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LETTER

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Disubstituted Bis-THF Moieties as New P2 Ligands in Nonpeptidal HIV-1 Protease Inhibitors

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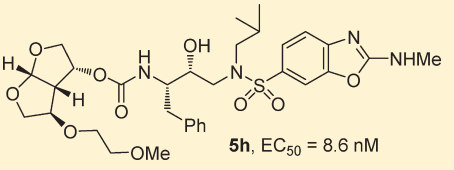
Supporting Information

ABSTRACT: A series of darunavir analogues featuring a substituted bis-THF ring as P2 ligand have been synthesized and evaluated. High affinity protease inhibitors (PIs) with an interesting activity on wild-type HIV and a panel of multi-PI resistant HIV-1 mutants containing clinically observed, primary mutations were identified using a cell-based assay. A number of PIs have been synthesized that show equivalent and greater activity for HIV-1 mutant strains as compared to wild-type HIV-1. The activity on the purified enzyme was confirmed for a selection of analogues.



KEYWORDS: HIV protease, inhibitors, darunavir, bis-THF bis-diol

The advent of protease inhibitors (PIs) into highly active antiretroviral therapy (HAART) regimens in 1996 marks a major innovation in AIDS chemotherapy.[1] Inhibition of the HIV-1 protease by PIs leads to immature, noninfectious viral particles and suppresses HIV-1 replication in the patient.[2] However, high replication rates, lack of HIV reverse transcriptase proofreading capacity, and therapeutic pressure produce mutant strains that are resistant to one or more PIs.[3] Other limitations arise from the peptidelike character of all first generation PIs. A major breakthrough in subsequent structure-based design was the development of a stereochemically defined fused bis-tetrahydrofuran (bis-THF) moiety as a nonpeptidal high-affinity P2 ligand, leading to the selection of darunavir (**1**, DRV, Figure 1) as a conceptually new HIV-1 PI.[4,5] DRV has proven to be highly effective against a broad spectrum of multi-PI resistant HIV mutant strains.[6] The X-ray crystal structure of DRV-bound HIV-1 protease revealed an extensive network of ligand−protein hydrogen-bonding interactions involving the enzyme backbone. Both oxygen atoms of the bis-THF moiety form strong hydrogen bonds with the backbone NH groups of Asp 29 and Asp 30.[7] Because the structure of the bis-THF ligand is very significant for the superior resistance profile of DRV, further research mainly focused on DRV analogues with alterations around the sulfonamide P2′ substituent and the P1 phenyl group.[8−10] However, our studies of the X-ray crystal structure of HIV-1 protease in complex with DRV and several recognition sequences (see the Supporting Information) led to the conclusion that substituents on the bis-THF B-ring could lead to additional interactions of the PI with the enzyme backbone. In particular, hydroxy, alkoxy, or alkoxyalkyl groups on the C4-position of the bis-THF B ring could form hydrogen-bonding interactions with the Gly48 amide group, with or without a water molecule. In addition, phenyl or benzyl groups were predicted to be able to fill a hydrophobic pocket in the proximity of the bis-THF moiety.

Ghosh reported the successful incorporation of an oxatricyclic tris-THF ligand into the PI structure (**2a**, Figure 1), in which the third THF moiety enables the formation of a water-mediated hydrogen bond with several conserved residues on the dimer interface as well as direct and water-mediated CH···O interactions with Gly48.[11] Recently, Ghosh et al. also reported the activity of the C4-alkoxylated bis-THF derivatives **2b**,**c** (as well as their corresponding diastereoisomer, not shown) on HIV-protease (HIV-1_{LA1}).[12] This prompts us to report our results on the synthesis and biological evaluation of novel DRV analogues with C4-*exo* substituted bis-THF ligands (**3a**−**j**[13] and **5a**−**i**, Figure 1).

While the C4-substituted DRV analogues could in principle be obtained via direct functionalization of the corresponding PI containing an OH group at that position (not shown), a conventional convergent strategy was chosen in which the PI analogues were obtained by coupling of a functionalized bis-THF derivative **6** with a number of amines **7** (Scheme 1). A few cases (analogues **a**) required functionalization postcoupling to arrive at the desired PI (see below). At this stage, the DRV *para*-aminophenyl-sulfonamide **7a**[5] and the (2-methylamino)-benzoxazole-sulfonamide **7b**[8] were selected as P2′ ligands based on their pronounced hydrogen-bonding abilities in the S2′ subsite.

The enantioselective synthesis of the substituted bis-THF fragments **6b**−**j** is shown in Scheme 2 and relies on the regioselective protection of a bis-THF diol **8**, as described earlier.[13,14] From **10**, functionalization with benzyl bromide or with a dimethyl sulfoxide (DMSO)/acetic anhydride/acetic acid mixture[15] led to fragments **12b**[13] and **12j**. The synthesis of the phenoxy-containing ligand **12c** was achieved via a three-step sequence starting with nucleophilic aromatic substitution with

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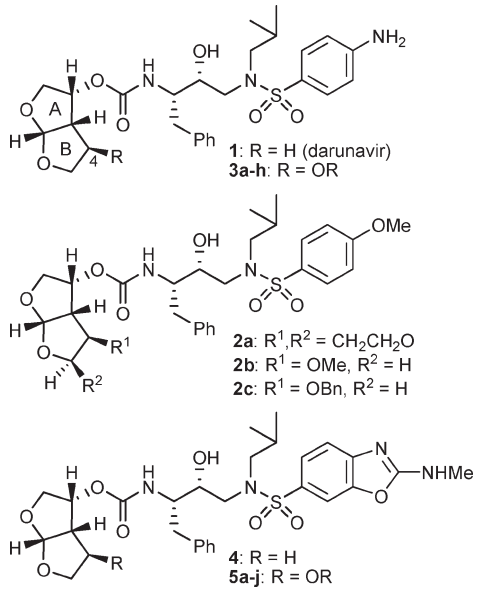
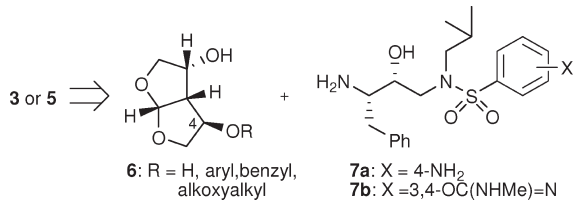
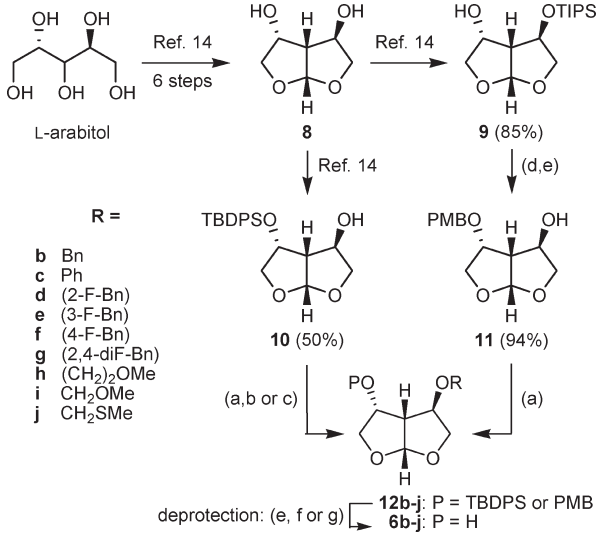


Figure 1. Structure of DRV (**1**) and PIs **2**−**5**.

Scheme 1. Retrosynthetic Analysis of Inhibitors 3 and 5



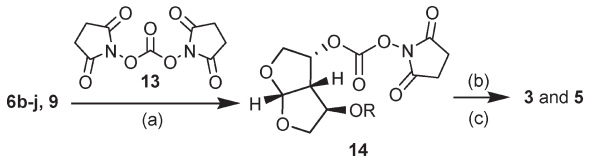
Scheme 2. Synthesis of Building Blocks 6b−j[a]



[a] Reagents and conditions: (a) NaH, DMF/THF; R-X, TBAI. (b) DMSO, Ac_2O, AcOH. (c) (i) F−C_6H_4−NO_2, KHMDS, THF; (ii) cyclohexene, $Pd(OH)_2$, EtOH; (iii) ^tBuONO, $BF_3 \cdot OEt_2$, CH_2Cl_2; Cu powder, EtOH. (d) NaHMDS, THF; PMBCl, TBAI. (e) TBAF, THF. (f) DDQ, CH_2Cl_2/H_2O. (g) H_2, Pd/C, EtOH.

4-fluoronitrobenzene followed by removal of the nitrogroup. However, the NaH-mediated benzylation suffered from competitive

Scheme 3. Synthesis of Inhibitors 1 and 2[a]



[a] Reagents and conditions: (a) Et_3N, CH_2Cl_2. (b) Compound **7a** or **7b**, Et_3N, CH_2Cl_2. (c) TBAF, THF (only for analogues b).

desilylation, which was avoided by using the *endo*-PMB ether **11**. Fluorobenzylation and alkylation afforded the bis-THF fragments **12d**−**i**. Deprotection of **12b**−**j** to bis-THF alcohols **6b**−**j** was achieved via TBAF-mediated desilylation, DDQ oxidation, or Pd-catalyzed hydrogenolysis. Silyl ether **9** also served as the precursor for the synthesis of PI analogues **3a** and **5a** (see below).

Finally, the synthesized ligands **6b**−**j** and silyl ether **9** were converted into the corresponding mixed carbonates **14** using disuccinimidyl carbonate (**13**, DSC)[16] and triethylamine (Scheme 3). Treatment of known amines **7a**[5] or **7b**[8] with the activated carbonates **14** in the presence of triethylamine afforded the PIs **3** and **5**. For the synthesis of PIs **3**/**5a** additional treatment with TBAF was required to remove the silyl protection on the *exo*-hydroxy group.

All compounds were tested for their antiviral activity against wild-type HIV-1 and a panel of recombinant clinical isolates, which were selected because of their high level of cross-resistance against several approved PIs.[3] Every isolate has 6−8 amino acid substitutions in the protease gene that are associated with resistance to PIs, including the primary mutations Met46 → Ile (M46I), G48V, I50V, L76V, V82A, I84V, and L90M (see the footnote of Table 1). Isolates M1 and M2 are of particular interest, as they were identified with primary mutations (I50V, I84V, and L76V) that are associated with resistance to DRV.[3] Atazanavir (entry 1), lopinavir (entry 2), DRV (**1**, entry 3), and its previously described benzoxazole analogue **4**[8] (entry 10) serve as reference compounds.

All three approved PIs (entries 1−3) inhibit HIV-1_{IIIB} at low nanomolar concentrations (EC_{50} = 6.6−13.9 nM) but exhibit a great variation in their resistance profile. Atazanavir and lopinavir (entries 1 and 2) showed a decreased activity against the majority of mutants. Interestingly, with atazanavir, an increased inhibition of mutant M2 was observed. DRV (**1**, entry 3) is equally potent against isolate M1. However, while mutant M3 was found to be hypersusceptible to DRV, a loss in activity was noted with isolate M2, which bears two primary mutations known to be associated with decreased susceptibility to DRV (I50V and L76V). Hypersusceptibility is defined as a lower fold-change on a mutant virus when being compared to the wild-type virus (FC < 1) and has only been reported for a few cases.[10,17] The benzoxazole analogue **4** (entry 8) is slightly more potent against HIV-1_{IIIB} and shows a better resistance profile against the mutant panel (EC_{50} = 0.9−3.2 nM). All newly synthesized compounds, apart from the free hydroxy analogues **3**/**5a**, inhibit wild-type HIV-1 at low nanomolar or even subnanomolar concentrations and show good cytotoxicity profiles ($CC_{50} \geq$ 9.8−32.3 μM), producing an acceptable selectivity index (CC_{50}/EC_{50}) of over 2000. The activity on HIV-1_{IIIB} was also measured in the presence of 50% human serum (HIV-1_{IIIB} + 50% HS) as an indication for the tendency of the compounds to bind to plasma proteins. The data show that there is no extensive plasma protein binding. PIs **3a** and **5a** (entries 4 and 9) were found to be only weak inhibitors of

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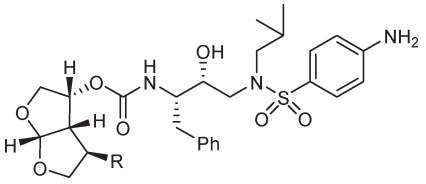
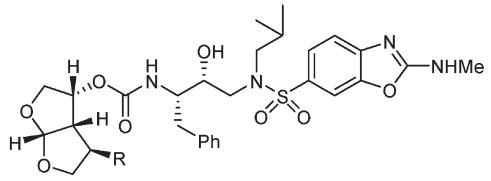
Table 1. Antiviral Activity (EC_{50}) of Compounds 3 and 5 against Wild-Type ($HIV\text{-}1_{IIIB}$) and Mutant Viruses[a]



Entry	compound	R	EC_{50} [nM][b] (*fold change relative to HIV-1_{IIIB}*)				
			$HIV\text{-}1_{IIIB}$	$HIV\text{-}1_{IIIB}$ + 50% HS	M1	M2	M3
1	atazanavir		6.9	13.3	58.0 (*8.4*)	3.2 (*0.5*)	60.5 (*8.8*)
2	lopinavir		13.2	63.2	114.6 (*8.7*)	307.3 (*23*)	32.1 (*2.4*)
3	**1**	H (darunavir)	6.6	17.2	11.3 (*1.7*)	51.0 (*7.8*)	2.3 (*0.4*)
4	**3a**	OH	772.9	814.7	112.3 (*0.1*)	204.5 (*0.3*)	40.4 (*<0.1*)
5	**3b**	OBn	1.5	12.1	4.7 (*3.1*)	18.0 (*12*)	1.7 (*1.1*)
6	**3c**	OPh	8.9	47.2	32.5 (*3.7*)	249.1 (*28*)	6.5 (*0.7*)
7	**3h**		15.5	37.0	11.9 (*0.8*)	71.4 (*4.6*)	6.8 (*0.4*)



8	**4**	H	2.3	8.3	2.7 (*1.2*)	3.2 (*1.4*)	0.9 (*0.4*)
9	**5a**	OH	137.7	446.3	125.4 (*0.9*)	108.0 (*0.8*)	104.1 (*0.8*)
10	**5b**	OBn	0.8	3.7	1.7 (*2.2*)	3.4 (*4.5*)	1.0 (*1.3*)
11	**5d**		0.7	4.0	1.3 (*2.0*)	2.1 (*3.1*)	0.9 (*1.3*)
12	**5e**		0.6	3.7	0.8 (*1.2*)	1.7 (*2.7*)	0.8 (*1.3*)
13	**5f**		0.6	4.0	1.1 (*1.8*)	2.3 (*3.8*)	1.0 (*1.6*)
14	**5g**		0.6	5.4	1.1 (*2.0*)	2.3 (*4.1*)	0.7 (*1.3*)
15	**5h**		8.6	24.0	6.9 (*0.8*)	10.8 (*1.3*)	4.3 (*0.5*)
16	**5i**		3.4	7.0	5.0 (*1.2*)	11.2 (*2.4*)	1.9 (*0.7*)
17	**5j**		1.3	6.7	3.9 (*3.0*)	9.3 (*7.1*)	2.2 (*1.7*)

[a] The following PI resistance-associated mutations were identified in the isolates. M1: L10I, **M46I**, I64V, **I84V**, **L90M**, I93L. M2: L10I, I13V, **M46I**, **I50V**, L63P, **L76V**. M3: L10I, K20R, M36I, **G48V**, I62V, A71V, **V82A**, I93L. Bold figures represent primary resistance mutations, and underlined figures are associated with resistance to DRV according to the IAS (ref 3). [b] The EC_{50} values were determined using MT4 cells, and all assays were conducted in quadruplicate. The numbers in parentheses represent the fold change in EC_{50} value for each isolate relative to the wild-type $HIV\text{-}1_{IIIB}$.

$HIV\text{-}1_{IIIB}$ but showed a good resistance profile toward the mutant panel. Indeed, all three isolates were found to be hypersusceptible to both compounds. For the other C4-substituted PIs, some general trends could be established. More lipophilic compounds (entries 5, 6, and 10−14) exhibit good activity against wild-type virus and clinical isolates M1/M3, but a loss in activity (FC = 2.7−28) was observed with mutant M2. The benzylated PI **3b** (entry 5) has also been tested previously against a different mutant panel, whereas similar resistance patterns were observed.[13] Although being slightly less active against $HIV\text{-}1_{IIIB}$ when compared to DRV or the benzylated analogues, a more balanced activity profile was observed for the methoxyethoxy-substituted PIs **3**/**5h** (entries 7 and 15). The clinical isolates M1 and M3 were found to be hypersusceptible to inhibitors **3**/**5h**, and only a 1−5-fold increase of EC_{50} was noted against isolate M2. A further analysis of the relationship between chain length and antiviral activity showed that a methyl linker (compounds **5i** and **5j**, entries 16 and 17) is slightly favored over the ethyl linker (compound **5h**) on $HIV\text{-}1_{IIIB}$. However, the methyl linker exhibits more variation in activity on the mutants,

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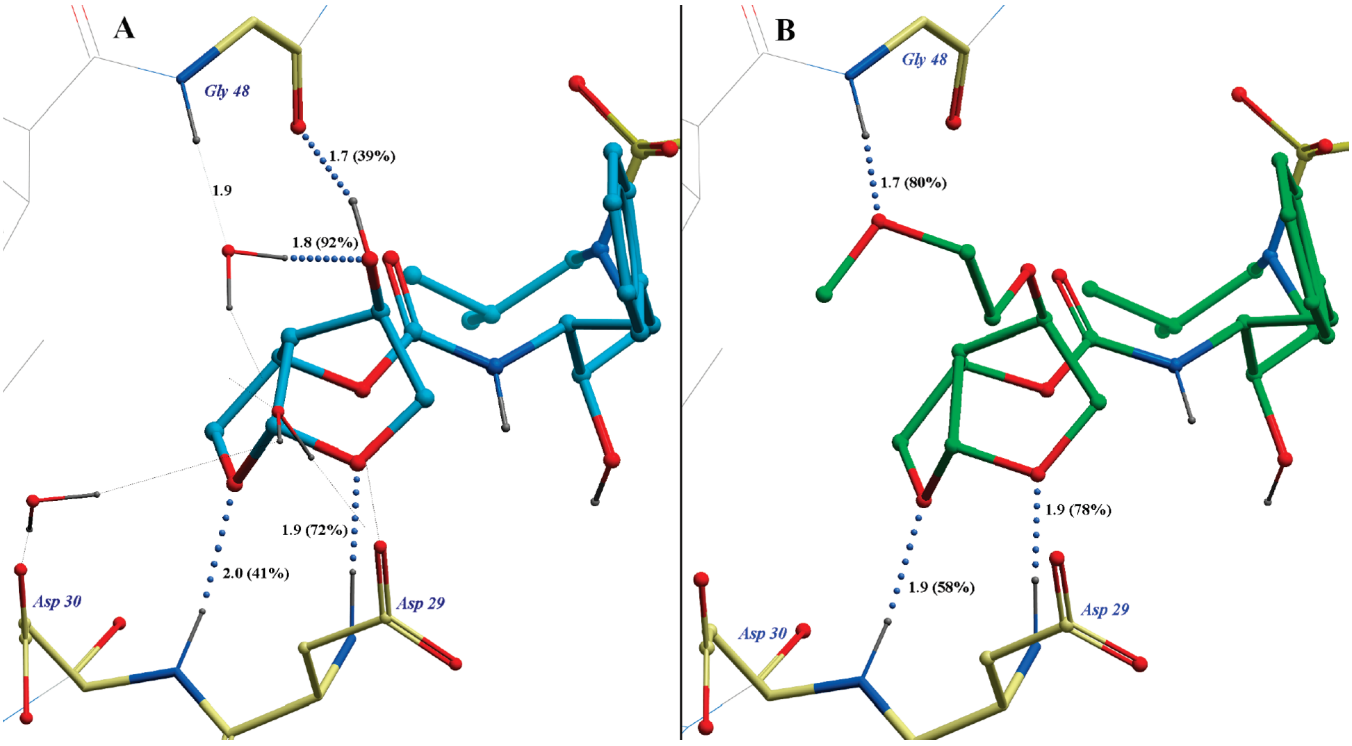

Figure 2. Three-dimensional depiction of possible binding modes of **3a** (A) and **3h** (B).

and the hypersusceptibility is lost. A direct interpretation of the hypersusceptibility remains challenging, since the observed effect might have multiple reasons. The most likely structural reason might be a changed protease or folding dynamics of the protease and substrates due to interference of the inhibitors with the flap region (containing Gly48 and Gly48′).

Unfortunately a direct comparison of these results with the data recently published by Ghosh[11,12] is difficult, as PIs having a *para*-methoxy sulfonamide-based P2′ ligand (as in **2a**) and *para*-amino sulfonamide- or benzoxazole-based P2′ ligands (as in **3** and **5**) have different interactions in the S2′ pocket of the protease resulting in a different resistance profile. In addition, the panel of mutants investigated by Ghosh for PI **2a** was focused on mutations mainly associated with resistance against other PIs, not including DRV as in our case. Although the tris-THF PI **2a** is approximately 10-fold more active against wild-type HIV-1 than DRV, activity was reduced against all of the selected multidrug-resistant mutants. A similar reduction of activity of DRV against their mutant panel was observed.

The enzymatic activity against wild-type HIV-1 protease was determined for the promising analogues **3h** (IC_{50} = 2.5 nM) and **5h** (IC_{50} = 1.5 nM) as well as the hydroxy analogue **3a** (IC_{50} = 2.9 nM) and benzyl derivative **3b** (IC_{50} = 3.5 nM).[18] All four PIs are highly active on the purified enzyme and show similar potency as DRV (**1**, IC_{50} = 3.8 nM). In fact, the enzymatic activity of PI **3a** is over 250-fold higher than in the cellular assay. This leads to the conclusion that additional ligand−protein interactions are present with PIs **3**/**5a**. However, the higher polar surface area and the increased number of hydrogen bond donors in the hydroxy analogues seem to limit the uptake into the cell, which explains the weak potency of **3**/**5a** against HIV-1_{IIIB} in the cellular assay.

In Figure 2, a 3D depiction of X-ray crystal structure-based modeling studies involving **3a** and **3h** is shown. On the basis of the 2HS1[19] coordinates, a R group extension in combination with a conformational search and subsequent energy minimizations was performed. In addition to the bonding distances, a percentage score is given, indicating the likeliness of a hydrogen bond based on statistical analysis as described by Clark and Labute.[20] The hydroxy group of PI **3a** appears to form a hydrogen bond with the backbone carbonyl of Gly48. This interaction is also observed in natural substrates of the PR (see the Supporting Information), which could contribute to the hypersusceptibility characteristic and competition against natural substrates of **3a**. Furthermore, the hydroxy group interacts with a water network, which itself is hydrogen bonding to amide NH of residue Gly48 and then extending to water-mediated interactions to the side chains of Asp29 and Asp30 (see Figure 2A). This analysis corresponds with the recently published crystal structure of the **2b**−HIV-1 protease complex,[11] in which the methoxy oxygen atom forms a water-mediated hydrogen bond with the amide NH of Gly48.

For **3**/**5h**, the methoxyethoxy moiety possibly displaces the water molecule and the second oxygen could form a direct hydrogen bond to the backbone NH of Gly48 (see Figure 2B). This interaction can also be found in some natural substrates, adding to the hypersusceptibility characteristic of those compounds. These direct backbone interactions, which are less influenced by residue mutations (side chain might change completely), furthermore explain the hypersusceptibility of the compounds **3**/**5h** and **3a**. The hydrogen-bonding distance is in both cases 1.7 Å but has in the case of **3**/**5h** a better orientation, which results in an 80% optimal hydrogen bond as calculated by Clark/Labute, while the contact in **3a** is only 39% optimal. A similar modeling study with fluorobenzyl analogue **5e** suggests weak C−H···π interactions between Gly48 and the aromatic ring (see the Supporting Information).

In conclusion, we designed and synthesized a series of disubstituted bis-THF containing HIV-1 PIs, which show

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interesting in vitro antiviral activity against wild-type virus and three multi-PI resistant virus strains. Many analogues display low nanomolar activity, and for some analogues, the hypersusceptibility of the mutants in a cellular context is noteworthy. On the basis of our modeling studies, it could be shown that PIs **3**/**5h** successfully extend the "backbone binding" concept,[21] which is crucial in combating multidrug resistance. Structural studies on the characterization of potential new ligand−enzyme interactions as well as further structure−activity relationship studies on disubstituted bis-THF ligands are currently underway.

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■ ASSOCIATED CONTENT

Supporting Information. Description of inhibition assay and experimental procedures and spectral data for compounds **3**, **5**, **6**, **9**, **11**, and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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