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Novel piperidinylpyrimidine derivatives as inhibitors of HIV-1 LTR activation

Norio Fujiwara [a,*], Takashi Nakajima [b], Yutaka Ueda [a], Hitoshi Fujita [a], Hajime Kawakami [c]

[a] Drug Research Division, Dainippon Sumitomo Pharma Co., Ltd, Enoki 33-94, Suita, Osaka 564-0053, Japan
[b] Drug Development Division, Dainippon Sumitomo Pharma Co., Ltd, Enoki 33-94, Suita, Osaka 564-0053, Japan
[c] Intellectual Property, Dainippon Sumitomo Pharma Co., Ltd, Enoki 33-94, Suita, Osaka 564-0053, Japan

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ABSTRACT

Piperidinylpyrimidine derivatives, previously prepared as inhibitors of TNF-α production, were evaluated for their inhibitory activity against HIV-1 LTR activation. Some of these derivatives inhibited activation of HIV-1 LTR-directed CAT gene expression induced by PMA in Jurkat cells. In this report, we describe SAR in this series of compounds and show that the 3,4-methylenedioxybenzoyl (piperonyloyl) group on the nitrogen of piperidine and lipophilic substitution at the C(6)-position of pyrimidine are important for this inhibitory activity. Some of the synthesized compounds also inhibited HIV-1 LTR transactivation induced by viral protein Tat. These results suggest that piperidinylpyrimidines are useful as potent AIDS therapeutics that directly inhibit HIV-1 LTR activation and indirectly suppress TNF-α production.

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) is a retrovirus that causes acquired immune deficiency syndrome (AIDS). Massive replication of HIV-1 results in a general decline in T cells population, which leads to collapse of the immune system, development of clinical manifestations of AIDS, and the ultimate death of the host. Recently, highly active antiretroviral therapy (HAART) has been shown to produce significant reduction of HIV-1 replication, improved quality of life, and increased survival. Current HAART regiments include inhibitors of two viral enzymes, reverse transcriptase and protease.[1] However, the efficacy of these inhibitors is limited by emergence of resistant viral strains and severe side effects.[2] Consequently, continuous efforts towards the discovery and development of novel inhibitors of HIV-1 infection and replication are still needed.[3]

As transcription of the HIV-1 provirus, governed by the viral long terminal repeat (LTR), is an essential step for viral replication,[4] HIV-1 LTR has been proposed as target for anti-HIV therapy.[5] Elsewhere, we have previously reported the synthesis and characterization of piperidinylpyrimidine derivatives as inhibitors of tumor necrosis factor-α (TNF-α) production.[6] TNF-α, a pleiotropic inflammatory cytokine produced mainly from activated macrophages, plays a critical role in HIV replication and disease progression.[7] In addition, increase in TNF-α production has been shown to play a considerable role in the phathogenesis of AIDS-associated cachexia and dementia.[8] Therefore, inhibition of TNF-α production and/or its role in HIV replication has been one of the strategies in AIDS therapy.[9] It is interesting that inhibitors of TNF-α production, such as pentoxifylline, thalidomide, SB203580, LMP-420, fluoroquinoline derivatives, and canventol derivatives are reported to down-regulate HIV-1 LTR transcription and/or HIV-1 replication,[10] although their mechanisms of action are different or unknown. These compounds would provide a new insight in AIDS therapy as they can act both directly (inhibition of HIV-1 LTR activation) and indirectly (inhibition of TNF-α production) against HIV-1, although the precise mechanism of this action is still unclear. In this study, we evaluated the inhibitory activity of piperidinylpyrimidine derivatives against HIV-1 LTR activation. Our findings based on compounds structure–activity relationships are discussed in this report.

2. Chemistry and biology

The compounds listed in Tables 1–5 were synthesized as outlined in Schemes 1–6. The 4-methoxybenzylpyrimidine analogs **1–21** were synthesized starting from the 1-benzoylpiperidine-4-carboxylic acid **48**[11] (Scheme 1). Claisen condensation of 4-methoxyphenylacetone with the acid chloride of **48** gave the chromatographically separable 1,3-diketone isomers **49** and **50** as a 1:1 mixture. Ring construction in **50** with guanidine gave the aminopyrimidine **1**, which was hydrolyzed with aqueous 6 N NaOH in EtOH to afford the secondary amine **51**. Subsequent alkylation or acylation on the piperidine nitrogen in the usual manner gave a series of substituted amino or amide compounds (**2–21**). The other 1,3-diketone isomer (**49**) could be processed to the desired piperonyloyl-substituted pyrimidine **34** in a similar way.

* Corresponding author. Tel.: +81 6 6337 5903; fax: +81 6 6337 6010.
E-mail address: norio-fujiwara@ds-pharma.co.jp (N. Fujiwara).

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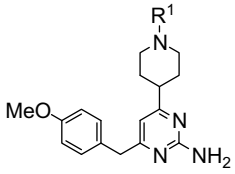
Table 1
Inhibitory activity of piperidinylpyrimidines against HIV-1 LTR activation and TNF-α production

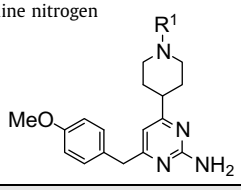


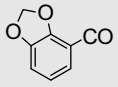
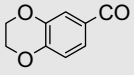
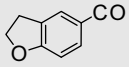
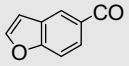
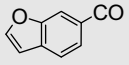
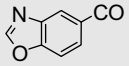
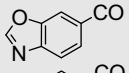
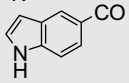
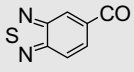
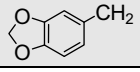
Compound	R^1	Inhibition of HIV-1 LTR IC_{50}[a] (μM)	Inhibition of TNF-α I C_{50}[b] (μM)
1	PhCO	>5 (19%)	1.4
2	4-MeOPhCO	>5 (−5%)	0.5
3	4-ClPhCO	>5 (21%)	0.6
4	4-O_2NPhCO	>5 (23%)	0.7
5	3,4-(OCH_2O) PhCO	0.6 (86%)	0.6
6	3,4-$(MeO)_2$ PhCO	>5 (14%)	0.9
7	3,4-Cl $_2$PhCO	>5 (17%)	0.7
8	3,4-$(MeO)_2$ $PhCH_2CO$	>5 (7%)	1.2
9	3,4-(OCH_2O) $PhCH_2CO$	>5 (16%)	1.8
10	Ac	>5 (0%)	6.1
11	$Me(CH_2)_6CO$	>5 (19%)	1.9

[a] Values within parenthesis indicate % inhibition at 5 μM.
[b] Inhibition in mouse macrophages.

The method in Scheme 1 was problematic for the synthesis of other substituted benzyl analogs, because condensation of benzyl methyl ketone was accompanied by the undesired 1,3-diketone regioisomer, which was the main product in some case.[12] Therefore, an alternative method to avoid production of the undesired isomer was applied (Scheme 2). Piperonyloyl rather than benzoyl protection on the nitrogen of isonipecotic acid was used for convenience. Activation of the carboxylic acid **54** with carbonyldiimidazole (CDI) and coupling with magnesium ethyl malonate[13] gave the β-ketoester **55**. Condensation of **55** with 4-methoxyphenylacetylchloride followed by deethoxycarbonylation of the resultant diketoester **56** under heating conditions in acetic acid gave the 1,3-diketone **57**, which was treated with guanidine to obtain the 4-meyhoxybenzylpyrimidine **5**. The other benzyl analogs **35**–**44** in Table 5 were made in a similar way (Scheme 3). This method can also be applicable to prepare other phenyl (**29**–**31**), phenethyl (**32**), and methyl (**33**) pyrimidine analogs. In the deethoxycarbony-

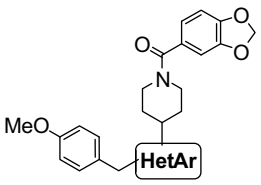
Table 2
Substitution on the piperidine nitrogen

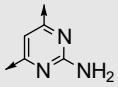
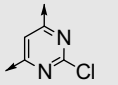
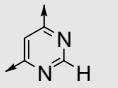
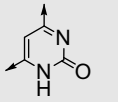
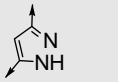
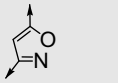
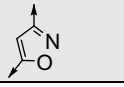


Compound	R	Inhibition of HIV-1 LTR IC_{50}[a] (μM)
12		>5 (13%)
13		>5 (7%)
14		>5 (−8%)
15		>5 (4%)
16		>5 (2%)
17		>5 (−7%)
18		>5 (−8%)
19		>5 (1%)
20		>5 (4%)
21		>5 (−2%)

[a] Values within parenthesis indicate% inhibition at 5 μM.

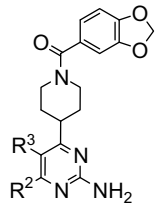
Table 3
Alternative heterocycles



Compound	HetAr	Inhibition of HIV-1 LTR IC_{50}[a] (μM)
5		0.6
22		0.7
23		0.5
24		>5 (−14%)
25		>5 (35%)
26		>5 (35%)
27		>5 (36%)

[a] Values within parenthesis indicate % inhibition at 5 μM.

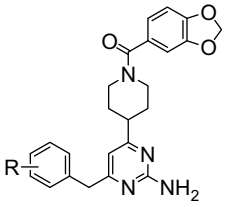
Table 4
Substitution on the pyrimidine



Compound	R^2	R^3	Inhibition of HIV-1 LTR IC_{50}[a] (μM)
5	4-$MeOPhCH_2$	H	0.6
28	–C=C–C=C–		2.6
29	Ph	H	>5 (47%)
30	4-ClPh	H	>5 (42%)
31	4-MeOPh	H	1.9
32	$Ph(CH_2)_2$	H	2.1
33	Me	H	>5 (10%)
34	Me	4-MeOPh	>5 (31%)

[a] Values within parenthesis indicate % inhibition at 5 μM.

Table 5
Substitution on the benzyl moiety: inhibitory activity against HIV-1 LTR activation (PMA or Tat-induced) and TNF-α production



Compound	R	HIV-1 LTR (PMA) IC_{50}[a] (μM)	HIV-1 LTR (Tat) IC_{50}[b] (μM)	TNF-α IC_{50}[c] (μM)
5	4-MeO	0.6	4.0	1.9
35	H	0.8	n.t.	3.2
36	3-MeO	0.3	n.t.	2.7
37	4-Cl	1.3	7.1	13
38	4-Br	0.7	n.t.	n.t.
39	4-BnO	1.2	2.1	n.t.
40	4-MeS	0.2	<1 (57%)	n.t.
41	3,4-$(MeO)_2$	0.6	n.t.	3.2
42	2,5-$(MeO)_2$	0.2	<1 (58%)	7.7
43	3,5-$(MeO)_2$	0.3	<1 (56%)	3.3
44	3,4-(OCH_2O)	0.7	n.t.	3.3
45	4-HO	>5 (36%)	n.t.	n.t.
46	4-MeS(O)	>5 (27%)	n.t.	n.t.
47	4-$MeSO_2$	>5 (37%)	n.t.	n.t.

[a] Values within parenthesis indicate % inhibition at 5 μM.
[b] Values within parenthesis indicate % inhibition at 1 μM.
[c] Inhibition in human PBMC.

lation step, it was useful to use DMSO–water[14] instead of acetic acid for reduction of reaction time.

Conversion into other functional groups, such as chloro, hydrogen, and sulfonyl could also be achieved (Scheme 4). Non-aqueous diazotization-replacement of the 2-aminopyrimidine **5** by the *tert*-butyl nitrite–$SbCl_3$ system[15] gave the 2-chloropyrimidine **22**, which was converted to **23** by hydrogenolysis. The benzyl moiety of compound **39** was removed by hydrogenolysis to give the phenol analog **45**. Oxidation of the methylthio analog **40** with hydrogen peroxide in the absence or presence of Na_2WO_4 afforded the sulfinyl (**46**) or sulfonyl (**47**) analog, respectively.

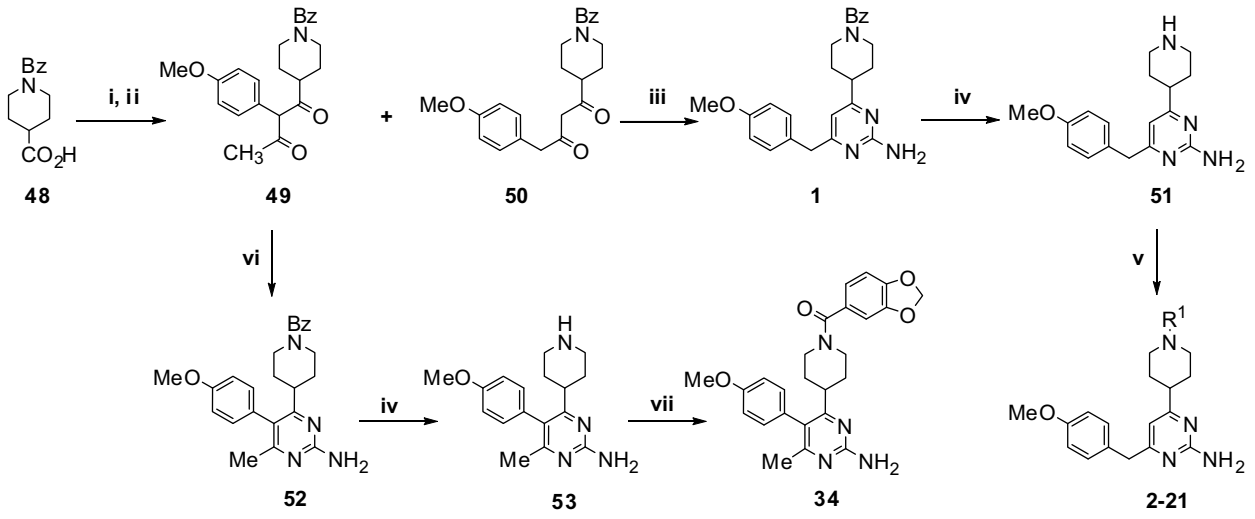
The 1,3-diketone **57** was also used for the synthesis of other heterocycles (Scheme 5). Condensation of **57** with urea and hydrazine gave the respective pyrimidine-2-one **24** and pyrazole **25**. Reaction of **57** with hydroxylamine gave a regioisomeric mixture of hydroxyisoxazolidines, which were separated by chromatography. Subsequent dehydration with methanesulfonyl chloride of each intermediate gave the isooxazoles **26** and **27**. The regiochemistry of the isooxaxoles was determined by ^1H NMR of the intermediate hydroxyisoxazolidines (see Section 4).

Selective ortho acylation[16] of aniline with the 1-benzoyl-4-cyanopiperidine **59**[17] in the presence of boron trichloride and aluminum trichloride gave the 2-aminobenzoyl piperidine **60**, which was reacted with cyanamide and taken through to the 2-aminoquinazoline **28** (Scheme 6).

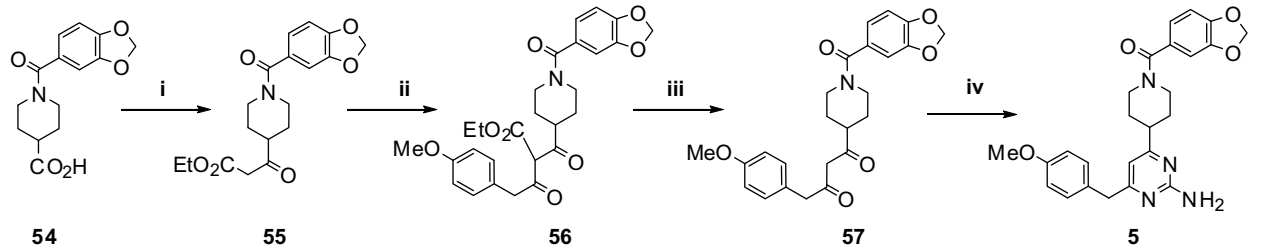
As treatment with phorbol 12-myristate 13-acetate (PMA) or cytokines, such as TNF-α and IL-1, is known to enhance HIV replication through activation of nuclear factor κB (NF-κB),[18] the synthesized compounds were evaluated for their ability to inhibit HIV-1 LTR-driven CAT activity induced by PMA stimulation in Jurkat cells. Introduction of plasmid DNA into cells was achieved as described previously.[19] Test results are given as % inhibition at 5 μM or as IC_{50} value (μM) from a single determination. Some of the synthesized compounds were evaluated for their effects on Tat-mediated activation in Jurkat cells co-transfected with a Tat-expression vector (pSVTat) and HIV-1 LTR CAT. Cell number was also measured using a Coulter counter to evaluate the cytotoxicity of the compounds, and inhibition of cell proliferation was not observed for the compounds tested. Compounds inhibitory activity on TNF-α production in mouse macrophages and human peripheral blood mono-nuclear cells (PBMC) stimulated with lipopolysaccharide (LPS) was also examined. IC_{50} values are given as average of two or three independent experiments.

3. Results and discussion

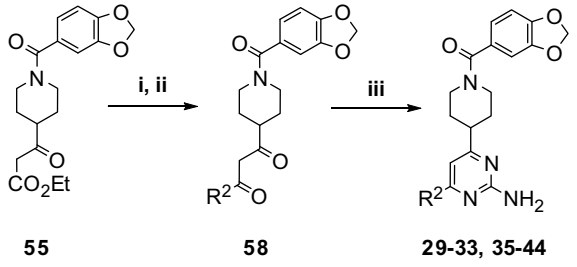
We first evaluated the inhibitory activity against HIV-1 LTR activation of piperidinylpyrimidine analogs that had been prepared as potent TNF-α inhibitors (**1**–**11**).[6] The results are summarized in Table 1. There was no correlation between the inhibitory activity against HIV-1 LTR activation and TNF-α production. At a concen-



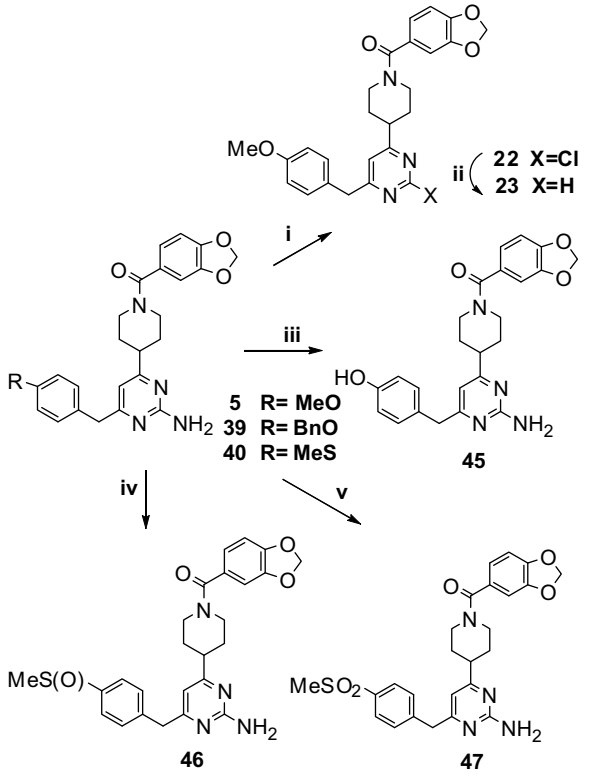
Scheme 1. Reagents and conditions: (i) $SOCl_2$, $CHCl_3$, reflux; (ii) 4-methoxyphenylacetone/LDA, THF, −70 °C; (iii) guanidine hydrochloride, K_2CO_3, pyridine, 110 °C; (iv) 6 N-NaOH, EtOH, reflux; (v) acid chloride, Et_3N, $CHCl_3$; carboxylic acid, HOBt, WSC, $CHCl_3$ or piperonal, $NaBH_3CN$, HCl/MeOH; (vi) guanidine hydrochloride, K_2CO_3, dioxane, 110 °C; (vii) piperonyloylchloride, Et_3N, $CHCl_3$.



Scheme 2. Reagents and conditions: (i) CDI, THF, room temperature, then $(EtO_2CCH_2CO_2)_2Mg$, 50 °C; (ii) 4-methoxyphenylacetylchloride, NaH, THF, 0 °C; (iii) AcOH, 110 °C; (iv) guanidine carbonate, pyridine, 110 °C.

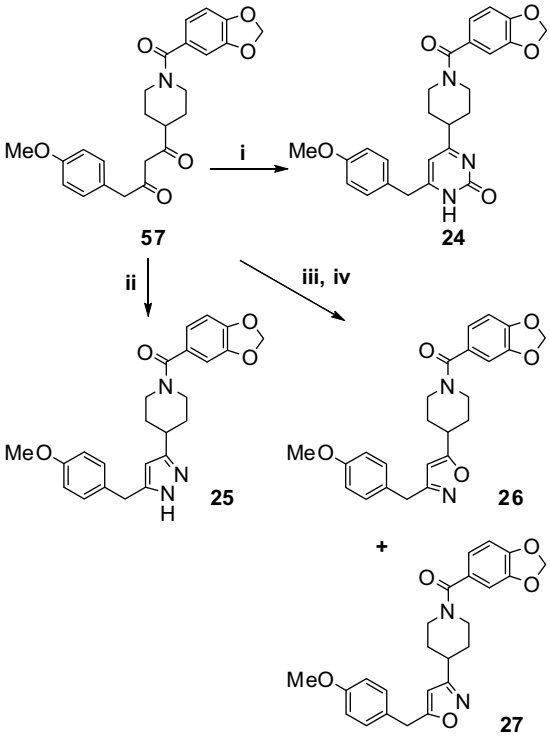


Scheme 3. Reagents and conditions: (i) R^2COCl, NaH, THF, 0 °C; (ii) AcOH, 110 °C, or DMSO, water, 110 °C; (iii) guanidine carbonate, pyridine, 110 °C.

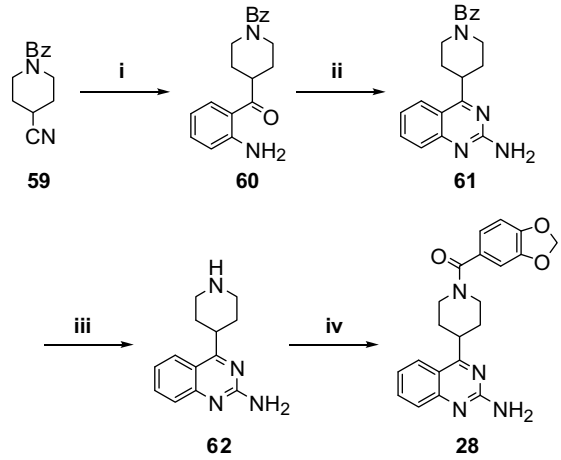


Scheme 4. Reagents and conditions: (i) *t*-BuONO, $SbCl_3$, $(CH_2Cl)_2$, reflux; (ii) H_2, Pd/C, AcONa, MeOH; (iii) H_2, Pd/C, MeOH; (iv) H_2O_2, AcOH, 60 °C; (v) H_2O_2, Na_2WO_4, AcOH, 50 °C.

tration of 5μM most of the compounds showed weak or no inhibitory activity against HIV-1 LTR activation compared with potent TNF-α inhibition. Of all compounds, only compound **5** with a piperonyloyl substituent on the piperidine nitrogen markedly inhibited HIV-1 LTR activation. It is of interest that compound **9**, with



Scheme 5. Reagents and conditions: (i) urea, 150 °C; (ii) hydrazine, EtOH; (iii) hydroxylamine hydrochloride, MeOH; (iv) MsCl, Et_3N, THF, 0 °C.



Scheme 6. Reagents and conditions: (i) aniline, BCl_3, $AlCl_3$, $(CH_2Cl)_2$, reflux; (ii) H_2NCN, 50 °C; (iii) 6 N-NaOH, EtOH, reflux; (iv) piperonyloylchloride, Et_3N, $CHCl_3$.

a homoanalog of piperonyloyl, had only weak inhibitory activity against HIV-1 LTR activation. Various fused-aryl analogs were further evaluated for their ability to replace the effect of the methylenedioxy moiety (Table 2). However, neither compound **12**, a regioisomer of piperonyloyl, nor other fused-benzoyl analogs **13**–**20** had anti-HIV-1 LTR activity. It is interesting to note that compound **20** did not give a promising result, although the benzothiadiazole is considered to be a bioisoster of methylenedioxyphenyl and is reported to be as potent as the methylenedioxyphenyl in antagonizing the endothelin receptor.[20] No inhibitory activity against HIV-1 LTR activation by the 3,4-methylenedioxybenzyl analog **21** suggests that this region prefers non-basic substituents. Since the piperonyloyl group is such an important contributor to the inhibitory activity against HIV-1 LTR activation, it was fixed for further SAR investigation.

Importance of the pyrimidine ring was investigated by replacement with alternative aromatic heterocycles (Table 3). Changing the aminopyrimidine to the structurally similar 2-chloro (**22**) or unsubstituted (**23**) pyrimidine retained the inhibitory activity against HIV-1 LTR activation, whereas the hydroxypyrimidine **24** had diminished activity. The lack of activity in **24** may be due to a preferred keto tautomeric form[21] and suggests that the nitrogen of the pyrimidine is important as a hydrogen-bond acceptor. The other five-membered heterocycles, that is, pyrazole (**25**) and isoxazoles (**26** and **27**) decreased the inhibitory activity against HIV-1 LTR activation, suggesting importance of the pyrimidine structure.

We next focused on substitution at the 5- and 6-positions of the pyrimidine ring (Table 4). The benzo (**28**), methoxyphenyl (**31**), and phenethyl (**32**) analogs were tolerated, whereas the simple methyl analog **33** had decreased inhibitory activity against HIV-1 LTR activation, indicating importance of the lipophilic group for good inhibitory activity. The 4-methoxy group was preferred to the unsubstituted or 4-chloro moiety at the position of the aromatic ring (**29**, **30** vs **31**), suggesting importance of a substituent on the phenyl ring. Bulky substituents, such as methoxyphenyl (**34**), at different position of the pyrimidine were not favorable. This is probably due to conformational change of the adjacent piperidine ring. Some of these findings are similar to those observed for test compounds inhibition of TNF-α production discussed elsewhere.[6]

Since the benzyl analog **5** showed good inhibitory activity against HIV-1 LTR activation, variation of the benzyl substituent was investigated (Table 5). Substitution patterns in number, position, and property affected the inhibitory activity, and 3-methoxy (**36**), 4-methylthio (**40**), 2,5-dimethoxy (**42**), and 3,5-dimethoxy (**43**) gave a threefold increase in the inhibitory activity compared to the unsubstituted benzyl compound **35**. The exceptions were 4-hydroxy (**45**), 4-methylsulfinyl (**46**), and methylsulfonyl (**47**), which markedly reduced the inhibitory activity. This is probably due to hydrophilicity of the substituents, indicating importance of a lipophilic structure in this region.

Regulation of HIV-1 replication is mediated by both cellular factors and viral proteins that play a critical role in transcription of the HIV-1 LTR promoter.[4c,4d] NF-κB is one of the cellular factors induced by stimulants, such as PMA and TNF-α. On the other hand, Tat protein is representative of viral proteins that play a pivotal role in several AIDS-associated conditions as well as in HIV replication, and is therefore a feasible target for anti-HIV therapy.[5e–g,22] In this study we found that the selected analogs **5**, **37**, **39**, **40**, **42**, and **43** also inhibited Tat-induced HIV-1 LTR transactivation with different potency (Table 5). Of these, **40**, **42** and **43** showed the most potent activity (IC_{50} <1 μM) against Tat stimulation. It is noted that the present piperidinylpyrimidines possess anti-Tat activity as well as anti-NF-κB activity. The inhibitory activity of these compound on NF-κB-dependent LTR transactivation is more potent than that of pentoxifylline (IC_{50} 0.5–1 mM)[10a] and similar to that of SB203580 (IC_{50} 0.1–1 μM).[10d] On the other hand, the inhibitory activity on Tat-dependent LTR transactivation is weaker than that of reported compounds such as fluoroquinoline K37 (IC_{50} <0.16 μM)[10f] and Ro5-3335 (IC_{50} 0.1–0.5 μM).[22b,22c] Tat can activate NF-κB by undefined pathways and both proteins may act synergistically in HIV transcription. It has been described that the anti-HIV effects of pentoxifylline were increased when it was combined with the Tat inhibitor.[22d] There is another evidence that some 4-phenylcoumarins display both anti-NF-κB and anti-Tat activities (IC_{50} ~25 μM) which correlate with a stronger inhibition of HIV replication.[5e] Piperidinylpyrimidines, and specially **40**, **42**, and **43**, therefore can be expected to have more potency against HIV replication with a presence of both activities.

Again, TNF-α acts as a key pathogenic mediator of AIDS as well as inflammatory diseases. HIV-1 infection dysregulates the immune system, leading to abnormal production of TNF-α in AIDS patients. Thus, inhibition of TNF-α production and/or effects has been one of the strategies in AIDS therapy. As mentioned above, inhibition of HIV-1 LTR activation by the piperydylpyrimidines investigated in this study did not correlate with these compounds inhibition of TNF-α production, indicating a different mechanism of action for each inhibition. However, some of the findings in the present SAR study, that is, importance of the pyrimidine structure, lipophilic substitution on the pyrimidine ring, and non-basic property of the piperidine nitrogen, are similar to those observed for test compounds inhibition of TNF-α production. The critical difference is the piperonyloyl group on the piperidine nitrogen, which is necessary for HIV-1 LTR inhibition, although a variety of acyl substituents are tolerated for inhibition of TNF-α production. Therefore, it can be expected that these piperidinylpyrimidine analogs have inhibitory activity against TNF-α production. Indeed, the selected analogs **5**, **35**–**37**, **41**–**44** inhibited TNF-α production with IC_{50} values in the micromolar order (Table 5). Human PBMC were used to evaluate the inhibitory activity, because human cells are reasonable to estimate the effect of the compounds than mouse derived cells. In fact there was a slight difference in activity between mouse and human cells for compound **5** (0.6 μM in mouse vs 1.9 μM in human).

In conclusion, we demonstrated in this study that some piperidinylpyrimidine derivatives effectively inhibit both PMA-or Tat-induced HIV-1 LTR transactivation and TNF-α production through different mechanisms. These results suggest that piperidinylpyrimidines are useful as a potent AIDS therapeutics that directly inhibit HIV-1 LTR activation and indirectly suppress TNF-α production. These compounds might also have beneficial effects on AIDS-associated diseases, such as dementia and chachexia through inhibition of TNF-α overexpression. Further studies, including the effectiveness of these compounds as anti-HIV drugs and their mechanism of action are in progress.

4. Experimental

4.1. Chemistry

Melting points were measured on a Thomas Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded at ambient temperature on a JEOL JNM AL-400 FT NMR spectrometer. Chemical shifts are expressed in δ values (ppm) relative to tetramethylsilane as an internal standard, and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). Mass spectra (MS) were measured on a Bruker Esquire 3000 Plus mass spectrometer using ES ionization modes (positive or negative mode). Elemental analyses were performed on a CE Instruments EA1110 elemental analyzer and a Yokogawa IC7000S Ion Chroma-

toanalyzer. In general, commercial reagents and solvents were of reagent grade and were used without further purification. Thin-layer chromatography (TLC) was performed on Merk Kieselgel 60 F254 precoated plates and components were visualized using UV light. Column chromatography was conducted using Merk Kieselgel 60 F254 or Cica-Reagent Silica Gel 60. The yields of all products were not optimized.

4.1.1. 2-(4-Methoxyphenyl)-1-[1-(phenylcarbonyl)piperidin-4-yl]butane-1,3-dione (49) and 4-(4-methoxyphenyl)-1-[1-(phenylcarbonyl)piperidin-4-yl]butane-1,3-dione (50)

A mixture of 1-benzoyl-isonipecotic acid **48**[11] (60 g, 0.257 mol), thionyl chloride (38 ml), and chloroform (360 ml) was refluxed for 3 h. Thereafter, the reaction mixture was concentrated in vacuo and toluene was added and concentrated to remove excess thionyl chloride and obtain 1-benzoyl-4-piperidinecarbonyl chloride as a crude oil. In a separate vessel, and under nitrogen atmosphere, *n*-BuLi (310 ml, 1.66 M in hexane) was added dropwise to a solution of $i\mathrm{Pr}_2\mathrm{NH}$ (72 ml, 0.514 mol) in THF (510 ml) at 0 °C over 1.5 h. The solution was stirred at 0 °C for 30 min and then cooled to −78 °C, and 4-methoxyphenylacetone (84.4 g, 0.514 mol) was added dropwise over 1.5 h. After stirring at the same temperature for another 30 min, a solution of 1-benzoyl-4-piperidinecarbonyl chloride in THF (510 ml) was added dropwise over 2 h. After 30 min, the mixture was acidified with concd HCl. EtOAc was added and the mixture was washed successively with water and brine, dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography using hexane/EtOAc (6:4, v/v) as eluent. Crystallization from EtOAc gave **49** (less polar, 35.3 g, 35%) as a white solid: mp 134–137 °C; ^{1}H NMR ($CDCl_3$) δ 16.85 (1H, s, enol), 7.40–7.34 (5H, m), 7.10–7.04 (2H, br m), 6.93 (2H, d, J = 7.3 Hz), 4.73–4.61 (1H, br m), 3.85 (3H, s), 3.78–3.67 (1H, br m), 2.81–2.51 (2H, br m), 2.46–2.38 (1H, m), 1.88 (3H, s), 1.81–1.59 (4H, br m); MS (ESI, positive) *m/z* 380 (M+1) and **50** (polar, 35.5 g, 35%) as an oil: ^{1}HNMR ($CDCl_3$) δ 15.44 (1H, s, enol), 7.42–7.37 (5H, m), 7.15 (2H, d, J = 8.9 Hz), 6.88 (2H, d, J = 8.9 Hz), 5.44 (1H, s), 4.77–4.67 (1H, br m), 3.84–3.75 (4H, m), 3.56 (2H, s), 3.05–2.78 (2H, br m), 2.46–2.38 (1H, m), 1.94–1.66 (4H, br m); MS (ESI, positive) *m/z* 380 (M+1).

4.1.2. 4-(4-Methoxybenzyl)-6-[1-(phenylcarbonyl)piperidin-4-yl]pyrimidin-2-amine (1)

A mixture of **50** (33 g, 0.084 mmol), guanidine hydrochloride (16 g, 0.168 mol) and potassium carbonate (23.18 g, 0.168 mol) in pyridine (210 ml) was heated at 110 °C for 8 h. The mixture was concentrated in vacuo and the residue was partitioned between $CHCl_3$ and water. The organic layer was washed with brine, dried over Na_2SO_4, and then evaporated. The residue was purified by silica gel column chromatography using EtOAc as eluent. Crystallization from EtOH gave **1** (15.34 g, 45%) as a white solid: mp 170–171 °C; ^{1}H NMR ($CDCl_3$) δ *7.42–7.38* (5H, m), 7.16 (2H, d, J = 8.5 Hz), 6.86 (2H, d, J = 8.5 Hz), 6.26 (1H, s), 4.98 (2H, s), 4.87–4.77 (1H, br m), 3.90–3.84 (1H, br m), 3.83 (2H, s), 3.80 (3H, s), 3.10–3.01, (1H, br m), 2.89–2.79 (1H, br m), 2.69–2.61 (1H, m), 1.95–1.72 (4H, m); MS (ESI, positive) *m/z* 403 (M+1); Anal. Calcd for $C_{24}H_{26}N_4O_2$: C, 71.62; H, 6.51; N, 13.92. Found: C, 71.67; H, 6.47; N, 13.98.

4.1.3. 4-(4-Methoxybenzyl)-6-piperidin-4-ylpyrimidin-2-amine (51)

A mixture of **1** (15 g, 37.3 mmol), EtOH (130 ml), and aqueous NaOH solution (6 N, 130 ml) was refluxed for 6 h under nitrogen atmosphere. Thereafter, ethanol was removed by evaporation and the residue was extracted with dichloromethane. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was crystallized from iPrOH–Et_2O to give **51** (8.81 g, 79%) as a white solid: mp 112–113 °C; ^{1}H NMR ($CDCl_3$) δ 7.16 (2H, d, J = 8.5 Hz), 6.85 (2H, d, J = 8.5 Hz), 6.28 (1H, s), 4.97 (2H, s), 3.83 (2H, s), 3.80 (3H, s), 3.16–3.12 (2H, m), 2.71–2.64 (2H, m), 2.55–2.42 (1H, m), 1.81–1.77 (2H, m), 1.62–1.51 (2H, m); MS (ESI, positive) *m/z* 299 (M+1); Anal. Calcd for $C_{17}H_{22}N_4O$: C, 68.43; H, 7.43; N, 18.78. Found: C, 68.35; H, 7.46; N, 18.90.

4.1.4. 4-(4-Methoxybenzyl)-6-{1-[(4-methoxyphenyl)carbonyl]-piperidin-4-yl}pyrimidin-2-amine (2)

To an ice cold solution of **51** (200 mg, 0.67 mmol) and Et_3N (0.1 ml, 0.70 mmol) in $CHCl_3$ (100 ml) was added 4-methoxybenzoyl chloride (120 mg, 0.70 mmol) under nitrogen atmosphere. After stirring for 1 h the mixture was washed successively with water and brine, dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by silica gel column chromatography using $CHCl_3$/MeOH (50:1, v/v) as eluent. Crystallization from EtOH gave **2** (286 mg, 99%) as a white solid: mp 169–170.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.39 (2H, d, J = 8.8 Hz), 7.16 (2H, d, J = 8.8 Hz), 6.91 (2H, d, J = 8.8 Hz), 6.86 (2H, d, J = 8.8 Hz), 6.27 (1H, s), 4.95 (2H, s), 4.83–4.62 (1H, br m), 4.11–3.90 (1H, br m), 3.83 (5H, s), 3.80 (3H, s), 3.08–2.78 (2H, br m), 2.69–2.61 (1H, m), 1.87–1.69 (4H, m); MS (ESI, positive) *m/z* 433 (M+1); Anal. Calcd for $C_{25}H_{28}N_4O_3{\cdot}0.25H_2O$: C, 68.71; H, 6.57; N, 12.82. Found: C, 68.93; H, 6.48; N, 12.93.

Compounds **3**–**7**, **10**, and **11** were prepared by using a procedure similar to that for the preparation of **2**.

4.1.5. 4-{1-[(4-Chlorophenyl)carbonyl]piperidin-4-yl}-6-(4-methoxybenzyl)pyrimidin-2-amine (3)

A white solid from MeOH (246 mg, 84%), mp 188–189 °C; ^{1}H NMR ($CDCl_3$) δ 7.39 (2H, d, J = 8.8 Hz), 7.36 (2H, d, J = 8.8 Hz), 7.16 (2H, d, J = 8.5 Hz), 6.86 (2H, d, J = 8.5 Hz), 6.26 (1H, s), 4.94 (2H, s), 4.82–4.74 (1H, br m), 3.88–3.83 (1H, br m), 3.83, (2H, s), 3.80 (3H, s), 3.11–3.01 (1H, br m), 2.89–2.78 (1H, br m), 2.69–2.61 (1H, m), 1.93–1.69 (4H, m); MS (ESI, positive) *m/z* 437 (M+1); Anal. Calcd for $C_{24}H_{25}ClN_4O_2$: C, 65.97; H, 5.77; N, 12.82; Cl, 8.11. Found: C, 65.85; H, 5.71; N, 12.88; Cl, 8.08.

4.1.6. 4-(4-Methoxybenzyl)-6-{1-[(4-nitrophenyl)carbonyl]-piperidin-4-yl}pyrimidin-2-amine (4)

A pale yellow solid from MeOH (200 mg, 67%), mp 178–179 °C; ^{1}H NMR ($CDCl_3$) δ 8.28 (2H, d, J = 8.5 Hz), 7.58 (2H, d, J = 8.5 Hz), 7.16 (2H, d, J = 8.8 Hz), 6.86 (2H, d, J = 8.8 Hz), 6.26 (1H, s), 4.95 (2H, s), 4.83–4.78 (1H, m), 3.83 (2H, s), 3.80 (3H, s), 3.70–3.66 (1H, m), 3.15–3.08 (1H, m), 2.91–2.84 (1H, m), 2.71–2.64 (1H, m), 1.98–1.67 (4H, m); MS (ESI, positive) *m/z* 448 (M+1); Anal. Calcd for $C_{24}H_{25}N_5O_4$: C, 64.42; H, 5.63; N, 15.65. Found: C, 64.37; H, 5.64; N, 15.78.

4.1.7. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (5)

A white solid from EtOH (858 mg, 96%), mp 170–171 °C; ^{1}H NMR ($CDCl_3$) δ 7.16 (2H, d, J *=8.5* Hz), 6.95–6.91 (2H, m), 6.86 (2H, d, J = 8.5 Hz), 6.82 (1H, d, J = 8.0 Hz), 6.26 (1H, s), 6.00 (2H, s), 4.94 (2H, s), 4.82–4.54 (1H, br m), 4.18–3.86 (1H, br m), 3.83 (2H, s), 3.80 (3H, s), 3.08–2.78 (2H, br m), 2.68–2.61 (1H, m), 1.90–1.66 (4H, m); MS (ESI, positive) *m/z* 447 (M+1); Anal. Calcd for $C_{25}H_{26}N_4O_4$: C, 67.25; H, 5.87; N, 12.55. Found: C, 67.06; H, 5.88; N, 12.78.

4.1.8. 4-{1-[(3,4-Dimethoxyphenyl)carbonyl]piperidin-4-yl}-6-(4-methoxybenzyl)pyrimidin-2-amine (6)

A white solid from MeOH (230 mg, 74%), mp 159–160 °C; ^{1}H NMR ($CDCl_3$) δ 7.16 (2H, d, J = 8.5 Hz), 7.00–6.98 (2H, m), 6.87–6.84 (3H, m), 6.27 (1H, s), 4.95 (2H, s), 4.80–4.64 (1H, br m), 4.16–3.98 (1H, br m), 3.91 (3H, s), 3.90 (3H, s), 3.83 (2H, s), 3.80

(3H, s), 3.04–2.84 (2H, br m), 2.69–2.62 (1H, m), 1.87–1.66 (4H, m); MS (ESI, positive) *m/z* 463 (M+1); Anal. Calcd for $C_{26}H_{30}N_4O_4$: C, 67.51; H, 6.54; N, 12.11. Found: C, 67.49; H, 6.49; N, 12.12.

4.1.9. 4-{1-[(3,4-Dichlorophenyl)carbonyl]piperidin-4-yl}-6-(4-methoxybenzyl)pyrimidin-2-amine (7)

A white solid from MeOH (272 mg, 86%), mp 195–196 °C; ^{1}H NMR ($CDCl_3$) δ *7.52–7.48* (2H, m), 7.26–7.24 (1H, m), 7.16 (2H, d, *J* = 8.8 Hz), 6.86 (2H, d, *J* = 8.8 Hz), 6.26 (1H, s), 4.95 (2H, s), 4.80–4.71 (1H, br m), 3.83 (2H, s), 3.80 (3H, s), 3.82–3.73 (1H, br m), 3.13–3.03 (1H, br m), 2.87–2.78 (1H, br m), 2.70–2.62 (1H, m), 1.94–1.69 (4H, br m); MS (ESI, positive) *m/z* 471 (M+1); Anal. Calcd for $C_{24}H_{24}Cl_2N_4O_2$: C, 61.15; H, 5.13; Cl, 15.04; N, 11.89. Found: C, 61.06; H, 5.10; Cl, 14.96; N, 11.94.

4.1.10. 4-(1-Acetylpiperidin-4-yl)-6-(4-methoxybenzyl)pyrimidin-2-amine (10)

A white solid from EtOH (227 mg, 99%), mp 203–205 °C, ^{1}H NMR ($CDCl_3$) δ 7.16 (2H, d, *J* = 8.8 Hz), 6.86 (2H, d, *J* = 8.8 Hz), 6.24 (1H, s), 4.96 (2H, s), 4.73–4.68 (1H, m), 3.91–3.86 (1H, m), 3.82 (2H, s), 3.80 (3H, s), 3.10 (1H, td, *J* = 13.0, 2.7 Hz), 2.64–2.55 (2H, m), 2.10 (3H, s), 1.89–1.81 (2H, m), 1.69–1.54 (2H, m); MS (ESI, positive) *m/z* 341 (M+1); Anal. Calcd for $C_{19}H_{24}N_4O_2{\cdot}0.25H_2O$: C, 66.16; H, 7.16; N, 16.24. Found: C, 65.92; H, 7.06; N, 16.07.

4.1.11. 4-(4-Methoxybenzyl)-6-(1-octanoylpiperidin-4-yl)pyrimidin-2-amine (11)

A white solid from hexane/EtOH (230 mg, 90%), mp 101–103 °C; ^{1}H NMR ($CDCl_3$) δ 7.16 (2H, d, *J* = 8.8 Hz), 6.86 (2H, d, *J* = 8.8 Hz), 6.24 (1H, s), 4.93 (2H, s), 4.75–4.70 (1H, m), 3.96–3.90 (1H, m), 3.82 (2H, s), 3.80 (3H, s), 3.10–3.02 (1H, m), 2.64–2.54 (2H, m), 2.32 (2H, t, *J* = 7.7 Hz), 1.90–1.81 (2H, m), 1.65–1.55 (4H, m), 1.32–1.28 (8H, m), 0.88 (3H, t, *J* = 7.0 Hz); MS (ESI, positive) *m/z* 425 (M+1); Anal. Calcd for $C_{25}H_{36}N_4O_2$: C, 70.72; H, 8.55; N, 13.20. Found: C, 70.61; H, 8.56; N, 13.16.

4.1.12. 4-{1-[(3,4-Dimethoxyphenyl)acetyl]piperidin-4-yl}-6-(4-methoxybenzyl)pyrimidin-2-amine (8)

To a solution of **51** (200 mg, 0.67 mmol), 3,4-dimethoxyphenylacetic acid (138 mg, 0.70 mmol), and 1-hydroxybenzotriazole (HOBt, 95 mg, 0.70 mmol) in $CHCl_3$ (100 ml) was added *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC·HCl, 135 mg, 0.70 mmol) at room temperature under nitrogen atmosphere. After stirring for 2 h, the mixture was washed successively with aqueous satd $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, and then evaporated. The residue was purified by silica gel column chromatography using $CHCl_3$/MeOH (50:1, v/v) as eluent. Crystallization from *i*PrOH gave **8** (308 mg, 96%) as a white solid: mp 140–140.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.15 (2H, d, *J* = 8.5 Hz), 6.85 (2H, d, *J* = 8.5 Hz), 6.82–6.79 (2H, m), 6.75 (1H, dd, *J* = 8.3, 2.0 Hz), 6.19 (1H, s), 4.94 (2H, s), 4.75–4.71 (1H, m), 3.97–3.93 (1H, m), 3.88 (3H, s), 3.86 (3H, s), 3.81 (2H, s), 3.80 (3H, s), 3.69 (2H, s), 3.05–2.98 (1H, m), 2.65–2.52 (2H, m), 1.84–1.70 (2H, m), 1.62–1.52 (1H, m), 1.44–1.33 (1H, m); MS (ESI, positive) *m/z* 477 (M+1); Anal. Calcd for $C_{27}H_{32}N_4O_4$: C, 68.05; H, 6.77; N, 11.76. Found: C, 67.96; H, 6.78; N, 11.78.

Compounds **9** and **12**–**20** were prepared using a procedure similar to that for the preparation of **8**.

4.1.13. 4-[1-(1,3-Benzodioxol-5-ylacetyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (9)

A white solid from *i*PrOH, (198 mg, 86%), 155–156 °C; ^{1}H NMR ($CDCl_3$) δ 7.15 (2H, d, *J* = 8.5 Hz), 6.86 (2H, d, *J* = 8.5 Hz), 6.77–6.74 (2H, m), 6.67 (1H, dd, *J* = 7.9, 1.6 Hz), 6.21 (1H, s), 5.94 (2H, s), 4.93 (2H, s), 4.74–4.69 (1H, m), 3.97–3.91 (1H, m), 3.82 (2H, s), 3.80 (3H, s), 3.64 (2H, s), 3.06–2.99 (1H, m), 2.66–2.53 (2H, m), 1.86–1.76 (2H, m), 1.61–1.40 (2H, m); MS (ESI, positive) *m/z* 461 (M+1); Anal. Calcd for $C_{26}H_{28}N_4O_4$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.62; H, 6.12; N, 12.18.

4.1.14. 4-[1-(1,3-Benzodioxol-4-ylcarbonyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (12)

A white solid from *i*PrOH (488 mg, 82%), mp 167–167.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.16 (2H, t, *J* = 5.9 Hz), 6.91–6.85 (5H, m), 6.26 (1H, s), 5.99 (2H, s), 4.95 (2H, s), 4.83–4.81 (1H, m), 3.83 (2H, s), 3.81–3.76 (1H, m), 3.12 (1H, t, *J* = 12.4 Hz), 2.82 (1H, t, *J* = 12.1 Hz), 2.68–2.61 (1H, m), 1.95–1.68 (4H, m); MS (ESI, positive) *m/z* 447 (M+1); Anal. Calcd for $C_{25}H_{26}N_4O_4$: C, 67.25; H, 5.87; N, 12.55. Found: C, 67.10; H, 5.88; N, 12.58.

4.1.15. 4-[1-(2,3-Dihydro-1,4-benzodioxin-6-ylcarbonyl)-piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (13)

A white solid from *i*PrOH (568 mg, 92%), mp 187–188 °C; ^{1}H NMR ($CDCl_3$) δ 7.16 (2H, d, *J* = 8.5 Hz), 6.96 (1H, d, *J* = 2.0 Hz), 6.92 (1H, dd, *J* = 8.0, 2.0 Hz), 6.87–6.85 (3H, m), 6.26 (1H, s), 4.93 (2H, s), 4.80–4.67 (1H, br m), 4.27 (4H, s), 4.08–3.91 (1H, br m), 3.83 (2H, s), 3.80 (3H, s), 3.03–2.85 (2H, br m), 2.68–2.60 (1H, m), 1.86–1.67 (4H, m); MS (ESI, positive) *m/z* 461 (M+1); Anal. Calcd for $C_{26}H_{28}N_4O_4$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.59; H, 6.14; N, 12.04.

4.1.16. 4-[1-(2,3-Dihydro-1-benzofuran-5-ylcarbonyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (14)

A white solid from *i*PrOH (581 mg, 98%), mp 162–264 °C; ^{1}H NMR ($CDCl_3$) δ 7.31 (1H, s), 7.20–7.15 (3H, m), 6.86 (2H, d, *J* = 8.5 Hz), 6.77 (1H, d, *J* = 8.0 Hz), 6.27 (1H, s), 4.94 (2H, s), 4.83–4.67 (1H, br m), 4.61 (2H, t, *J* = 8.7 Hz), 4.29–3.93 (1H, br m), 3.83 (2H, s), 3.80 (3H, s), 3.23 (2H, t, *J* = 8.7 Hz), 3.02–2.86 (2H, br m), 2.68–2.61 (1H, m), 1.87–1.67 (4H, m); MS (ESI, positive) *m/z* 445 (M+1); Anal. Calcd for $C_{26}H_{28}N_4O_3$: C, 70.25; H, 6.35; N, 12.60. Found: C, 70.47; H, 6.38; N, 12.63.

4.1.17. 4-[1-(1-Benzofuran-5-ylcarbonyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (15)

A white solid from EtOH (459 mg, 77%), mp 188–188.5 °C; ^{1}H NMR ($CDCl_3$) δ *7.69–7.67* (2H, m), 7.52 (1H, d, *J* = 8.3 Hz), 7.37 (1H, dd, *J* = 8.3, 1.5 Hz), 7.17 (2H, d, *J* = 8.8 Hz), 6.86 (2H, d, *J* = 8.8 Hz), 6.80 (1H, dd, *J* = 2.2, 1.0 Hz), 6.27 (1H, s), 4.96 (2H, s), 4.84–4.78 (1H, br m), 3.96–3.88 (1H, br m), 3.83 (2H, s), 3.80 (3H, s), 3.05–2.88 (2H, br m), 2.70–2.62 (1H, m), 1.88–1.72 (4H, br m); MS (ESI, positive) *m/z* 443 (M+1); Anal. Calcd for $C_{26}H_{26}N_4O_3$: C, 70.57; H, 5.92; N, 12.66. Found: C, 70.50; H, 5.95; N, 12.69.

4.1.18. 4-[1-(1-Benzofuran-6-ylcarbonyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (16)

A white solid from MeOH (520 mg, 88%), mp 186–187 °C; ^{1}H NMR ($CDCl_3$) δ 7.70 (1H, d, *J* = 2.2 Hz), 7.63–7.59 (2H, m), 7.31 (1H, dd, *J* = 7.9, 1.1 Hz), 7.17 (2H, d, *J* = 8.5 Hz), 6.86 (2H, d, *J* = 8.5 Hz), 6.80 (1H, dd, *J* = 2.2, 1.1 Hz), 6.28 (1H, s), 4.94 (2H, s), 4.85–4.76 (1H, br m), 4.00–3.91 (1H, br m), 3.84 (2H, s), 3.80 (3H, s), 3.05–2.92 (2H, br m), 2.69–2.63 (1H, m), 1.88–1.73 (4H, br m); MS (ESI, positive) *m/z* 443 (M+1); Anal. Calcd for $C_{26}H_{26}N_4O_3$: C, 70.57; H, 5.92; N, 12.66. Found: C, 70.36; H, 5.95; N, 12.69.

4.1.19. 4-[1-(1,3-Benzoxazol-5-ylcarbonyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (17)

A white solid from MeOH (524 mg, 88%), mp 178–179 °C; ^{1}H NMR ($CDCl_3$) δ 8.16 (1H, s), 7.86 (1H, d, *J* = 1.5 Hz), 7.63 (1H, d, *J* = 8.5 Hz), 7.50 (1H, dd, *J* = 8.5, 1.5 Hz), 7.17 (2H, d, *J* = 8.5 Hz), 6.86 (2H, d, *J* = 8.5 Hz), 6.27 (1H, s), 4.96 (2H, s), 4.90–4.77 (1H,

br m), 3.92–3.86 (1H, br m), 3.84 (2H, s), 3.80 (3H, s), 3.11–2.88 (2H, br m), 2.71–2.63 (1H, m), 1.94–1.72 (4H, m); MS (ESI, positive) *m/z* 444 (M+1); Anal. Calcd for $C_{25}H_{25}N_5O_3$: C, 67.70; H, 5.68; N, 15.79. Found: C, 67.42; H, 5.70; N, 15.85.

4.1.20. 4-[1-(1,3-Benzoxazol-6-ylcarbonyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (18)

A white solid from MeOH (492 mg, 83%), mp 185–186 °C; ^{1}H NMR ($CDCl_3$) δ 8.17 (1H, s), 7.82 (1H, d, J = 8.0 Hz), 7.69 (1H, d, J = 1.2 Hz), 7.44 (1H, dd, J = 8.0, 1.2 Hz), 7.16 (2H, d, J = 8.5 Hz), 6.86 (2H, d, J = 8.5 Hz), 6.27 (1H, s), 4.99 (2H, s), 4.86–4.76 (1H, br m), 3.92–3.86 (1H, br m), 3.83 (2H, s), 3.80 (3H, s), 3.09–2.89 (2H, m), 2.70–2.63 (1H, m), 1.95–1.79 (4H, m); MS (ESI, positive) *m/z* 444 (M+1); Anal. Calcd for $C_{25}H_{25}N_5O_3{\cdot}0.25H_2O$: C, 67.02; H, 5.74; N, 15.63. Found: C, 67.22; H, 5.72; N, 15.52.

4.1.21. 4-[1-(1H-Indol-5-ylcarbonyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (19)

A white solid from MeOH/H_2O (550 mg, 93%): mp 146–147 °C; ^{1}H NMR ($CDCl_3$) δ 8.36 (1H, br s), 7.74 (1H, s), 7.40 (1H, d, J = 8.3 Hz), 7.29–7.26 (2H, m), 7.17 (2H, d, J = 8.5 Hz), 6.86 (2H, d, J = 8.5 Hz), 6.60–6.58 (1H, m), 6.28 (1H, s), 4.95 (2H, s), 4.85–4.69 (1H, br m), 4.19–3.99 (1H, br m), 3.84 (2H, s), 3.80 (3H, s), 3.06–2.88 (2H, br m), 2.70–2.62 (1H, m), 1.85–1.71 (4H, m); MS (ESI, positive) *m/z* 442 (M+1); Anal. Calcd for $C_{26}H_{27}N_5O_2$: C, 70.73; H, 6.16; N, 15.86. Found: C, 70.49; H, 6.21; N, 15.79.

4.1.22. 4-[1-(2,1,3-Benzothiadiazol-5-ylcarbonyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (20)

A white solid from MeOH (518 mg, 84%), mp 164–165 °C; ^{1}H NMR ($CDCl_3$) δ *8.08–8.05* (2H, m), 7.65 (1H, dd, J = 8.8, 1.7 Hz), 7.17 (2H, d, J = 8.5 Hz), 6.86 (2H, d, J = 8.5 Hz), 6.27 (1H, s), 4.95 (2H, s), 4.89–4.81 (1H, br m), 3.92–3.86 (1H, br m), 3.84 (2H, s), 3.80 (3H, s), 3.21–3.09 (1H, br m), 2.97–2.85 (1H, br m), 2.72–2.65 (1H, m), 1.98–1.74 (4H, br m); MS (ESI, positive) *m/z* 461 (M+1); Anal. Calcd for $C_{24}H_{24}N_6O_2S$: C, 62.59; H, 5.25; N, 18.25; S, 6.96. Found: C, 62.48; H, 5.20; N, 18.32, S, 6.91.

4.1.23. 4-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (21)

To a solution of **51** (700 mg, 2.35 mmol), piperonal (706 mg, 4.70 mmol), and aqueous HCl (4N, 0.6 ml) in MeOH (70 ml) was added $NaBH_3CN$ (74 mg, 1.18 mmol) and the mixture was stirred at room temperature over night. After evaporation of the solvent, the residue was diluted with aqueous satd $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, and then evaporated. The residue was purified by silica gel column chromatography using $CHCl_3$/MeOH (30:1, v/v) as eluent. Crystallization from *i*PrOH gave **21** (400 mg, 39%) as a white solid: mp 126–127 °C; ^{1}H NMR ($CDCl_3$) δ 7.15 (2H, d, J = 8.5 Hz), 6.86–6.83 (3H, m), 6.74 (2H, s), 6.28 (1H, s), 5.94 (2H, s), 4.91 (2H, s), 3.82 (2H, s), 3.80 (3H, s), 3.40 (2H, s), 2.96–2.93 (2H, m), 2.41–2.33 (1H, m), 2.02–1.95 (2H, m), 1.81–1.67 (4H, m); MS (ESI, positive) *m/z* 433 (M+1); Anal. Calcd for $C_{25}H_{28}N_4O_3$: C, 69.42; H, 6.53; N, 12.95. Found: C, 69.31; H, 6.57; N, 13.20.

4.1.24. 5-(4-Methoxyphenyl)-4-methyl-6-[1-(phenylcarbonyl)-piperidin-4-yl]pyrimidin-2-amine (52)

A mixture of **49** (30 g, 0.076 mol), guanidine hydrochloride (72.6 g, 0.76 mol), and potassium carbonate (105 g, 0.76 mol) in dioxane (300 ml) was heated at 100 °C for 10 h. The reaction mixture was filtered off and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (19:1, v/v) as eluent. Crystallization from EtOH gave **52** (2.14 g, 7%) as a white solid: mp 226–227 °C; ^{1}H NMR ($CDCl_3$) δ *7.42–7.36* (5H, m), 7.07–6.93 (4H, m), 4.92 (2H, s), 4.76–4.69 (1H, br m), 3.87 (3H, s), 3.78–3.70 (1H, br m), 2.87–2.76 (1H, br m), 2.68–2.53 (2H, m), 2.08 (3H, s), 1.95–1.81 (2H, br m), 1.73–1.47 (2H, m); MS (ESI, positive) *m/z* 403 (M+1); Anal. Calcd for $C_{24}H_{26}N_4O_2$: C, 71.62; H, 6.51; N, 13.92. Found: C, 71.52; H, 6.48; N, 14.03.

The yield of **52** was very low, because the major product of this reaction was deacetylated ketone, 2-(4-methoxyphenyl)-1-[1-(phenylcarbonyl)piperidin-4-yl]ethanone, which was obtained by retro-Claisen cleavage (16.15 g, 57% as an oil): ^{1}H NMR ($CDCl_3$) δ *7.41–7.36* (5H, m), 7.11 (2H, d, J = 8.8 Hz), 6.87 (2H, d, J = 8.8 Hz), 4.71–4.55 (1H, br m), 3.80 (3H, s), 3.79–3.71 (1H, br m), 3.70 (2H, s), 3.04–2.68 (2H, br m), 2.76–2.68 (1H, m), 1.97–1.57 (4H, m).

4.1.25. 5-(4-Methoxyphenyl)-4-methyl-6-piperidin-4-ylpyrimidin-2-amine (53)

Compound **53** was prepared from **52** (1.9 g, 4.7 mmol) using a procedure similar to that for the preparation of **51**. Crystallization from EtOH gave **53** (1.24 g, 88%) as a white solid: mp 191–192 °C; ^{1}H NMR ($CDCl_3$) δ 7.03 (2H, d, J = 8.8 Hz), 6.96 (2H, d, J = 8.8 Hz), 4.89 (2H, s), 3.87 (3H, s), 3.04 (2H, dt, J = 12.6, 2.7 Hz), 2.56–2.49 (1H, m), 2.43 (2H, dt, J = 12.6, 2.7 Hz), 2.07 (3H, s), 1.80–1.69 (2H, m), 1.53–1.48 (2H, m); MS (ESI, positive) *m/z* 299 (M+1); Anal. Calcd for $C_{17}H_{22}N_4O{\cdot}0.1EtOH$: C, 68.18; H, 7.52; N, 18.49. Found: C, 67.92; H, 7.38; N, 18.79..

4.1.26. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-5-(4-methoxyphenyl)-6-methylpyrimidin-2-amine (34)

Compound **34** was prepared from **53** (200 mg, 0.67 mmol) using a procedure similar to that for the preparation of **2**. Crystallization from EtOH gave **34** (289 mg, 97%) as a white solid: mp 220–222 °C; ^{1}H NMR ($CDCl_3$) δ 7.03 (2H, d, J = 7.8 Hz), 6.96 (2H, d, J = 8.8 Hz), 6.93–6.90 (2H, m), 6.80 (1H, d, J = 7.8 Hz), 5.99 (2H, s), 4.91 (2H, s), 4.78–4.49 (1H, br m), 3.98–3.73 (4H, m), 2.82–2.51 (3H, m), 2.08 (3H, s), 1.91–1.82 (2H, m), 1.62–1.50 (2H, m); MS (ESI, positive) *m/z* 447 (M+1); Anal. Calcd for $C_{25}H_{26}N_4O_4{\cdot}0.25H_2O$: C, 66.58; H, 5.92; N, 12.42. Found: C, 66.68; H, 5.87; N, 12.72.

4.1.27. 1-(1,3-Benzodioxol-5-ylcarbonyl)piperidine-4-carboxylic acid (54)

To an ice cold solution of isonipecotic acid (119.77 g, 0.927 mol) and potassium carbonate (256.32 g, 1.855 mol) in water (900 ml) was added piperonyloyl chloride (155.6 g, 0.843 mol) in THF (900ml) with vigorous stirring over 2 h. The reaction temperature was kept below 10 °C during the addition, and the mixture was stirred for an additional 30 min. The reaction mixture was acidified with concd HCl and extracted with chloroform. The organic layer was washed with water, dried over anhydrous $MgSO_4$, and then evaporated. The residue was crystallized from methanol to give **54** (210.81 g, 90%) as a white solid: mp 156–158 °C; ^{1}H NMR (DMSO-d_6) δ 12.30 (1H, s), 6.96–6.94 (2H, m), 6.88 (1H, dd, J = 8.0, 1.5 Hz), 6.07 (2H, s), 4.29–3.54 (2H, br m), 3.09–2.88 (2H, br m), 2.55–2.49 (1H, m), 1.88–1.79 (2H, br m), 1.54–1.45 (2H, m).

4.1.28. Ethyl 3-[1-(1,3-benzodioxol-5-ylcarbonyl)piperidin-4-yl]-3-oxopropanoate (55)

To a solution of **54** (110 g, 0.397 mol) in THF (2 L) was added *N*,*N*′-carbonyldiimidazole (70.8 g, 0.437 mol) in several portions at room temperature under nitrogen atmosphere. After stirring for 3.5 h, magnesium ethyl malonate[13] (125.12 g, 0.437 mol) was added and the mixture was refluxed for 3 h. The mixture was then concentrated, diluted with aqueous satd $NaHCO_3$ solution (1.5 L), and extracted with ethyl acetate (3 L). The organic layer was washed with brine twice, dried over anhydrous $MgSO_4$, and then evaporated. The residue was purified by column chromatography using hexane/EtOAc (1:1, v/v) as eluent to give the ketoester **55** (132.40 g, 96.1%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 6.92–6.88

(2H, m), 6.82 (1H, d, J = 8.0 Hz), 6.00 (2H, s), 4.64–4.37 (1H, br m), 4.20 (2H, q, J = 7.2 Hz), 4.15–3.86 (1H, br m), 3.51 (2H, s), 3.04–2.94 (2H, m), 2.82–2.74 (1H, m), 1.97–1.84 (2H, m), 1.69–1.61 (2H, m), 1.28 (3H, t, J = 7.2 Hz); MS (ESI, positive) m/z 348 (M+1).

4.1.29. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2-amine (5)

To an ice cooled solution of **55** (3.47 g, 10 mmol) in THF (17 ml) was added sodium hydride (60% oil dispersion, 840 mg, 21 mmol) in several portions under nitrogen atmosphere. The reaction temperature was kept below 5 °C during the addition and the mixture was further stirred for 15 min. To this mixture was added 4-meyhoxyphenylacetyl chloride (2.03 g, 11 mmol) in THF (7 ml) dropwise over 15 min. After stirring for 15 min at 5 °C, the mixture was acidified with aqueous HCl (1 N) and extracted with EtOAc. The organic layer was washed with water, dried over Na_2SO_4, and then evaporated to give ethyl 2-{[1-(1,3-benzodioxol-5-ylcarbonyl)piperidin-4-yl]carbonyl}-4-(4-methoxyphenyl)-3-oxobutanoate (**56**, 5.67 g) as a crude oil. The diketoester **56** was used in the following reaction without further purification. A mixture of **56** (5.67 g) and AcOH (44 ml) was refluxed for 11 h. The mixture was then poured into water and extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4, and then evaporated. The residue was purified by silica gel column chromatography using hexane/EtOAc (1:1, v/v) as eluent to give 1-[1-(1,3-benzodioxol-5-ylcarbonyl)-piperidin-4-yl]-4-(4-methoxyphenyl)butane-1,3-dione **57** (3.13 g, 74%) as an oil: ^{1}H NMR ($CDCl_3$) δ 15.44 (1H, s, enol), 7.15 (2H, d, J = 8.5 Hz), 6.92–6.86 (4H, m), 6.81 (1H, d, J = 7.6 Hz), 6.00 (2H, s), 5.44 (1H, s), 4.70–4.39 (1H, br m), 4.20–3.90 (1H, br m), 3.81 (3H, s), 3.55 (2H, s), 2.96–2.83 (2H, m), 2.45–2.37 (1H, m), 1.86–1.78 (2H, m), 1.66–1.59 (2H, m); MS (ESI, positive) m/z 424 (M+1).

A mixture of this 1,3-diketone (**57**, 1 g, 2.36 mmol), guanidine carbonate (425 mg, 2.36 mmol), and pyridine (5 ml) was heated at 110 °C for 4.5 h. The mixture was then concentrated in vacuo, and the residue was partitioned between $CHCl_3$ and aqueous satd $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4, and then evaporated. Crystallization from ethanol gave **5** (750 mg, 71%). ^{1}H NMR spectrum of the prepared compound was identical to that of **5** prepared by an alternative method.

Compounds **41, 42** and **44** were prepared using a procedure similar to that for the preparation of **5**.

4.1.30. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(3,4-dimethoxybenzyl)pyrimidin-2-amine (41)

A white solid from MeOH/Et_2O (366 mg, 55% for three steps from **55**), mp 150–151 °C; ^{1}H NMR ($CDCl_3$) δ: 6.95–6.91 (2H, m), 6.84–6.77 (4H, m), 6.27 (1H, s), 5.99 (2H, s), 4.97 (2H, s), 4.83–4.55 (1H, br m), 4.29–3.96 (1H, br m), 3.87 (3H, s), 3.86 (3H, s), 3.83 (2H, s), 3.04–2.82 (2H, br m), 2.68–2.61 (1H, m), 1.90–1.80 (2H, m), 1.75–1.68 (2H, m); MS (ESI, positive) m/z 477 (M+1); Anal. Calcd for $C_{26}H_{28}N_4O_5$: C, 65.53; H, 5.92; N, 11.76. Found: C, 65.45; H, 5.91; N, 11.80.

4.1.31. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(2,5-dimethoxybenzyl)pyrimidin-2-amine (42)

A white solid from MeOH/Et_2O (397 mg, 60% for three steps from **55**), mp 150–152 °C; ^{1}H NMR ($CDCl_3$) δ *6.94–6.91* (2H, m), 6.84–6.75 (4H, m), 6.25 (1H, s), 6.00 (2H, s), 4.93 (2H, s), 4.80–4.58 (1H, br m), 4.16–3.91 (1H, br m), 3.88 (2H, s), 3.76 (3H, s), 3.76 (3H, s), 3.03–2.81 (2H, br m), 2.67–2.60 (1H, m), 1.87–1.80 (2H, m), 1.74–1.67 (2H, m); MS (ESI, positive) m/z 477 (M+1); Anal. Calcd for $C_{26}H_{28}N_4O_5$: C, 65.53; H, 5.92; N, 11.76. Found: C, 65.31; H, 5.91; N, 11.76.

4.1.32. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(1,3-benzodioxol-5-ylmethyl)pyrimidin-2-amine (44)

A white solid from MeOH/Et_2O (187 mg, 18% for three steps from **55**), mp 197–198 °C; ^{1}H NMR ($CDCl_3$) δ *6.95–6.91* (2H, m), 6.82 (1H, d, J = 8.0 Hz), 6.77–6.69 (3H, m), 6.28 (1H, s), 6.00 (2H, s), 5.95 (2H, s), 4.96 (2H, s), 4.81–4.59 (1H, br m), 4.17–3.91 (1H, br m), 3.79 (2H, s), 3.07–2.78 (2H, br m), 2.69–2.61 (1H, m), 1.91–1.81 (2H, m), 1.76–1.69 (2H, m); MS (ESI, positive) m/z 461 (M+1); Anal. Calcd for $C_{25}H_{24}N_4O_5$: C, 65.21; H, 5.25; N, 12.17. Found: C, 64.92; H, 5.23; N, 12.16.

4.1.33. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(4-bromobenzyl)pyrimidin-2-amine (38)

To an ice cooled solution of **55** (7 g, 20 mmol) in THF (35 ml) was added sodium hydride (60% oil dispersion, 1.68 g, 42 mmol) in several portions under nitrogen atmosphere. The reaction temperature was kept below 5 °C during the addition and the mixture was further stirred for 30 min. To this mixture was added 4-bromophenylacetyl chloride (5.18 g, 22 mmol) in THF (15 ml) dropwise over 1 h. After stirring for 30 min, the mixture was acidified with aqueous HCl (4 N) and extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4, and then evaporated to give a diketoester as a crude oil. A mixture of this diketoester, water (1.6 ml), and DMSO (18 ml) was heated at 110 °C for 7.5 h, poured into water, and extracted with EtOAc. The organic layer was washed with water, dried over Na_2SO_4, and then evaporated. The residue was purified by silica gel column chromatography using hexane/EtOAc (1:1, v/v) as eluent to give 1-[1-(1,3-benzodioxol-5-ylcarbonyl)piperidin-4-yl]-4-(4-bromophenyl)butane-1,3-dione (5.54 g, 58.2%) as an oil: ^{1}H NMR ($CDCl_3$) δ 15.38 (1H, s, enol), 7.46 (2H, d, J = 8.2 Hz), 7.12 (2H, d, J = 8.2 Hz), 6.92–6.87 (2H, m), 6.82 (1H, d, J = 7.6 Hz), 6.00 (2H, s), 5.44 (1H, s), 4.73–4.42 (1H, br m), 4.17–3.91 (1H, br m), 3.57 (2H, s), 3.01–2.83 (2H, br m), 2.46–2.38 (1H, m), 1.89–1.77 (2H, m), 1.71–1.61 (2H, m); MS (ESI, positive) m/z 472 (M+1).

A mixture of this 1,3-diketone (5.3 g, 11 mmol), guanidine carbonate (2.02 g, 11 mmol), and pyridine (25 ml) was heated at 110 °C for 8 h. The mixture was then concentrated in vacuo, and the residue was partitioned between $CHCl_3$ and aqueous satd $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4, and then evaporated. Crystallization from ethanol gave **38** (3.88 g, 69.8%) as a white solid: mp 162–162.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.44 (2H, d, J = 8.3 Hz), 7.13 (2H, d, J = 8.3 Hz), 6.95–6.91 (2H, m), 6.82 (1H, d, J = 7.8 Hz), 6.26 (1H, s), 6.00 (2H, s), 4.95 (2H, s), 4.80–4.58 (1H, br m), 4.10–3.89 (1H, br m), 3.83 (2H, s), 3.07–2.81 (2H, br m), 2.70–2.62 (1H, m), 1.87–1.67 (4H, m); MS (ESI, positive) m/z 495 (M+1); Anal. Calcd for $C_{24}H_{23}BrN_4O_3$: C, 58.19; H, 4.68; Br, 16.13; N, 11.31. Found: C, 58.19; H, 4.71; Br, 16.07; N, 11.23.

Compounds **29**–**33**, **35**–**37**, **39**, **40**, and **43** were prepared using a procedure similar to that for the preparation of **38**.

4.1.34. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-phenylpyrimidin-2-amine (29)

A white solid from MeOH/Et_2O (272 mg, 29% for three steps from **55**), mp 201–202 °C; 1H NMR ($CDCl_3$) δ *8.00–7.96* (2H, m), 7.49–7.46 (3H, m), 6.98–6.92 (3H, m), 6.84 (1H, d, J = 7.8 Hz), 6.01 (2H, s), 5.08 (2H, s), 4.88–4.67 (1H, br m), 4.20–3.93 (1H, br m), 3.09–2.92 (2H, br m), 2.85–2.77 (1H, m), 2.00–1.80 (4H, m); MS (ESI, positive) m/z 403 (M+1); Anal. Calcd for $C_{23}H_{22}N_4O_3 \cdot 0.25H_2O$: C, 67.88; H, 5.57; N, 13.77. Found: C, 67.89; H, 5.63; N, 13.78.

4.1.35. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(4-chlorophenyl)pyrimidin-2-amine (30)

A white solid from MeOH/Et_2O (490 mg, 49% for three steps from **55**), mp 103–106 °C; ^{1}H NMR ($CDCl_3$) δ 7.94 (2H, d,

J = 8.3 Hz), 7.44 (2H, d, *J* = 8.3 Hz), 6.99–6.93 (2H, m), 6.89 (1H, s), 6.84 (1H, d, *J* = 7.8 Hz), 6.01 (2H, s), 5.04 (2H, s), 4.86–4.67 (1H, br m), 4.18–3.94 (1H, br m), 3.09–2.91 (2H, br m), 2.85–2.77 (1H, m), 2.01–1.75 (4H, m); MS (ESI, positive) *m/z* 437 (M+1); Anal. Calcd for $C_{23}H_{21}ClN_4O_3$: C, 63.23; H, 4.84; Cl, 8.11; N, 12.82. Found: C, 63.18; H, 4.91; Cl, 8.05; N, 12.84.

4.1.36. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(4-methoxyphenyl)pyrimidin-2-amine (31)

A white solid from MeOH/Et_2O (450 mg, 45% for three steps from **55**), mp 103–106 °C; ^{1}H NMR ($CDCl_3$) δ 7.96 (2H, d, *J* = 8.8 Hz), 6.99–6.94 (4H, m), 6.87 (1H, s), 6.83 (1H, d, *J* = 7.8 Hz), 6.00 (2H, s), 5.01 (2H, s), 4.85–4.68 (1H, br m), 4.15–3.98 (1H, br m), 3.87 (3H, s), 3.08–2.95 (2H, br m), 2.82–2.75 (1H, m), 1.99–1.78 (4H, m); MS (ESI, positive) *m/z* 433 (M+1); Anal. Calcd for $C_{24}H_{24}N_4O_4$: C, 66.65; H, 5.59; N, 12.96. Found: C, 66.67; H, 5.70; N, 12.93.

4.1.37. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(2-phenylethyl)pyrimidin-2-amine (32)

A white solid from EtOH (298 mg, 30% for three steps from **55**), mp 158–158.5 °C; ^{1}H NMR ($CDCl_3$) δ *7.30–7.27* (2H, m), 7.22–7.17 (3H, m), 6.96–6.92 (2H, m), 6.83 (1H, d, *J* = 8.0 Hz), 6.27 (1H, s), 6.01 (2H, s), 4.95 (2H, s), 4.80–4.64 (1H, br m), 4.09–3.92 (1H, br m), 3.02–2.93 (4H, m), 2.85 (2H, t, *J* = 8.2 Hz), 2.71–2.63 (1H, m), 1.89–1.69 (4H, br m); MS (ESI, positive) *m/z* 431 (M+1); Anal. Calcd for $C_{25}H_{26}N_4O_3$: C, 69.75; H, 6.09; N, 13.01. Found: C, 69.57; H, 6.12; N, 13.05.

4.1.38. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-methylpyrimidin-2-amine (33)

A white solid from EtOH (347 mg, 41% for three steps from **55**), mp 171–173 °C; ^{1}H NMR ($CDCl_3$) δ *6.96–6.92* (2H, m), 6.83 (1H, d, *J* = 7.8 Hz), 6.38 (1H, s), 6.00 (2H, s), 4.90 (2H, s), 4.80–4.64 (1H, br m), 4.23–3.88 (1H, br m), 3.07–2.88 (2H, br m), 2.73–2.65 (1H, m), 2.33 (3H, s), 1.93–1.69 (4H, m); MS (ESI, positive) *m/z* 341 (M+1); Anal. Calcd for $C_{18}H_{20}N_4O_3$: C, 63.52; H, 5.92; N, 16.46. Found: C, 63.59; H, 5.97; N, 16.62.

4.1.39. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-benzylpyrimidin-2-amine (35)

A white solid from MeOH (302 mg, 39% for three steps from **55**), mp 199–200 °C; ^{1}H NMR ($CDCl_3$) δ *7.34–7.30* (2H, m), 7.26–7.23 (3H, m), 6.95–6.91 (2H, m), 6.82 (1H, d, *J* = 7.8 Hz), 6.28 (1H, s), 6.00 (2H, s), 4.95 (2H, s), 4.80–4.58 (1H, br m), 4.19–3.95 (1H, br m), 3.89 (2H, s), 3.06–2.82 (2H, br m), 2.69–2.61 (1H, m), 1.87–1.67 (4H, m); MS (ESI, positive) *m/z* 417 (M+1); Anal. Calcd for $C_{24}H_{24}N_4O_3$: C, 69.21; H, 5.81; N, 13.45. Found: C, 69.10; H, 5.77; N, 13.56.

4.1.40. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(3-methoxybenzyl)pyrimidin-2-amine (36)

A white solid from MeOH/Et_2O (239 mg, 23% for three steps from **55**), mp 167–168 °C; ^{1}H NMR ($CDCl_3$) δ 7.23 (1H, d, *J* = 8.3 Hz), 6.94–6.91 (2H, m), 6.85–6.79 (4H, m), 6.29 (1H, s), 5.99 (2H, s), 4.96 (2H, s), 4.83–4.59 (1H, br m), 4.14–3.91 (1H, br m), 3.86 (2H, s), 3.80 (3H, s), 3.05–2.84 (2H, br m), 2.69–2.61 (1H, m), 1.90–1.79 (2H, m), 1.75–1.69 (2H, m); MS (ESI, positive) *m/z* 447 (M+1); Anal. Calcd for $C_{25}H_{26}N_4O_4$: C, 67.25; H, 5.87; N, 12.55. Found: C, 67.14; H, 5.84; N, 12.61.

4.1.41. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(4-chlorobenzyl)pyrimidin-2-amine (37)

A white solid from MeOH/Et_2O (343 mg, 33% for three steps from **55**), mp 162–163 °C; ^{1}H NMR ($CDCl_3$) δ 7.28 (2H, d, *J* = 8.3 Hz), 7.18 (2H, d, *J* = 8.3 Hz), 6.95–6.91 (2H, m), 6.82 (1H, d, *J* = 8.0 Hz), 6.26 (1H, s), 6.00 (2H, s), 4.96 (2H, s), 4.80–4.56 (1H, br m), 4.24–3.95 (1H, br m), 3.85 (2H, s), 3.04–2.81 (2H, br m), 2.69–2.62 (1H, m), 1.88–1.82 (2H, m), 1.74–1.68 (2H, m); MS (ESI, positive) *m/z* 451 (M+1); Anal. Calcd for $C_{24}H_{23}ClN_4O_3$: C, 63.93; H, 5.14; Cl, 7.86; N, 12.43. Found: C, 63.69; H, 5.11; Cl, 7.73; N, 12.43.

4.1.42. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-[4-(benzyloxy)benzyl]pyrimidin-2-amine (39)

A white solid from EtOH (425 mg, 21% for three steps from **55**), mp 178–179 °C; ^{1}H NMR ($CDCl_3$) δ *7.44–7.31* (5H, m), 7.16 (2H, d, *J* = 8.5 Hz), 6.94–6.91 (4H, m), 6.82 (1H, d, *J* = 7.8 Hz), 6.27 (1H, s), 6.00 (2H, s), 5.06 (2H, s), 4.94 (2H, s), 4.81–4.68 (1H, br m), 4.09–3.92 (1H, br m), 3.83 (2H, s), 3.01–2.83 (2H, br m), 2.69–2.61 (1H, m), 1.86–1.68 (4H, m); MS (ESI, positive) *m/z* 523 (M+1); Anal. Calcd for $C_{31}H_{30}N_4O_4$: C, 71.25; H, 5.79; N, 10.72. Found: C, 71.12; H, 5.80; N, 10.77.

4.1.43. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-[4-(methylthio)benzyl]pyrimidin-2-amine (40)

A white solid from MeOH (1.56g, 36% for three steps from **55**), mp 185.5–186.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.21 (2H, d, *J* = 8.3 Hz), 7.17 (2H, d, *J* = 8.3 Hz), 6.95–6.91 (2H, m), 6.82 (1H, d, *J* = 7.8 Hz), 6.27 (1H, s), 6.00 (2H, s), 4.96 (2H, s), 4.80–4.61 (1H, br m), 4.12–3.91 (1H, br m), 3.84 (2H, s), 3.03–2.84 (2H, br m), 2.69–2.61 (1H, m), 2.48 (3H, s), 1.86–1.70 (4H, m); MS (ESI, positive) *m/z* 463 (M+1); Anal. Calcd for $C_{25}H_{26}N_4O_3S$: C, 64.91; H, 5.67; N, 12.11 S, 6.93. Found: C, 64.94; H, 5.72; N, 12.17; S, 6.87.

4.1.44. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(3,5-dimethoxybenzyl)pyrimidin-2-amine (43)

A white solid from MeOH (344 mg, 52% for three steps from **55**), mp 176–178 °C; ^{1}H NMR ($CDCl_3$) δ *6.95–6.91* (2H, m), 6.82 (1H, d, *J* = 7.8 Hz), 6.40–6.35 (3H, m), 6.30 (1H, s), 6.00 (2H, s), 4.97 (2H, s), 4.80–4.63 (1H, br m), 4.10–3.89 (1H, br m), 3.82 (2H, s), 3.78 (6H, s), 3.04–2.81 (2H, br m), 2.69–2.61 (1H, m), 1.88–1.71 (4H, m); MS (ESI, positive) *m/z* 477 (M+1); Anal. Calcd for $C_{26}H_{28}N_4O_5$: C, 65.53; H, 5.92; N, 11.76. Found: C, 65.46; H, 5.95; N, 11.83.

4.1.45. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-2-chloro-6-(4-methoxybenzyl)pyrimidine (22)

To a suspension of **5** (4.46 g, 10 mmol) and $SbCl_3$ in 1,2-dichloroethane (250 ml) was added *tert*-butylnitrate (6.2 ml, 52 mmol) and heated at 60 °C for 2 h under nitrogen atmosphere. To the mixture was added aqueous satd $NaHCO_3$ and the mixture was filtered off and filtrate was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography, using $CHCl_3$/EtOAc (9:1, v/v) as eluent to give **22** (1.17 g, 25%) as a foam: ^{1}H NMR ($CDCl_3$) δ 7.17 (2H, d, *J* = 8.8 Hz), 6.95–6.87 (4H, m), 6.83–6.81 (2H, m), 6.00 (2H, s), 4.82–4.47 (1H, br m), 4.33–4.04 (1H, br m), 4.02 (2H, s), 3.81 (3H, s), 3.07–2.90 (2H, br m), 2.89–2.80 (1H, m), 1.93–1.85 (2H, m), 1.77–1.68 (2H, m); MS (ESI, positive) *m/z* 466 (M+1); Anal. Calcd for $C_{25}H_{24}ClN_3O_4 \cdot 0.2H_2O \cdot 0.2CHCl_3$: C, 61.34; H, 5.03; Cl, 11.50; N, 8.52. Found: C, 61.47; H, 5.02; Cl, 11.13; N, 8.52.

4.1.46. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidine (23)

A mixture of **22** (200 mg, 0.43 mmol), NaOAc (53 mg, 0.65 mmol), methanol (1.5 ml), and benzene was hydrogenated under 1 atm in the presence of 10% Pd/C (67 mg) for 8 h. The catalyst was filtered over Celite, washed with MeOH, and the filtrate was concentrated in vacuo. The residue was purified with preparative thin layer chromatography using $CHCl_3$/MeOH (19:1, v/v) as

eluent. Crystallization from hexane/Et_2O gave **23** (137 mg, 74%) as a white solid: mp 101–102 °C; ^1H NMR ($CDCl_3$) δ 9.05 (1H, s), 7.18 (2H, d, J = 8.5 Hz), 6.96–6.92 (3H, m), 6.88 (2H, d, J = 8.5 Hz), 6.82 (1H, d, J = 7.8 Hz), 6.00 (2H, s), 4.87–4.57 (1H, m), 4.44–4.13 (1H, m), 4.04 (2H, s), 3.81 (3H, s), 3.10–2.92 (2H, br m), 2.89–2.81 (1H, m), 1.96–1.88 (2H, m), 1.81–1.71 (2H, m); MS (ESI, positive) m/z 432 (M+1); Anal. Calcd for $C_{25}H_{25}N_3O_4$: C, 69.59; H, 5.84; N, 9.74. Found: C, 69.33; H, 5.79; N, 9.75.

4.1.47. 4-({2-Amino-6-[1-(1,3-benzodioxol-5-ylcarbonyl)-piperidin-4-yl]pyrimidin-4-yl}methyl)phenol (45)

A solution of **39** (230 mg, 0.44 mmol) in MeOH (3 ml) and THF (3 ml) was hydrogenated under 1 atm in the presence of 10% Pd/C (20 mg) for 7 h. The catalyst was filtered over Celite, and the filtrate was concentrated. The residue was purified by silica gel column chromatography using $CHCl_3$/MeOH (50:1, v/v) as eluent. Crystallization from MeOH/H_2O gave **45** (160 mg, 84%) as a white solid: mp 136.5–137 °C; ^1H NMR ($CDCl_3$) δ 7.05 (2H, d, J = 8.5 Hz), 6.95–6.91 (2H, m), 6.82 (1H, d, J = 7.8 Hz), 6.73 (2H, d, J = 8.5 Hz), 6.60 (1H, br s), 6.30 (1H, s), 6.00 (2H, s), 5.01 (2H, s), 4.83–4.60 (1H, br m), 4.15–3.92 (1H, br m), 3.81 (2H, s), 3.05–2.84 (2H, br m), 2.71–2.63 (1H, m), 1.92–1.84 (2H, m), 1.75–1.66 (2H, m); MS (ESI, positive) m/z 433 (M+1); Anal. Calcd for $C_{24}H_{24}N_4O_4{\cdot}0.75H_2O$: C, 64.63; H, 5.76; N, 12.56. Found: C, 64.87; H, 5.74; N, 12.65.

4.1.48. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-[4(methylsulfinyl)benzyl]pyrimidin-2-amine (46)

A mixture of **40** (200 mg, 0.43 mmol), MeOH (12 ml), and 34% H_2O_2 solution (0.2 ml) was heated at 60 °C. After 2.5 h, H_2O_2 (0.2 ml) was added, and the mixture was further heated for 3 h. Water was then added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography using $CHCl_3$/MeOH (30:1, v/v) as eluent. Crystallization from methanol gave **46** (190 mg, 92%) as a white solid: mp 183.5–184 °C; ^1H NMR ($CDCl_3$) δ 7.60 (2H, d, J = 8.3 Hz), 7.42 (2H, d, J = 8.3 Hz), 6.95–6.91 (2H, m), 6.82 (1H, d, J = 7.8 Hz), 6.30 (1H, s), 6.00 (2H, s), 4.97 (2H, s), 4.82–4.59 (1H, br m), 4.18–3.99 (1H, br m), 3.94 (2H, s), 3.06–2.83 (2H, br m), 2.73 (3H, s), 2.70–2.63 (1H, m), 1.89–1.68 (4H, m); MS (ESI, positive) m/z 479 (M+1); Anal. Calcd for $C_{25}H_{26}N_4O_4S$: C, 62.74; H, 5.48; N, 11.71; S, 6.70. Found: C, 62.59; H, 5.52; N, 11.80; S, 6.57.

4.1.49. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-[4-(methylsulfonyl)benzyl]pyrimidin-2-amine (47)

A mixture of **40** (200 mg, 0.43 mmol), $Na_2WO_4{\cdot}2H_2O$ (10 mg, 0.03 mmol), MeOH (12 ml), and 34% H_2O_2 solution (0.4 ml) was heated at 50 °C for 1 h. Water was then added, and the mixture was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography using $CHCl_3$/MeOH (30:1, v/v) as eluent. Crystallization from MeOH gave **47** (175 mg, 82%) as a white solid: mp 201–202.5 °C; ^1H NMR ($CDCl_3$) δ 7.89 (2H, d, J = 8.3 Hz), 7.46 (2H, d, J = 8.3 Hz), 6.95–6.91 (2H, m), 6.82 (1H, d, J = 7.8 Hz), 6.31 (1H, s), 6.00 (2H, s), 4.97 (2H, s), 4.81–4.63 (1H, br m), 4.20–4.04 (1H, br m), 3.96 (2H, s), 3.05 (3H, s), 3.01–2.87 (2H, br m), 2.72–2.64 (1H, m), 1.87–1.69 (4H, m); MS (ESI, positive) m/z 495 (M+1); Anal. Calcd for $C_{25}H_{26}N_4O_5S$: C, 60.71; H, 5.30; N, 11.33; S, 6.48. Found: C, 60.52; H, 5.30; N, 11.32; S, 6.41.

4.1.50. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]-6-(4-methoxybenzyl)pyrimidin-2(1H)-one (24)

A mixture of **57** (3 g, 7.08 mmol) and urea (8.58 g, 0.142 mol) was heated at 150 °C with vigorous stirring for 4 h. To this mixture was added water and the whole was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and then evaporated. The residue was purified by silica gel column chromatography using $CHCl_3$/MeOH (50:1, v/v) as eluent. Crystallization from methanol gave **24** (795 mg, 25%) as a white solid: mp 197–198 °C (dec); ^1H NMR ($CDCl_3$) δ 13.48 (1H, br s), 7.22 (2H, d, J = 8.5 Hz), 6.94–6.92 (2H, m), 6.88 (2H, d, J = 8.5 Hz), 6.81 (1H, d, J = 7.8 Hz), 6.02 (1H, s), 5.99 (2H, s), 4.85–4.53 (1H, br m), 4.19–3.94 (1H, br m), 3.91 (2H, s), 3.81 (3H, s), 3.07–2.85 (2H, br m), 2.80–2.70 (1H, m), 1.90–1.69 (4H, m); MS (ESI, positive) m/z 448 (M+1); Anal. Calcd for $C_{25}H_{25}N_3O_5$: C, 67.10; H, 5.63; N, 9.39. Found: C, 66.90; H, 5.65; N, 9.47.

4.1.51. 1-(1,3-Benzodioxol-5-ylcarbonyl)-4-[5-(4-methoxybenzyl)-1H-pyrazol-3-yl]piperidine (25)

A mixture of **57** (1.0 g, 2.26 mmol), hydrazine monohydrate (473 mg, 9.44 mmol), and MeOH (35 ml) was stirred at room temperature for 2 h. The mixture was then concentrated, diluted with water, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography using $CHCl_3$/MeOH (50:1, v/v) as eluent to give **25** (763 mg, 77%) as a foam: ^1H NMR ($CDCl_3$) δ 7.14 (2H, d, J = 8.8 Hz), 6.94–6.90 (2H, m), 6.86 (2H, d, J = 8.8 Hz), 6.81 (1H, d, J = 8.0 Hz), 6.00 (2H, s), 5.87 (1H, s), 4.77–4.50 (1H, br m), 4.20–3.93 (1H, br m), 3.92 (2H, s), 3.80 (3H, s), 3.12–2.96 (2H, br m), 2.93–2.86 (1H, m), 2.05–1.92 (2H, m), 1.74–1.60 (2H, m); MS (ESI, positive) m/z 420 (M+1); Anal. Calcd for $C_{24}H_{25}N_3O_4{\cdot}0.9H_2O$: C, 66.16; H, 6.20; N, 9.64. Found: C, 66.08; H, 5.81; N, 9.62.

4.1.52. 1-(1,3-benzodioxol-5-ylcarbonyl)-4-[3-(4-methoxybenzyl)isoxazol-5-yl]piperidine (26) and 1-(1,3-Benzodioxol-5-ylcarbonyl)-4-[5-(4-methoxybenzyl)isoxazol-3-yl]piperidine (27)

A mixture of **57** (1.0 g, 2.26 mmol), hydroxylamine hydrochloride (329 mg, 4.72 mmol), iPr$_2$NEt (610 mg, 4.72 mmol), and DMF (16 ml) was stirred at room temperature. After stirring for 5 h, the mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography using hexane/EtOAc (1:2, v/v) as eluent to give 5-[1-(1,3-benzodioxol-5-ylcarbonyl)piperidin-4-yl]-3-(4-methoxybenzyl)-4,5-dihydroisoxazol-5-ol (less polar, 414 mg, 40%): ^1H NMR ($CDCl_3$) δ 7.13 (2H, d, J = 8.5 Hz, ArH), 6.90–6.86 (4H, m, ArH), 6.81 (1H, d, J = 7.8 Hz, ArH), 5.99 (2H, s, OCH_2O), 4.81–4.53 (1H, br m, NCH_AH$_B$CH$_2$CH), 4.23–3.87 (1H, br m, NC$H_{A'}$H$_{B'}$CH$_2$CH), 3.80 (3H, s, OCH_3), 3.67 (1H, d, J = 15.1Hz, CH$_A$$H_B$PhOCH$_3$), 3.62 (1H, d, J = 15.1 Hz, CH$_{A'}$$H_{B'}$PhOCH$_3$), 3.07–2.72 (4H, m, 1H is D_2O exchangeable, OH, NCH$_A$$H_BCH_2CH, NCH_{A'}$$H_{B'}CH_2$CH, N=CC$H_AH_B$), 2.63 (1H, d, J = 17.8 Hz, N=CCH$_A$$H_B$), 2.00–1.72 (3H, m, NCH$_2CH_2CH$, NCH$_2CH_AH_B$CH), 1.37–1.25 (2H, m, NCH$_2$CH$_A$$H_B$CH); MS (ESI, negative) m/z 437 (M−1) and its isomer 3-[1-(1,3-benzodioxol-5-ylcarbonyl)piperidin-4-yl]-5-(4-methoxybenzyl)-4,5-dihydroisoxazol-5-ol (polar, 217 mg, 21%) as a foam: ^1H NMR ($CDCl_3$) δ 7.25 (2H, d, J = 8.5 Hz, ArH), 6.91–6.80 (5H, m, ArH), 5.99 (2H, s, OCH_2O), 4.62–4.23 (1H, br m, NCH_AH$_B$CH$_2$CH), 4.17–3.85 (1H, br m, NC$H_{A'}$H$_{B'}$CH$_2$CH), 3.80 (3H, s, OCH_3), 3.15 (2H, s, CH_2PhOCH$_3$), 3.04–2.93 (3H, m, 1H is D_2O exchangeable, OH, NCH$_A$$H_BCH_2CH, NCH_{A'}$$H_{B'}CH_2$CH), 2.89 (1H, d, J = 17.6 Hz, N=CCH_AH$_B$), 2.81 (1H, d, J = 17.6 Hz, N=CCH$_A$$H_B$), 2.67–2.60 (1H, m, NCH$_2CH_2CH$), 1.89–1.83 (2H, m, NCH$_2CH_AH_B$CH), 1.65–1.52 (2H, m, NCH$_2$CH$_A$$H_B$CH); MS (ESI, negative) m/z 437 (M−1).

To an ice cooled solution of 5-[1-(1,3-benzodioxol-5-ylcarbonyl)piperidin-4-yl]-3-(4-methoxybenzyl)-4,5-dihydroisoxazol-5-ol (less polar, 200 mg, 0.46 mmol) and Et_3N (0.19 ml, 1.38 mmol) in CH_2Cl_2 (5 ml) was added MsCl (39 μl, 0.64 mmol). After stirring for 2 h, the mixture was diluted with water and extracted with

EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography using hexane/EtOAc (1:1, v/v) as eluent. Crystallization from ether gave **26** (168 mg, 88%) as a white solid: mp 121–122 °C; ^{1}H NMR ($CDCl_3$) δ 7.16 (2H, d, J = 8.5 Hz, Ar*H*), 6.92–6.81 (5H, m, Ar*H*), 6.00 (2H, s, OCH_2O), 5.74 (1H, s, C=C*H*), 4.68–4.33 (1H, br m, NCH_AH$_B$CH$_2$CH), 4.19–3.95 (1H, br m, NC$H_{A'}$H$_{B'}$CH$_2$CH), 3.91 (2H, s, CH_2PhOCH$_3$), 3.79 (3H, s, OCH_3), 3.07–2.96 (3H, m, NCH$_2$CH$_2$C*H*, NCH$_A$H$_B$CH$_2$CH, NCH$_{A'}$H$_{B'}$CH$_2$CH), 2.07–2.01 (2H, m, NCH$_2$CH$_A$H$_B$CH), 1.71–1.62 (2H, m, NCH$_2$CH$_A$H$_B$CH); MS (ESI, positive) *m/z* 421 (M+1); Anal. Calcd for $C_{24}H_{24}N_2O_5$: C, 68.56; H, 5.75; N, 6.66. Found: C, 68.52; H, 5.74; N, 6.75.

Compound **27** was prepared in the same way as **26** using the other isomer 3-[1-(1,3-benzodioxol-5-ylcarbonyl)piperidin-4-yl]-5-(4-methoxybenzyl)-4,5-dihydroisoxazol-5-ol (polar, 150 mg, 0.34 mmol). Crystallization from ether gave **27** (140 mg, 97%) as a white solid: mp 96–96.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.17 (2H, d, J = 8.8 Hz, Ar*H*), 6.93–6.86 (4H, m, Ar*H*), 6.81 (1H, d, J = 7.8 Hz, Ar*H*), 6.00 (2H, s, OCH_2O), 5.74 (1H, s, C=C*H*), 4.70–4.40 (1H, br m, NCH_AH$_B$CH$_2$CH), 4.31–4.01 (1H, br m, NC$H_{A'}$H$_{B'}$CH$_2$CH), 3.98 (2H, s, CH_2PhOCH$_3$), 3.81 (3H, s, OCH_3), 3.09–2.93, (3H, m, NCH$_2$CH$_2$C*H*, NCH$_A$H$_B$CH$_2$CH, NCH$_{A'}$H$_{B'}$CH$_2$CH), 1.99–1.92 (2H, m, NCH$_2$CH$_A$H$_B$CH), 1.73–1.63 (2H, m, NCH$_2$CH$_A$H$_B$CH); MS (ESI, positive) *m/z* 421 (M+1); Anal. Calcd for $C_{24}H_{24}N_2O_5$: C, 68.56; H, 5.75; N, 6.66. Found: C, 68.47 H, 5.73; N, 6.71.

4.1.53. 1-(Benzoyl)piperidine-4-carbonitrile (59)

To an ice cold solution of ethyl 4-cyanopiperidine[17] (40 g, 0.363 mol) and Et_3N (37.47 g, 0.37 mol) in THF (250 ml) was added dropwise benzoyl chloride (52.05 g, 0.37 mol) over 2 h under nitrogen atmosphere. The reaction mixture was then concentrated in vacuo and the residue was partitioned between dichloromethane and water. The organic layer was dried over $MgSO_4$, and evaporated. Crystallization from *i*PrOH gave **59** (73.53 g, 95%) as a white solid: mp 94–96 °C; ^{1}H NMR ($CDCl_3$) δ 7.45–7.37 (5H, m), 3.95–3.40 (4H, m), 2.97–2.91 (1H, m), 2.03–1.76 (4H, br m); MS (ESI, positive) *m/z* 215 (M+1).

4.1.54. (2-Aminophenyl)[1-(phenylcarbonyl)piperidin-4-yl]methanone (60)

To an ice cold solution of boron trichloride (26.3 ml, 0.303 mol) in 1,2-dichloroethane (300 ml) was added dropwise aniline (28.21 g, 0.303 mol) over 15 min under a nitrogen atmosphere. To this mixture was added portion wise 1-benzoyl-4-cyanopiperidine (50 g, 0.233 mol) and aluminum chloride (40.39 g, 0.303 mol) successively. After stirring at room temperature for 15 min, the mixture was refluxed for 14 h, acidified with aqueous HCl (1 N) with ice cooling, and then refluxed for 1 h. The reaction mixture was basified with aqueous NaOH (3 N), and the resulting slurry was filtered over Celite. The filtrate was then extracted with dichloromethane. The organic layer was dried over Na_2SO_4, and the solvent was removed in vacuo. The residue was crystallized from EtOH to give **60** (51.4 g,71%) as an off-white solid: mp 138–138.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.74 (1H, dd, J = 8.2, 1.3 Hz), 7.45–7.37 (5H, m), 7.30–7.26 (1H, m), 6.69–6.64 (2H, m), 6.31 (2H, br s), 4.82–4.72 (1H, br m), 3.92–3.82 (1H, br m), 3.60–3.52 (1H, m), 3.17–2.94 (2H, br m), 2.02–1.76 (4H, m); MS (ESI, positive) *m/z* 309 (M+1).

4.1.55. 4-[1-(Phenylcarbonyl)piperidin-4-yl]quinazolin-2-amine (61)

A mixture of **60** hydrochloride (17 g, 0.049 mol) and cyanamide (4.12 g, 0.098 mol) was warmed in an oil bath. At about 50 °C, vigorous exothermic reaction occurred. After completion of the reaction, water was added, and the mixture was basified with sodium hydrogen carbonate. The mixture was then extracted with dichloromethane. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography using hexane/EtOAc (1:1, v/v) as eluent. Crystallization from EtOH gave **61** (15.85 g, 96%) as a white solid: mp182–183 °C; ^{1}H NMR ($CDCl_3$) δ 7.94 (1H, d, J = 8.3 Hz), 7.71–7.66 (1H, m), 7.60 (1H, d, J = 8.5 Hz), 7.48–7.41 (5H, m), 7.30–7.28 (1H, m), 5.16 (2H, s), 4.96–4.83 (1H, br m), 4.01–3.91 (1H, br m), 3.74–3.66 (1H, m), 3.26–2.98 (2H, br m), 2.10–1.82 (4H, m); MS (ESI, positive) *m/z* 333 (M+1); Anal. Calcd for $C_{20}H_{20}N_4O$: C, 72.27; H, 6.06; N, 16.86. Found: C, 72.01; H, 6.01; N, 16.80.

4.1.56. 4-Piperidin-4-ylquinazolin-2-amine (62)

A mixture of **61** (24 g, 0.072 mol), EtOH (250 ml) and 6 N aqueous NaOH (250 ml) was refluxed for 7 h under a nitrogen atmosphere. Thereafter ethanol was removed by evaporation and the residue was extracted with dichloromethane. The organic layer was washed with brine, dried over magnesium sulfate, and evaporated. The residue was crystallized from *i*PrOH to give **62** (13.0 g, 82%) as a white solid: mp 184–186 °C; ^{1}H NMR ($CDCl_3$) δ 7.96 (1H, d, J = 7.6 Hz), 7.69–7.64 (1H, m), 7.58 (1H, d, J = 7.8 Hz), 7.28–7.24 (1H, m), 5.11 (2H, s), 3.60–3.52 (1H, m), 3.26 (2H, dt, J = 12.2, 3.0 Hz), 2.86 (2H, dt, J = 12.2, 3.0 Hz), 1.99–1.84 (4H, m); MS (ESI, positive) *m/z* 229 (M+1); Anal. Calcd for $C_{13}H_{16}N_4$: C, 68.39; H, 7.06; N, 24.54. Found: C, 68.21; H, 7.04; N, 24.61.

4.1.57. 4-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-yl]quinazolin-2-amine (28)

The titled compound was prepared from **62** (600 mg, 2.6 mmol) using a procedure similar to that for the preparation of **2**. Crystallization from MeOH gave **28** (418 mg, 45%) as a white solid: mp 197–198 °C; ^{1}H NMR ($CDCl_3$) δ 7.94 (1H, d, J = 8.3 Hz), 7.71–7.67 (1H, m), 7.60 (1H, d, J = 8.3 Hz), 7.29 (1H, d, J = 8.0 Hz), 7.01–6.97 (2H, m), 6.84 (1H, d, J = 8.0 Hz), 6.01 (2H, s), 5.14 (2H, s), 4.88–4.70 (1H, br m), 4.20–4.03 (1H, br m), 3.73–3.66 (1H, m), 3.23–3.01 (2H, m), 2.10–1.89 (4H, m); MS (ESI, positive) *m/z* 377 (M+1); Anal. Calcd for $C_{21}H_{20}N_4O_3$: C, 67.01; H, 5.36; N, 14.88. Found: C, 66.78; H, 5.33; N, 14.87.

4.2. Biology

4.2.1. Measurement of HIV-1 LTR-driven CAT gene expression

Plasmids: p469, HIV-1 LTR-driven CAT and pSV-Tat, Tat-expression vector were kindly provided by Nabel.[18b] Human T lymphoma cell line, Jurkat, was cultured in RPMI 1640 medium (Life Technologies, Grand Island, NY, USA) supplemented with heat-inactivated fetal calf serum (FBS; Sigma, St. Louis, MO, USA). Introduction of plasmid DNA into cells was carried out according to the method described by Li et al.[19a] Briefly, the cells were suspended in the culture medium at 2×10^7 cells/ml, and 1 μg of p469 and 0.5 μg of DEAE-dextran (Sigma) were added. In some experiments the cells were co-transfected with 0.5 μg of pSV-Tat. After 10 min incubation at room temperature with occasional mixing, the cells were pulsed with an electroporation apparatus at 140 V. The cells were then re-suspended in fresh culture medium and cultured for 20 h at 37 °C.

Test compounds were dissolved in DMSO at 100 mM as a stock solution. Aliquots of the stock solution were kept frozen at −20 °C. Jurkat cells transfected with p469 were treated with 10 ng/ml of phorbol 12-myristate 13-acetate (PMA; Sigma) to activate HIV-1-LTR-directed CAT gene transcription in the presence of each test compound. In the case of cells transfected with p469 and pSV-Tat, they were simply treated with the test compound. After the cells were cultured for an additional 24 h at 37 °C, they were washed twice with phosphate-buffered saline (PBS) and lysed in

50 mM Tris–HCl containing 15% glycerol with repeated cycles of freeze and thaw. The cell lysates were centrifuged, and protein concentration in each lysate was determined by Bradford method (Bio-Rad, Hercules, CA, USA). Measurement of CAT activity in cell lysates was carried out according to a method similar to that described by Fridovich-Keil et al.[19b] In brief, an equal amount (30 μg of protein) of cell lysates was incubated at 37 °C with 35 μg of acetyl co-enzyme A (Gibco/BRL, Grand Island, MO, USA) and 0.1 μCi of ^{14}C-chloramphenicol (Dupont, NEN Boston, MA, USA) in 42 ml solution for 2 hours. Acetyl-^{14}C-chloramphenicol was then extracted with 9 volumes of EtOAc and fractionated on a TLC gel with 1:19 (v/v) in MeOH/$CHCl_3$. CAT activity was detected by autoradiography and quantified by an Imaging Densitometer (model GS-700; Bio-Rad).

4.2.2. Measurement of TNF-α production from mouse macrophages and PBMC

LPS (*Escherichia coli* o111B4, Difco Laboratories, Detroit, MI, USA) was dissolved in PBS (pH 7.4, 1 mg/ml) and stored at −20 °C as working stock. Mouse peritoneal macrophages were prepared as previously described.[23] Briefly, 5-week-old female BALB/c mice (Charles River Japan, Kanagawa, Japan) were injected intraperitoneally with 1 ml of 2.4% thioglycolate broth. After 4 days, peritoneal exudate cells were recovered from the peritoneal cavity with 4 ml of minimum essential medium (MEM). After three washes with MEM, the cells were inoculated into 96-well culture plates (Costar, Cambridge, MA, USA) at 2×10^5 cells/ well, and incubated at 37 °C for 1 h in a CO_2 incubator. Non-adherent cells were removed by extensively washing the wells with warmed MEM. The adherent cells were supplemented with MEM containing 10% FBS (Lifetechnologies, Grand Island, NY, USA) and used as peritoneal macrophages. Human PBMC were prepared by Ficoll–Paque centrifugation from blood of healthy donors and cultured in RPMI 1640 medium supplemented with heat-inactivated 10% FBS and L-glutamine. The cells were inoculated into 96-well culture plates at 1×10^5 cells/well. LPS was diluted with the culture medium as described above and added to macrophages and PBMC at a final concentration of 10 μg/ml and 0.1 μg/ml, respectively. Test compounds were dissolved in DMSO and added to the culture simultaneously with LPS. Final DMSO concentration was below 0.1%. After 20 h, the supernatants were collected and stored at −20 °C.

TNF-α levels in mouse macrophages were determined with an in-house ELISA system. Ninety-six-well plates (Nunc, Roskilde, Denmark) were coated with anti-mouse TNF-α monoclonal antibody (Pharmingen, San Diego, CA, USA) in 0.5 M bicarbonate buffer (pH 8.5) at 4 °C overnight and blocked with PBS containing 10% FBS (block solution) for 3 h. The supernatants, which were diluted appropriately with the block solution and a standard mouse recombinant TNF-α (10^4 U/μg; Genzyme, Cambridge, MA, USA), were applied to the wells and the plates were incubated at 4 °C overnight. After four washes, a biotinylated anti-mouse TNF-α monoclonal antibody was added and incubation continued for another 45 min at room temperature. After six washes, the wells were incubated with streptavidin-conjugated peroxidase (KPL, Gaithersburg, ML, USA) for 30 min, and then washed eight times. The enzyme reaction was initiated by adding TMB peroxidase substrate (KPL) for 10 min at room temperature and terminated by adding a stop solution. The developed color was measured for absorbance at 450 nm and TNF-α levels in the supernatants were quantified using the standard curve of rTNF-α. Detection limit of the assay was about 250 pg/ml of rTNF-α. TNF-α levels in PBMC were measured with commercially available ELISA kits (Genzyme) according to the manufacturer's instructions.

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