in a growing number of patients, the development of multidrug-resistant viruses compromises HAART efficacy and limits therapeutic options.[6] There is an urgent need of new drugs that could combat the current resistance. New classes of antiviral drugs, especially against novel targets, will provide the only hope of treatment for an increasing number of patients.[7,8]

During our effort to develop new anti-HIV drugs,[9–13] we discovered a series of benzylaminopyrimidines (Fig. 1) that exhibited a range of inhibitory activities against HIV replication. Although 4-aminopyrimidines related to compound **1** have been extensively studied as pesticides,[14–18] no antiviral activity for these pyrimidines has been reported to date. One of our main goals for our cell-based anti-HIV screening platform was to identity potential novel targets for HIV inhibition. Biochemical inhibition studies suggested that these compounds are neither HIV NRTI's nor integrase inhibitors. As HIV-1 protease is not part of the recombinant HIV cell model used for screening, it can be excluded as a target of these inhibitors. We were intrigued by the novel structural features of these HIV inhibitors, and believed that these compounds could serve as a molecular probe to identify novel targets for HIV inhibition. Affinity chromatography is one of the most commonly used techniques in target identification.[19,20] It requires the attachment of the ligand of unknown target to a solid support, in a manner as to maintain the affinity for the potential target after the ligand is attached to the support. We wish to report in this communication our effort to establish the preliminary SAR of these pyrimidines as novel HIV inhibitors and the identification of the attachment site for target identification.

A representative synthesis of these amino pyrimidines is shown in Scheme 1, which calls for the preparation of a dichloro intermediate **4**. Pyrimidine **4** could be easily prepared from methyl 2-chloro-3-oxo-pentanoate (**2**) by sequential pyrimidine ring formation with formamidinium chloride and chlorination of the resulting hydroxypyrimidine **3** with phosphoryl chloride.[21] In parallel, nitrobenzylamine **5** was first protected with Boc_2O and the nitro group was then reduced to give aniline **6**. Acetylation of the aniline followed by the removal of the Boc group with trifluoroacetic acid provided benzylamine **8** for the final coupling with pyrimidine

Keywords: Aminopyrimidine; HIV-1 inhibitor.
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doi:10.1016/j.bmcl.2006.09.047

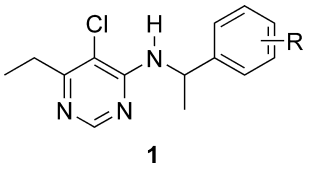

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Figure 1.

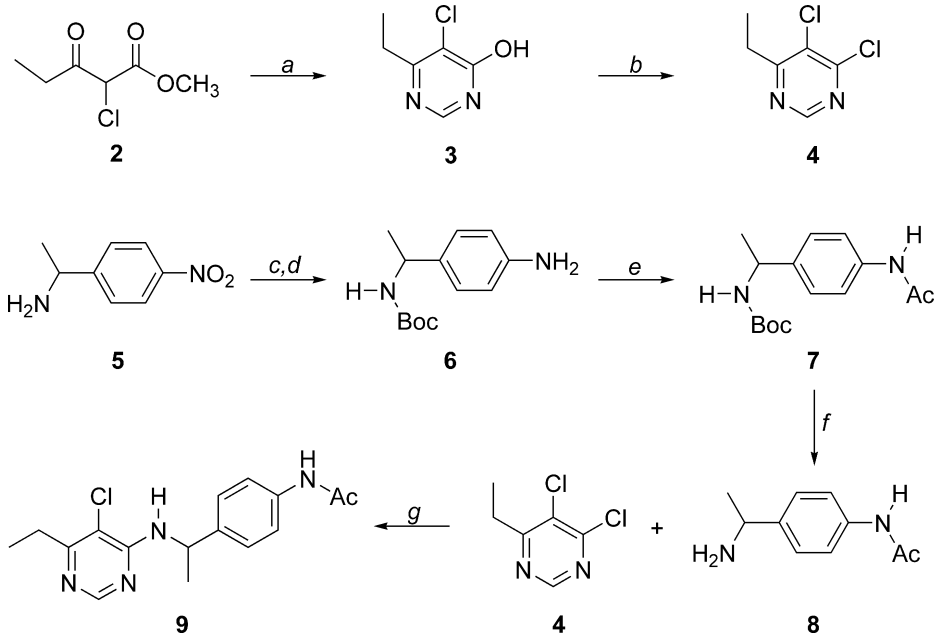
chloride **4**. Under the influence of potassium carbonate, pyrimidine **9** was conveniently obtained in a moderate yield.

Using similar chemistry, a large number of aminopyrimidines could be easily synthesized for SAR studies (Fig. 2). These compounds were tested in the cell-based HIV inhibition assay and their inhibitory activities are summarized in Table 1. It was clear that the anti-HIV activities of the pyrimidines are sensitive to structural perturbations. Compounds **10a**–**10e** have the common benzylaminopyrimidine core but with varying R groups at the 4 position of the phenyl ring. The antiviral activities of these analogs are shown in Table 1. Data indicate that a hydrophobic R moiety is preferred, and the biphenyl analog **10d** exhibited a 17 nM inhibitory activity. The quinolinoxy analog **10e** also had an IC_{50} of 48 nM. This is very useful information, as it suggests that a large space is available to accommodate substitution at the 4 position of the phenyl ring. On the other hand, small and polar substitutions at the 4 position resulted in the reduction in potency, and **10a** and **10b** have IC_{50}s of 2.659 μM and 341 nM, respectively.

To explore the SAR in this region, various heterocycles (**11a**–**11j**) were introduced to replace the phenyl moiety in **10**. Data suggested that this scaffold tolerates a number of heterocycles in this region, and the majority of these modifications maintained good anti-HIV activity. In particular, compounds **11a**, **11c**, **11f**, and **11g** showed double digit nM IC_{50}s. These results again suggest that this region of the molecule might be a suitable position for linker attachment for target identification.

The anti-HIV activity of **12a**–**12c** suggested that a small hydrophobic functionality like a methyl group is favored at the benzylic position, as it is almost twenty-fold more potent than the unsubstituted analog **12a**, and about twofold more potent than the corresponding ethyl analog **12c**.

To explore the SAR in the pyrimidine region of this scaffold, a number of analogs with various substitutions on the pyrimidine ring were examined (Fig. 2 and Table 1). For the purpose of this study, the 4-fluorophenoxy benzyl moiety was held constant. Our studies suggest that a slightly larger propyl group at position 6 (R^2 in **13**) is preferred over the methyl group for anti-HIV activity (**13a** vs. **13b**). The methoxymethyl group (**13c**) leads to a further improvement in potency. The combination of a bromo R^1 and an ethyl R^2 gave an extremely potent HIV inhibitor (**13d**) with an IC_{50} of 3 nM. However, a bromo R^1 and a propyl R^2 are not compatible, resulting in only a moderately potent analog **13e** (IC_{50} = 760 nM vs. 96 and 3 nM). These data suggest that space around positions 5 and 6 is limited, and sensitive to the size of the substituent. Further substitution of **13d** with a methyl group at position 2 also gave a potent analog (**13f**) with an IC_{50} of 18 nM. More interestingly, the 5-amino analog **13g** also exhibited potent antiviral activity (18 nM). The combined data of **13c** and **13g** suggest that the 5 and 6 positions of the pyrimidines ring are not very sensitive to polarity perturbations.



Scheme 1. Synthesis of benzylaminopyrimidine **9**. Reagents and conditions: (a) $H_2NCH{=}NH{\cdot}HCl$, CH_3ONa, MeOH; (b) $POCl_3$, toluene, 77%; (c) BOC_2O, $NaHCO_3$, CH_2Cl_2/H_2O, 25 °C, 12 h, 92%; (d) Ni, H_2, N_2H_4, EtOH, 25 °C, 24 h, 50%; (e) Ac_2O, Py, DMAP, CH_2Cl_2, 0–25 °C, 3 h, 88%; (f) CF_3COOH, CH_2Cl_2, 25 °C, 3 h, 88%; (g) **4**, DMF, K_2CO_3, 80 °C, 5 h, 45%.

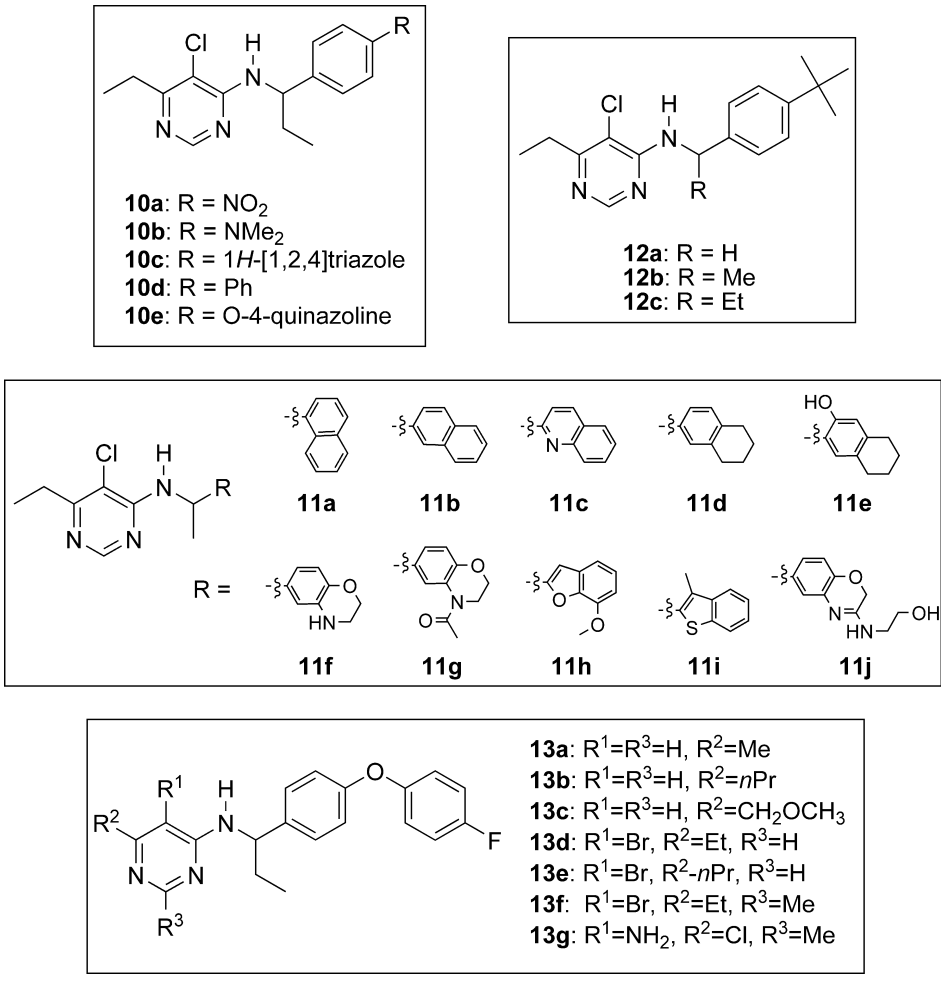
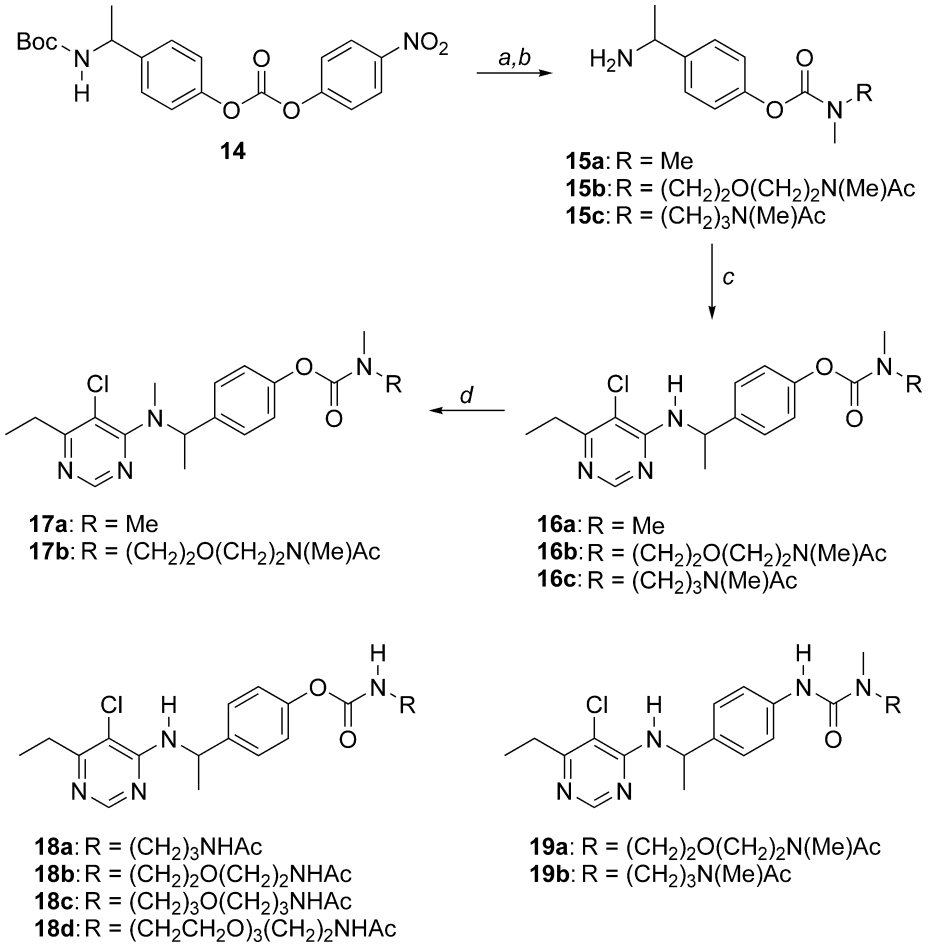


Figure 2. Structures of aminopyrimidines **10**–**13**.

Table 1. Anti-HIV activities of 4-aminopyridines[22]

Compound	EC_{50}[23] (μM)	CC_{50}[24] (μM)	Compound	EC_{50} (μM)	CC_{50} (μM)
Efavirenz	0.0005	~10	**12c**	0.028	>10
Nevirapine	0.050	>10	**13a**	0.324	>10
9	1.188	>10	**13b**	0.096	>10
10a	2.659	>10	**13c**	0.031	>10
10b	0.341	>10	**13d**	0.003	>10
10c	0.091	>10	**13e**	0.760	>10
10d	0.017	>10	**13f**	0.018	>10
10e	0.048	>10	**13g**	0.015	>10
11a	0.095	>10	**16a**	0.004	>10
11b	0.139	>10	**16b**	0.004	>10
11c	0.042	>10	**16c**	0.327	>10
11d	0.170	>10	**17a**	>10	>10
11e	0.778	>10	**17b**	>10	>10
11f	0.029	>10	**18a**	0.190	>10
11g	0.035	>10	**18b**	0.005	>10
11h	0.691	>10	**18c**	0.005	>10
11i	0.816	>10	**18d**	0.190	>10
11j	0.353	>10	**19a**	3.020	>10
12a	0.331	>10	**19b**	4.889	>10
12b	0.016	>10	**21**	0.622	>10

These preliminary SAR studies clearly indicate that the phenyl moiety in **1** could tolerate a large variety of structural modifications and is the most promising position for attaching a linker for the purpose of target identification. To test this hypothesis, a number of analogs with potential linkers were prepared (**16**–**19**, Scheme 2).

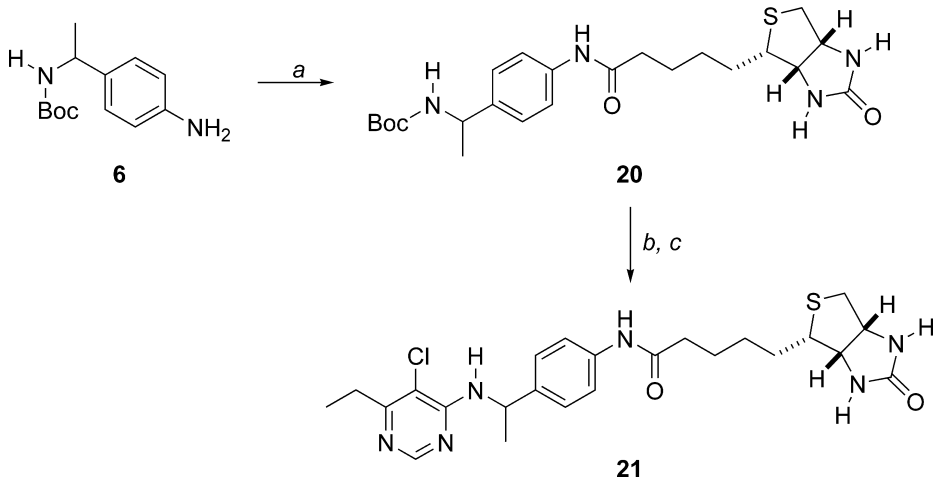


Scheme 2. Synthesis of benzylaminopyrimidines **16**–**19**. Reagents and conditions: (a) $NH(CH_3)R$, CH_2Cl_2, 25 °C, 30 min, 92%; (b) TFA, CH_2Cl_2, 25 °C, 3 h, 84%; (c) **4**, K_2CO_3, DMF, 25 °C, 4 h, 70%; (d) MeI, K_2CO_3, DMF, 38 °C, 12 h, 90–95%.

Reaction of the activated 4-nitrophenoxyl carbonate **14** with various amines gave the carbamates, which were easily converted to the corresponding benzyl amines **15** after the removal of the Boc group. Selective displacement of the chlorine at the 4 position in **4** with amines **15a**–**15c** furnished the amino pyrimidines **16a**–**16c**. We were pleased to find out that both the simple dimethylamine (**16a**) and the extended amine analog (**16b**) exhibited an IC_{50} of 4 nM. Interestingly, the other extended analog **16c** has significantly reduced activity, suggesting some sensitivity in the linker region in the maintenance of the antiviral potency. Treatment of **16a** and **16b** with methyl iodide in the presence of potassium carbonate furnished the corresponding N-methylated analogs **17a** and **17b** in excellent yields (Scheme 2). As suspected, both **17a** and **17b** lost all anti-viral activity, suggesting the critical role of the N–H hydrogen in **1** in the interaction with the target. More importantly, the N-methylated analogs like **17b** could serve as a negative control for target identification.

To further explore the effect of various potential linkers on anti-HIV activity, additional analogs **18a**–**18d**, **19a**, and **19b** were synthesized using similar chemistry (Scheme 2). All these compounds incorporate an NH in the carbamate linker, which could potentially result in increased water solubility compared to the corresponding *N*-methyl analogs (**16a**–**16c**). To our delight, the removal of the *N*-methyl groups in **16b** had no negative impact on the anti-viral activity. Similar to **16c**, the 1,3-diamine analog **18a** showed sub-micromolar activity. On the other hand, analog **18b** fully maintained the potent antiviral activity when compared to its counterpart **16b**. The slightly extended analog **18c** is still a potent HIV inhibitor. Although the further extended analog **18d** lost some antiviral activity, it still had an IC_{50} of 190 nM, which could be sufficient as a ligand for affinity chromatography. Two urea analogs **19a** and **19b** were also prepared for SAR analysis (Scheme 2), and both compounds exhibited only micromolar activity, confirming that a carbamate linker is superior for antiviral activity.

A biotinated analog **21** was designed to evaluate its potential as a probe for affinity chromatography (Scheme 3). It was easily synthesized from the Boc-protected aniline **6** by (1) standard amide coupling; (2) removal of the Boc protecting group; and (3) selective displacement of dichloropyrimidine (**4**) with the resulting amine. Our data demonstrate that **21** is still a potential HIV inhibitor with an IC_{50} of 622 nM, confirming that these compounds could serve as suitable probes for identifying the molecular target of this series of novel HIV inhibitors.



Scheme 3. Synthesis of biotin conjugated benzylaminopyrimidine **21**. Reagents and conditions: (a) (+)-biotin, HATU, DIEA, THF, 25 °C, 24 h, 58%; (b) CF_3COOH, CH_2Cl_2, 25 °C, 3 h, 82%; (c) **4**, DMF, K_2CO_3, 80 °C, 4 h, 45%.

In summary, we have identified a series of 4-aminopyrimidines as novel HIV inhibitors through high-throughput screening and systematic structural modifications. A number of these pyrimidines possessed single digit nanomolar potency. These pyrimidines were believed to act on unknown anti-HIV target(s). Analog synthesis was directed toward establishing a SAR, with a particular focus to identify a handle on the molecule for linking to the solid support for target identification. Our studies suggest that the phenyl ring in **1** is very amenable for chemical modifications and that various potential linkers could be attached to the 4 position of the phenyl ring while maintaining potent anti-HIV activity. (+)-Biotin was also linked via an amide bond at this position, and the linked molecule **21** showed submicromolar inhibitory activity in the cell-based assay. The synthesis and utilization of optimal molecular probes in identifying the unknown target are on-going, and the results will be reported in due course.

Acknowledgments

The authors thank Karen Wolff and Hong Yin for performing the HIV inhibition assays.

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22. All compounds except **21** are racemic. Compound **21** is a mixture of two diastereoisomer.
23. HEK 293T cells are routinely cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS, 1× Pen/Strep/glutamine. The protocol is as follows: 293T cells are seeded in the 1536-well format at 700 cells/well (5 μL volume) using an Aquamax (Molecular Devices) liquid dispenser. Cells are cultured at 37 °C under 5% CO_2 for 24 h. Fifty nanoliter of each compound (serially diluted in DMSO) is transferred using the PinTool (GNF). After a 1 h at 37 °C incubation, HIV reporter virus is transferred to the cells using the Aquamax in a volume of 2 μL corresponding to a multiplicity of infection (MOI) of approximately 1.0. The treated and infected cells are cultured for an additional 48 h at 37 °C. Luciferase activity is monitored by addition of Bright-Glo (Promega, Cat. Nos. E263B and E264B) luciferase reagent (5 μL/well, Aquamax) followed by plate reading on the

CLIPR apparatus (Molecular Devices) using a 20 s shuttle speed.
24. 293T cells are seeded in the 1536-well format at 700 cells/well (5 μL volume) using an Aquamax (Molecular Devices) liquid dispenser. Cells are cultured at 37 °C under 5% CO_2 for 24 h. Fifty nanoliter of each compound (serially diluted in DMSO) is transferred using the PinTool (GNF). The treated and uninfected cells are cultured for an additional 48 h at 37 °C. Cell viability is assessed by addition of 1 μL of Alamar Blue (Promega, Cat. No. 00-100) diluted 1:1 in DMEM. Cells are further incubated for 4 h at room temperature and subsequent fluorescence intensity is read using an Acquest (TREK systems) with a 50/50 beam splitter.