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Exploration of piperidine-4-yl-aminopyrimidines as HIV-1 reverse transcriptase inhibitors. *N*-Phenyl derivatives with broad potency against resistant mutant viruses

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ABSTRACT

Further investigation of the recently reported piperidine-4-yl-aminopyrimidine class of non-nucleoside reverse transcriptase inhibitors (NNRTIs) has been carried out. Thus, preparation of a series of *N*-phenyl piperidine analogs resulted in the identification of 3-carboxamides as a particularly active series. Analogs such as **28** and **40** are very potent versus wild-type HIV-1 and a broad range of NNRTI-resistant mutant viruses. Synthesis, structure–activity relationship (SAR), clearance data, and crystallographic evidence for the binding motif are discussed.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are an important part of the successful combination therapy for HIV-1 known as HAART (highly active anti-retroviral therapy).[1] The efficacy of NNRTIs results from their binding to an allosteric site of HIV reverse transcriptase (HIV-RT), which dramatically decreases the ability of that enzyme to function in reproduction of the virus.[2] Efavirenz and Nevirapine are two very effective first generation NNRTIs, but a major problem with them has been the development of viral resistance. This results from a low genetic barrier of the RT enzyme to mutation and several viable mutant viruses can survive treatment with these agents.[3,4] Thus, there is a need for new NNRTIs with activity against the clinically significant mutant strains as well as the wild-type HIV-1 virus.[5]

Etravirine (TMC125), which was recently approved for use in treatment-experienced HIV patients,[6] and the benzophenone GW-678248[7] are examples of second generation NNRTIs (Fig. 1). Etravirine has a broad range of activity against clinically important mutant RT viruses but its development as a drug was hindered by poor solubility.[8] GW-678248 displays a good mutant profile along with good pharmacokinetic properties (PK) and was developed as a prodrug.[9] We recently reported our efforts to combine aspects of these two chemotypes, which resulted in the discovery of piperidine-4-yl-aminopyrimidine structures such as **1**–**3** (Fig. 1). Our

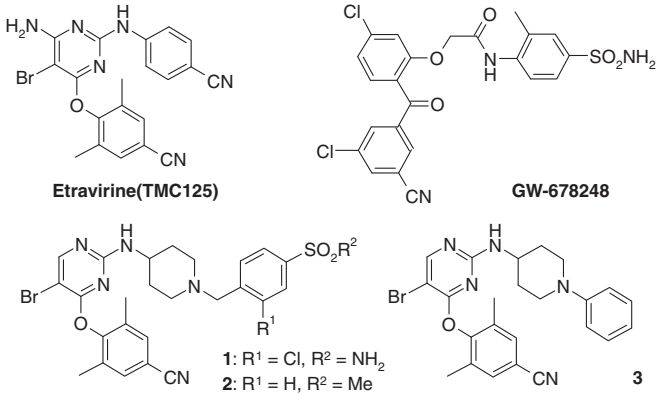

Figure 1. Structures of Etravirine, GW-678248, and aminopyrimidine leads **1**–**3**.

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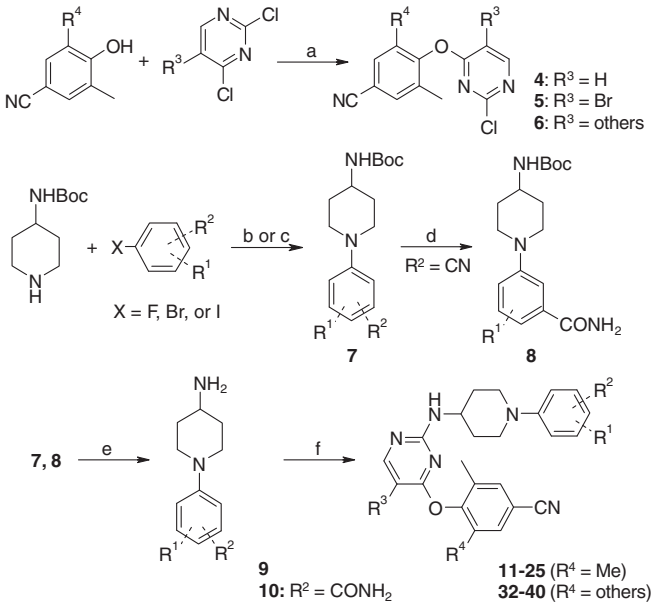


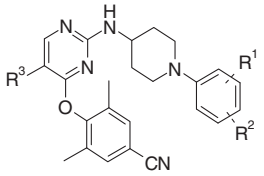
Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, rt; (b) K_2CO_3, DMSO, 140 °C (X = F); (c) K_2CO_3, CuI, proline, DMSO, 90 °C (X = Br, or I); (d) NaOH, H_2O_2, EtOH, 40 °C; (e) TFA, CH_2Cl_2, rt; (f) **4** or **5**, DIPEA, NMP, 100 °C.

publication describing that work focused on optimization of the *N*-benzyl piperidines, which led to compounds **1** and **2** as highly active examples of the series.[10] In the present effort we have explored a series of *N*-phenyl analogs for which compound **3** was the lead structure. As will be described below, that effort has yielded compounds with potency profiles well surpassing those of the initial derivatives **1**–**3**.

The first phase of our new explorations involved determining the best substitution pattern for the *N*-phenyl 'head' ring. For this purpose we kept the 2,6-dimethyl-4-cyanophenyl 'tail' ring constant and used either Br or H as a 5′-substituent on the pyrimidine ring. The syntheses of analogs **12**–**25** required the initial preparation of the intermediates **4**, **5**, **7**, and **8** as shown in Scheme 1. Thus, the treatment of 2,4-dichloropyrimidines with 3,5-dimethyl- 4-cyano-phenol gave phenyl ethers **4** and **5** in excellent yields. Separately, the use of 4-Boc-aminopiperidine to displace the fluorine atom from an appropriately substituted aryl ring gave the *N*-phenylpiperidine intermediates **7**. In an alternative procedure, the intermediates **7** could also be made by copper catalyzed couplings of aryl iodides or bromides with the same aminopiperidine reagent. Compounds wherein R^2 was to be carboxamide were made by hydrolysis of the corresponding nitriles **7** to give intermediates **8**. Deprotections of **7** and **8** gave amine products **9** and **10**, and their further couplings with the 2-chloropyrimidine reagents **4** and **5** gave the desired compounds of structures **11–25**.[11] The inhibitory activities of these products were measured against recombinant wild-type and mutant RT enzymes, and results for the wild-type (WT), the K103N/Y181C double mutant (KY) and the Y188L mutant are given in Table 1.[12]

The preferred substitution pattern for the *N*-benzyl piperidine series represented by **1** and **2** had been a polar substituent at the 4-position of the head group together with a small lipophilic 2-substituent.[10] It was appreciated that the best pattern for the *N*-phenyl piperidines would likely vary from the *N*-benzyl series due to differences in geometry and available vectors. As shown in Table 1, a polar substituent on the *N*-phenyl head group was indeed needed for the best activity in this series as well, but the 3-position was the preferred location. Thus, the 3-sulfonamide **12** and the 3-carboxamides **15** and **16** were among the most potent

Table 1
Enzyme inhibitory potency of *N*-phenyl piperidines **11–25**

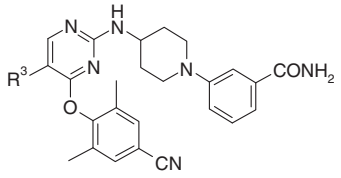


11-25

ID #	R^1	R^2	R^3	WT[a] IC_{50} (nM)	KY[a] IC_{50} (nM)	Y188L[a] IC_{50} (nM)
3	H	H	Br	192	112	334
11	H	4-SO_2NH_2	H	24	>1000	>1000
12	H	3-SO_2NH_2	Br	19	72	105
13	H	3-SO_2NH_2	H	15	622	194
14	H	2-$CONH_2$	Br	32	967	—
15	H	3-$CONH_2$	Br	19	30	70
16	H	3-$CONH_2$	H	7	16	34
17	H	4-$CONH_2$	Br	24	>1000	—
18	H	3-SO_2CH_3	Br	42	100	831
19	H	3-SO_2CH_3	H	11	238	—
20	H	3-CN	Br	103	352	—
21	H	3-CN	H	51	772	—
22	H	3-CO_2H	Br	16	>1000	—
23	4-Cl	3-$CONH_2$	H	9	192	—
24	5-Cl	3-$CONH_2$	H	28	265	—
25	6-Cl	3-$CONH_2$	Br	76	>1000	874
Etravirine	—	—	—	26	19	108

[a] Values are the mean of at least two independent heteropolymeric enzyme inhibition assays (see Ref. 12).

Table 2
Enzyme inhibitory potency of 5′-substituted analogs



ID #	R^3	WT[a]	KY[a] IC_{50} (nM)	Y188L[a] IC_{50} (nM)
15	Br	19	30	70
16	H	7	16	34
26	Cl	24	13	203
27	F	14	14	35
28	CH_3	12	15	—
29	CF_3	86	41	—
30	NH_2	19	17	118
31	NO_2	109	64	—

[a] Values are the mean of at least two independent heteropolymeric enzyme inhibition assays (see Ref. 12).

inhibitors of the WT, KY, and Y188L enzymes. In fact, the carboxamide was clearly the superior 3-substituent over the sulfonamide, cyano, sulfone, and carboxylic acid groups exemplified in derivatives **12–13** and **18–22**. Addition of a second head-ring substituent in compounds **23–25** did not add activity in this series. The superior potencies associated with the 3-carboxamide in this data set were sufficiently striking that this substituent was kept constant in our further structural modifications.

The next variable we examined was the 5′-substituent on the pyrimidine core ring. Our crystal structures and modeling studies had suggested that such substituents could occupy a small hydrophobic pocket formed by G138, V179 and Y181, and modulate the potency of these molecules. Thus, several analogs were made

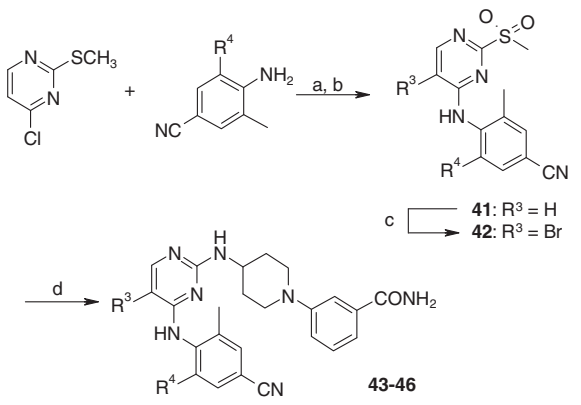
according to the chemistry of Scheme 1 by employing intermediates **6** where R^3 is other than H or Br, and activities of the products **26**–**31** are summarized in Table 2. Although no single substituent was clearly superior, it did appear that small hydrophobic groups (R^3 = H, F or CH_3) are preferable at this position.

The two final SAR variables we investigated were substituents on the tail ring and the nature of the linker to that ring. The 2,6-bis-substitution pattern of the tail ring appears necessary to maintain the orthogonal relationship of that ring with the core, which seems to be a characteristic of Etravirine-like structures.[10] The flanking methyl groups are suspected as a metabolic liability, but other substituents can be tolerated in at least one of the two positions.[13] Exchange of the ether linkage for an amino (NH) might be expected to affect activity via effects on the conformation and polarity of the molecule.

Phenyl ethers **32–40** where tail ring substituent R^4 is other than methyl were prepared from the appropriate intermediates **4–6** by the chemistry of Scheme 1. A different method was used for synthesis of the NH-linked compounds in order to achieve regioselectivity in the coupling reactions. Thus, as shown in Scheme 2, the treatment of 4-chloro-2-methylsulfanyl pyrimidine with 2,6-disubstituted-4-cyanoanilines followed by an MCPBA oxidation gave methylsulfones **41**. The 5′-Br intermediates **42** were prepared by bromination of **41** with NBS. Displacement of the methylsulfone groups of **41** and **42** with amine **10** then afforded the desired NH-linked products **43–46**.[11]

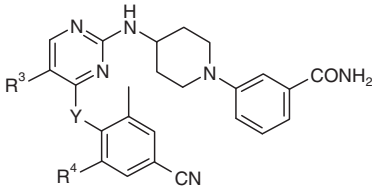
Enzyme inhibitory activities are presented in Table 3 for representative products containing the R^4-groups Cl, OCH_3, CH_3, and F, the linkers O and NH, and various 5′-substituents. Most of these combinations seemed well tolerated. Indeed, several compounds had quite excellent enzyme potencies, with **32** and **40** demonstrating single digit nanomolar activities versus wild-type and KY mutant enzymes.

At this point antiviral data was collected for several of the most promising compounds. Thus, inhibitory potencies were measured for the WT, KY, and Y188L viruses, as well as for two other frequently encountered mutant strains L101I/K103N and V106A.[14] Their protein shifted potencies against the WT virus were also determined.[15] This data along with human liver microsome clearance data (HLM) for selected analogs and reference NNRTIs is presented in Table 4. It is evident from the table that activity profiles significantly superior to the reference compounds have been achieved with the present scaffold. The most potent compounds are **28**, **32**, **35**, and **46**, and the profile of **28** is particularly impressive when the low protein shift is noted. Unfortunately, these most potent compounds were also cleared rapidly in HLM assays. The facile microsomal clearance is not totally unexpected given the lipophilicity of the molecules and the known susceptibility of aromatic methyl groups to oxidative metabolism. Indeed, metabolite ID studies confirmed the oxidation of the tail ring methyl groups, and somewhat unexpectedly, they also found products of degradation via N-dephenylation.[16]



Scheme 2. Reagents and conditions: (a) neat, 160 °C; (b) MCPBA, CH_2Cl_2, 20 °C; (c) NBS, CH_2Cl_2, 20 °C; (d) **10**, DIPEA, DMSO, 150 °C.

Table 3
Enzyme inhibitory potency of compounds with tail ring and linker modifications



ID #	R^3	R^4	Y	WT[a] IC_{50} (nM)	KY[a] IC_{50} (nM)	Y188L[a] IC_{50} (nM)
16	H	CH_3	O	7	16	34
28	CH_3	CH_3	O	12	15	—
32	H	Cl	O	8	6	—
33	Br	Cl	O	28	17	413
34	Cl	Cl	O	20	13	48
35	CH_3	Cl	O	6	20	—
36	H	OCH_3	O	18	54	64
37	Br	OCH_3	O	56	28	—
38	Cl	OCH_3	O	19	50	101
39	CH_3	OCH_3	O	21	11	66
40	Br	F	O	6	5	29
43	H	CH_3	NH	23	18	—
44	Br	CH_3	NH	19	16	75
45	H	Cl	NH	36	17	—
46	Br	Cl	NH	15	10	42

[a] Values are the mean of at least two independent heteropolymeric enzyme inhibition assays (see Ref. 12).

A second set of analogs of interest includes **40**, **43**, and **44**, which all show reasonable clearance as well as good antiviral activity. Compound **44** in particular has a mutant profile very similar to that of Etravirine, and **40** has even better potential when the protein shift is considered. Although it is difficult to generalize about the antiviral SAR of the *N*-phenyl piperidine scaffold, it does appear that the small, lipophilic 5′-substituents (H, Br or CH_3) on the core ring are most consistent with good potency, and a variety of R^4-groups are tolerated in place of methyl as a tail ring flanking substituents. Also, a source of extra polarity in the molecule seems helpful in reducing HLM clearance. This is seen with the 2-fluoro tail ring compound **40** and the NH-linked analogs **43** and **44**, although not with **46**.

Co-crystals of 3-substituted analogs with wild-type HIV-1 enzyme could not be prepared in good enough quality for X-ray diffraction work. However, it was possible to determine a crystal structure for the 4-sulfamoylphenyl piperidine **11**,[17] and an overlay with our previously reported structure of the 4-methanesulfonyl *N*-benzyl piperidine **2**[10] is shown in Figure 2. It is clear that a 3-substituent on the *N*-phenyl group would occupy essentially the same space as the 4-substituent of the *N*-benzyl ring, and this is evidently the preferred locus as implied by the SAR of both series. It places the polar head group near the solvent–protein interface and it is also well positioned for interaction with the V106 backbone NH. Further noteworthy interactions include the edge-to-face approach of the tail ring to W229, H-bonding of the pyrimidinyl NH with the K101 backbone carbonyl, and the stacking of the head-ring with P236. Also of interest is the nearly identical placement of the piperidine ring nitrogens of **11** and **2**. The latter had been shown to make a hydrogen bond with the backbone NH of K103 via a water molecule,[10] and a similar interaction is implied for **11**. The diffraction density for the bound water is not as conclusive in the case of **11**, but crystal structure distances and modeling support its presence in essentially the same position as noted for **2**.

Table 4
Antiviral potencies of several aminopyrimidine derivatives compared to reference compounds

Analog	R[3a]	R[4a]	Y[a]	WT[b]	WT, 40% HuS[c]	Protein shift[c]	K103N/Y181C[b]	Y188L[b]	K103N/L100I[b]	V106A[b]	HLM[d]
28	CH_3	CH_3	O	1.1	4.5	4.0	3.8	<1.2	6.7	0.6	730
32	H	Cl	O	0.4	7.5	17.4	5.3	1.6	42.0	0.4	274
35	CH_3	Cl	O	1.6	14.2	8.6	5.5	1.4	7.4	0.7	580
40	Br	F	O	7.3	18.2	2.5	28.0	5.5	18.4	3.9	82
43	H	CH_3	NH	0.5	13.9	27.1	15.2	3.7	>100	0.8	31
44	Br	CH_3	NH	1.5	14.2	9.8	11.7	2.7	10.8	0.9	42
46	Br	Cl	NH	1.2	9.9	8.6	4.9	<1.2	2.9	0.5	418
1	—	—	—	6.1	48.7	8.0	7.3	2.8	5.9	2.0	151
Etravirine	—	—	—	2.1	25.9	12.2	9.2	3.1	9.5	2.0	29
Efavirenz	—	—	—	1.8	18.1	10.1	82.8	>100	>100	5.7	—

[a] Substituents R^3, R^4, and Y are as defined in Table 3.
[b] Cell based antiviral assay in the presence of 10% FBS, IC_{50}'s in nM (see Ref. 14).
[c] WT activity in the presence of 40% human serum (HuS) indicates protein shifted activity, also given as ratio (see Ref. 15).
[d] Human liver microsome clearance in μl/min/mg.

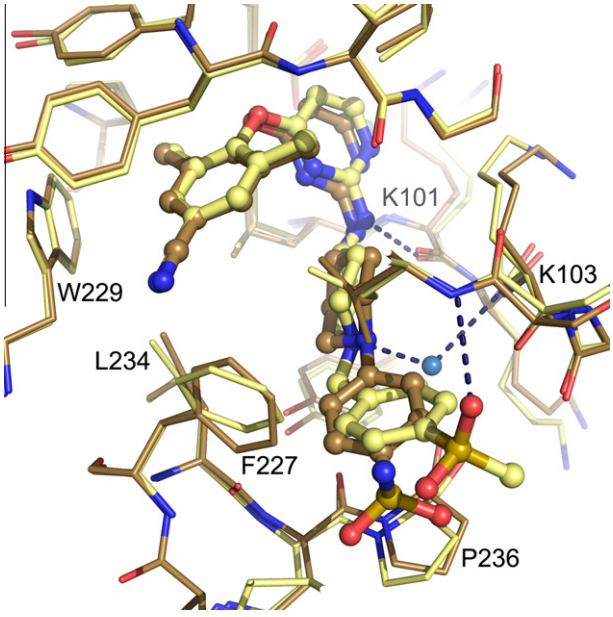


Figure 2. Overlay of crystal structures of *N*-phenyl piperidine **11** (bronze) and the *N*-benzyl piperidine **2** (ivory).

In summary, we have developed a new *N*-phenyl piperidinyl aminopyrimidine scaffold combining facets of the K101 and K103 binding classes of NNRTIs. Representative compounds **28** and **32** have achieved remarkable potency and mutant profiles, but they are readily cleared metabolically. Other compounds like **40** and **44** have reasonable clearance and possess lesser but still very good antiviral potencies. It appears that having the major interactions made with conserved residues and the protein backbone, combined with the opportunity for extra interactions in the P236/V106 pocket and with K103, have afforded these molecules a remarkable immunity to RT mutant variability. It also seems the potential of very high potency with adequate metabolic stability may yet be realized from the N-substituted piperidinyl aminopyrimidine motif. Continued efforts towards these goals have included modifying the overall polarity of the molecules and further investigations of the SAR, and the results of those studies will be the subject of future reports.

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References and notes

1. De Clercq, E. *Chem. Biodivers.* **2004**, *1*, 44.
2. Balzarini, J. *Curr. Top. Med. Chem.* **2004**, *4*, 921.
3. Wainberg, M. A. *J. Acquir. Immune Defic. Syndr.* **2003**, *34*, S2.
4. Delaugerre, C.; Rohban, R.; Simon, A.; Mouroux, M.; Tricot, C.; Agher, R.; Huraux, J. M.; Katlama, C.; Calvez, V. *J. Med. Virol.* **2001**, *65*, 445.
5. Sweeney, Z. K.; Klumpp, K. *Curr. Opin. Drug Discov. Devel.* **2008**, *11*, 458.
6. Madruga, J. V.; Cahn, P.; Grinsztejn, B.; Haubrich, R.; Lalezari, J.; Mills, A.; Pialoux, G.; Wilkin, T.; Peeters, M.; Vingerhoets, J.; de Smedt, G., et al *Lancet* **2007**, *370*, 29.
7. Ren, J.; Chamberlain, P. P.; Stamp, A.; Short, S. A.; Weaver, K. L.; Romines, K. R.; Hazen, R.; Freeman, A.; Ferris, R. G.; Andrews, C. W.; Boone, L.; Chan, J. H.; Stammers, D. K. *J. Med. Chem.* **2008**, *51*, 5000.
8. Davies, S. L.; Castaner, J.; Silvestre, J. S.; Bayes, M. *Drugs Future* **2005**, *30*, 462.
9. Sahlberg, C.; Zhou, X. *Anti-infect. Agents Med. Chem.* **2008**, *7*, 101.
10. Kertesz, D. J.; Brotherton-Pleiss, C.; Yang, M.; Wang, Z.; Lin, X.; Qui, Z.; Hirschfeld, D. R.; Gleason, S.; Mirzadegan, T.; Dunten, P. W.; Harris, S. F.; Villaseñor, A.; Hang, J. Q.; Heilek, G. M.; Klumpp, K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4025.
11. For a more detailed description of these syntheses see: Kertesz, D. J.; Brotherton-Pleiss, C.; Yang, M. US 2008/0146595.
12. *Enzymatic assay*: IC_{50} values were obtained from a SPA heteropolymeric assay. RNA-dependent DNA polymerase activity was measured using a biotinylated primer oligonucleotide and tritiated dNTP substrate. Newly synthesized DNA was quantified by capturing the biotinylated primer molecules on streptavidin coated Scintillation Proximity Assay (SPA) beads (Amersham). Each value represents the average of at least two independent assays.
13. Ludovici, D. W.; De Corte, B. L.; Kukla, M. J.; Ye, H.; Chih, Y. H.; Lichtenstein, M. A.; Kavash, R. W.; Koen, A.; de Bethune, M. P.; Azijn, H.; Oauwels, R.; Lewi, P. J.; Heeres, J.; Koymans, L. M. H.; de Jonge, M. R.; Van Aken, K. J. A.; Daeyaert, F. F. D.; Das, K.; Arnold, E.; Janssen, P. A. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2235.
14. *Replication assay*: EC_{50} values were obtained following incubation of compound with infected MT4 cells in the presence of 10% fetal bovine serum. The assay assesses the reduction in the cytopathic effect of HIV-1 on MT4 cells in the presence of HIV-RT inhibitors. Each value represents the average of at least two independent assays. For a detailed description of the antiviral assay see: Pauwels, R.; De, C. E.; Desmyter, J.; Balzarini, J.; Goubau, P.; Herdewijn, P.; Vanderhaeghe, H.; Vandeputte, M. *J. Virol. Methods*, **1987**, *16*, 171.
15. Determination of potency versus wild type virus was repeated in the presence of 40% human serum (HS) for these compounds. The resulting 'protein shifted potency' as compared to the result with 10% FBS (see Ref. 14) is considered an indicator of potential for in vivo activity.
16. Wang, J.; DeMaio, W.; Chandrasekaran, A.; Shen, L.; Bach, A., II; Scatina, J.; Talaat, R. *Curr. Drug Discovery Technol.* **2006**, *3*, 101.
17. Atomic coordinates for the structure of **11** bound to HIV-RT were deposited with the RCSB Protein Data Bank (PDB) under the access code 3 NBP.