Analysis of HIV protease (HIVp) dimerization using immobilized monomeric HIVp. A buffer containing 0.1 M sodium acetate (pH 5.0), 1 mM EDTA and 3% DMSO v/v was used as a running buffer. It has been previously established that the maximum enzymatic activity of HIVp is observed at pH 5.0, and the monomeric and dimeric forms of HIVp reach equilibrium in solution (8,9) thus, analytes containing different concentrations of HIVp, were injected on to the chip surface with immobilized HIVp monomer. The calculated value of the apparent dissociation constant (K_d) was ~$1.0x10^{-7}$ M. In such a model system, the process of HIVp dimerization in the absence (control) or presence of phenanthridine derivatives was further studied. The control analytes contained 250 nM protease protein and 3% DMSO (v/v). The test analyte contained 250 nM protease protein and 100 μM compound in 3% DMSO (v/v). Control and test analytes were pre-incubated for 15 min on ice before injection. Subsequently, analytes were injected for 5 min at a flow rate of 20 μl/min (n=3). Table SII shows the optical biosensor output signals on a comparative assessment of the re-association of a dimeric form of HIVp chip in the absence (control) and in the presence of phenanthridine derivatives.

Figure S1. Surface plasmon resonance binding sensograms of different concentrations of compound 2a to the (A) monomeric and (B) dimeric form of HIV protease covalently immobilized on a CM5 optical chip. The resulting sensograms are the biosensor signal difference between the working (with immobilized HIV protease protein) and control flow cell (without protein immobilization). 1, 5 μM; 2, 15 μM; 3, 50 μM; RU, resonance units.

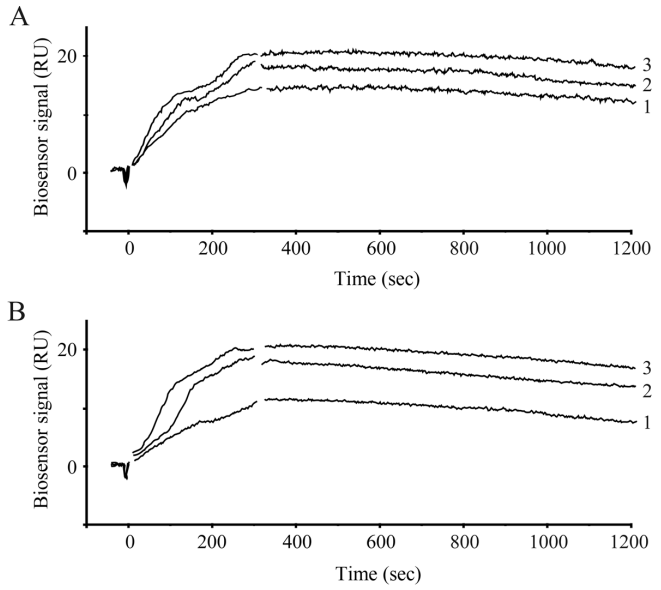


Figure S2. Inhibition curves of the enzymatic activity of HIV protease in the presence of different concentrations of compound 2a. The experiments were performed in duplicate. 1, control (without compound); 2, 9 μM; 3, 18 μM; 4, 36 μM; 5, 72 μM; 6, 108 μM.

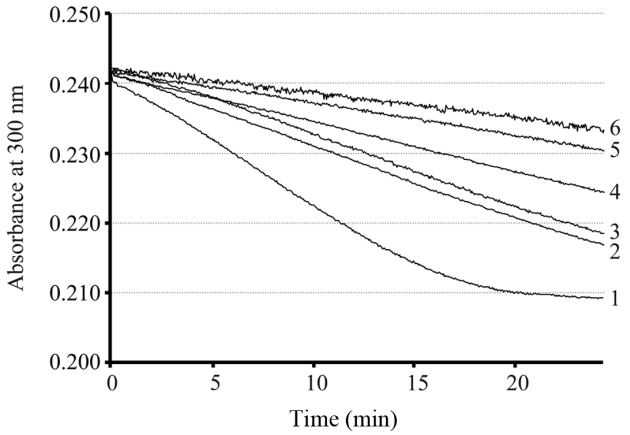
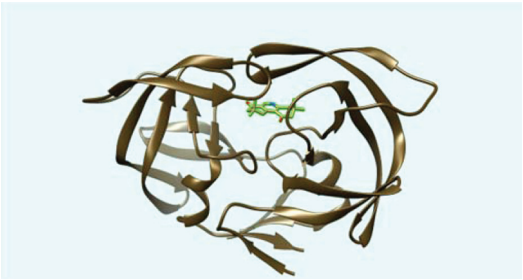
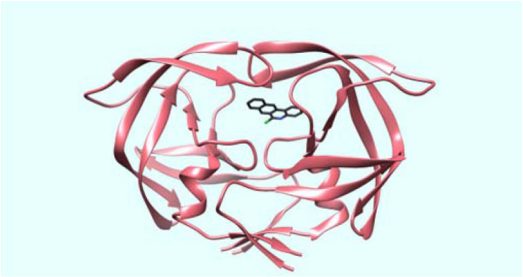
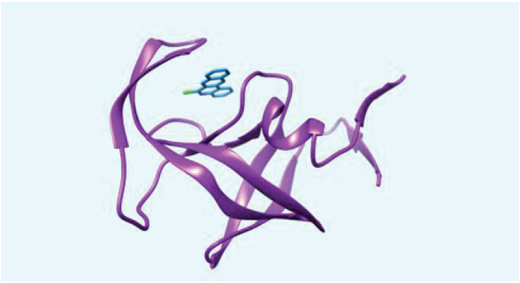
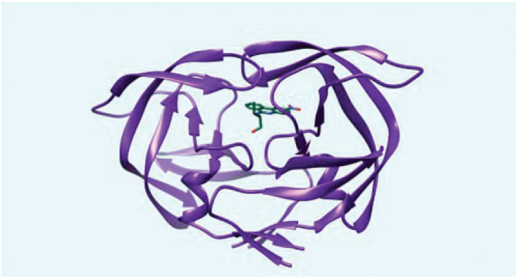
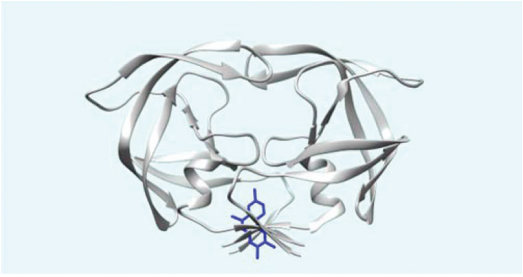
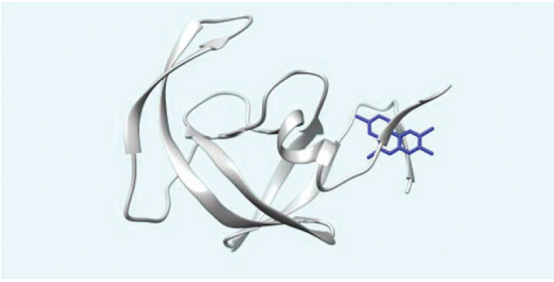


Table SI. The optimal docking conformations for the predicted complexes of phenanthridine derivatives with dimeric or monomeric forms of HIVp.

Compound	HIVp dimeric form	HIVp monomeric form[a]
2a	3,3,9,9, -tetramethyl-3,4,9,10-tetrahydro-2H, 8H-phenanthridine-1,7 dione	
Predicted binding structure		
4a	6-chloro-benzo(J)phenenthridine	
Predicted binding structure		
5a	2-(2-nitro-6-phenanthridinilamino)-1-butanol	
Predicted binding structure		
	2,3,6,8-tetrachlorophenanthridine (HIVp dimerization inhibitor)	
Predicted binding structure		

[a]HIVp monomer is rotated counterclockwise 90º relative to the dimer form.

Table SII. Surface plasmon resonance assessment of the effect of the phenanthridine derivatives on the dimerization of HIV1 protease.

Compound	Control	1a	2a	3a	4a	5a	6a	7a	8a
Biosensor signal, RU	365±10	372±12	375±9	367±10	382±12	370±8	366±8	373±10	362±14

RU, resonance units.