Identification of small molecule compounds targeting the interaction of HIV-1 Vif and human APOBEC3G by virtual screening and biological evaluation

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Table S1. The similarity analysis between IMB-301 and other active compounds. The similarity was calculated using Open Babel.

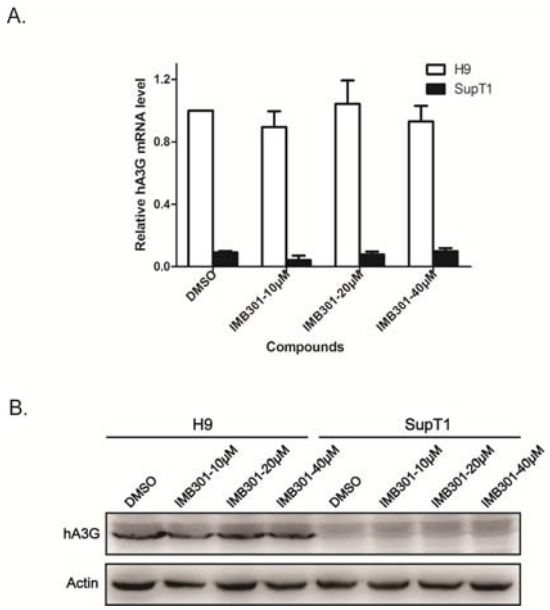


Figure S1. H9 and SupT1 cells were seeded in 6-well-plate, and treated with IMB301 at different concentrations (10μM, 20μM, and 40μM). 48 hours later, cells were collected for (A) qRT-PCR and (B) western blots assay. hA3G primers for qRT-PCR:
F'-TTACCTGCTTCACCTCCTGG
R'-TCATCTAGTCCATCCCAGGG

A.

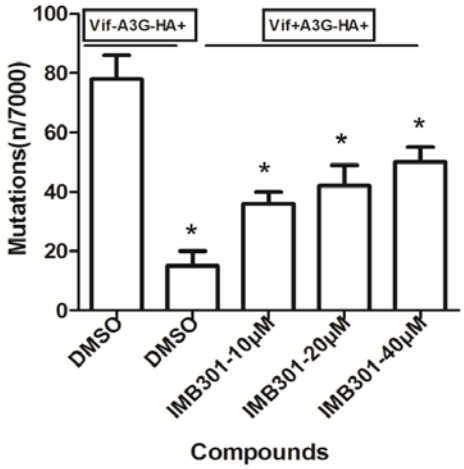
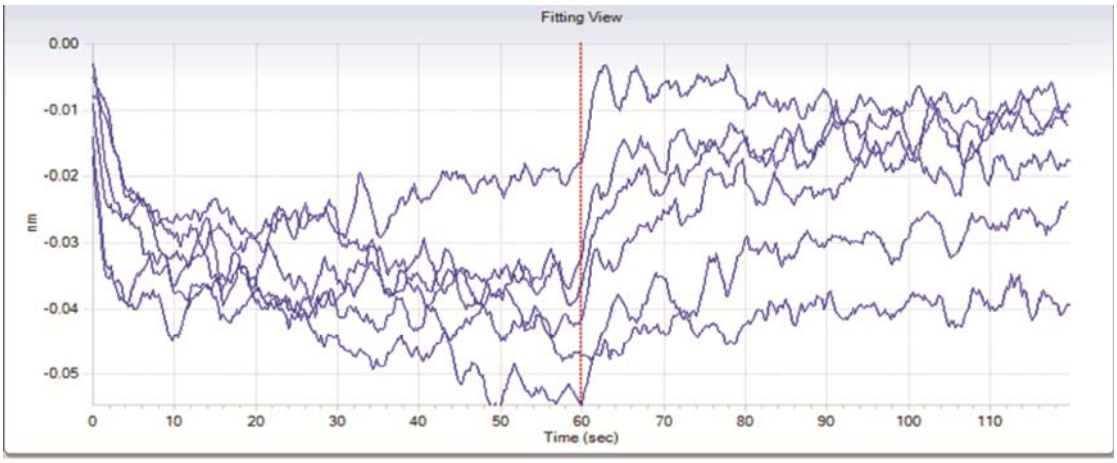
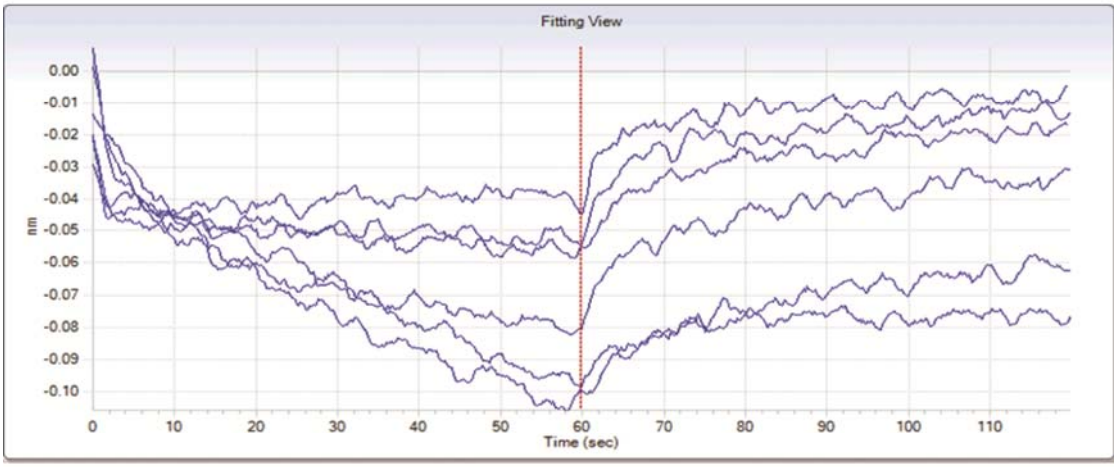


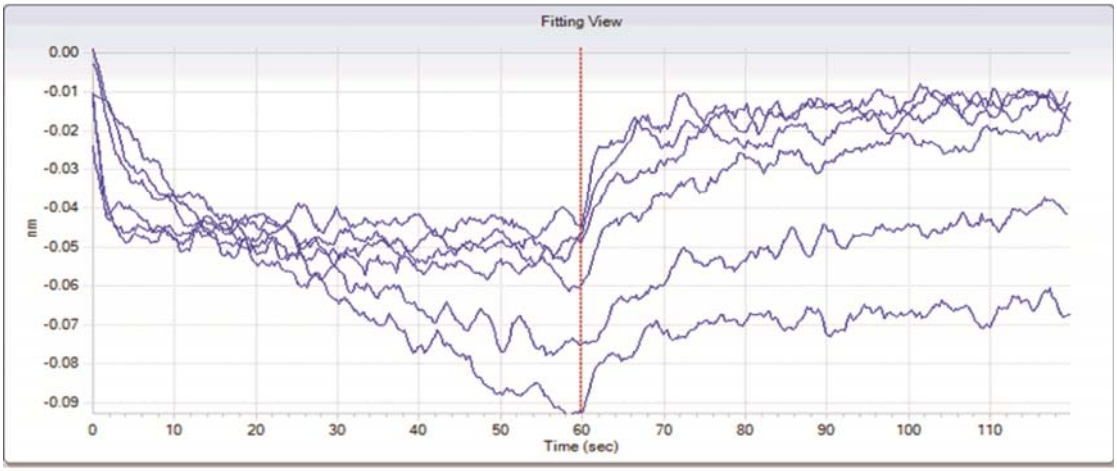
Figure S2. 293T cells were co-transfected with 300ng pNL4-3(R-E-)Vif+/-, 200ng VSVG and 200ng hA3G-HA (or pcDNA3.1) in 6-well-plate. After 12 h, the media was changed and IMB-301 was added at different concentrations (10μM, 20μM, 40μM). 36h later, the supernatants were collected and then used to infect the TZM-bl cells (1×105) in 6-well plates. 48 hours later, total DNA was isolated using DNeasy DNA isolation kit (Qiagen). About 700 bp DNA fragment of HIV-1 was amplified with Taq DNA polymerase (Invitrogen) using the primers HIV-1-F, 5'-AGGCAGCTGTAG ATATTAGCCAC, and HIV-1-R, 5'-GTATGAGGGATCTCTAGCTACCA36. The PCR products were cloned into the TA-cloning vector (Invitrogen). The nucleotide sequences of individual clones from each infected culture sample were determined. *Statistical significance was determined using the GraphPad Prism software ($P<0.05$)



IMB-293



IMB-350



IMB-945

Figure S3. Octet binding analysis of IMB-293, IMB-350, IMB-945 and hA3G has been performed respectively. There is no binding signal shown.

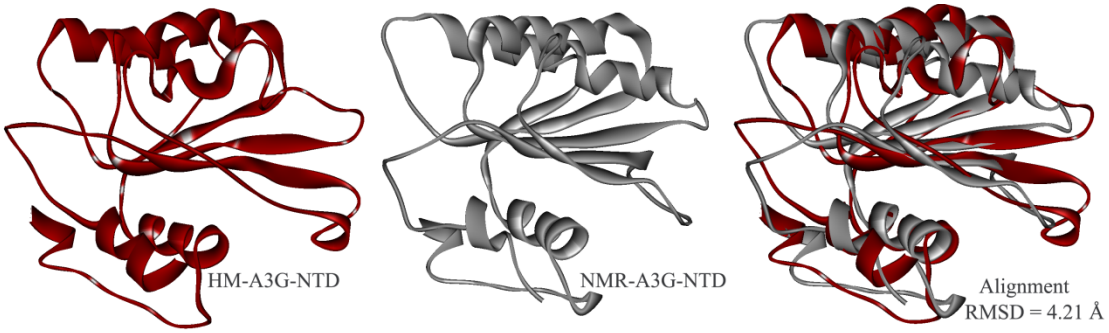


Figure S4. The superimposition of the homology structure of A3G-NTD (HM-A3G-NTD) and NMR structure of A3G-NTD (NMR-A3G-NTD) in Discovery Studio gives a RMSD value of 4.21 Å for overall structure.

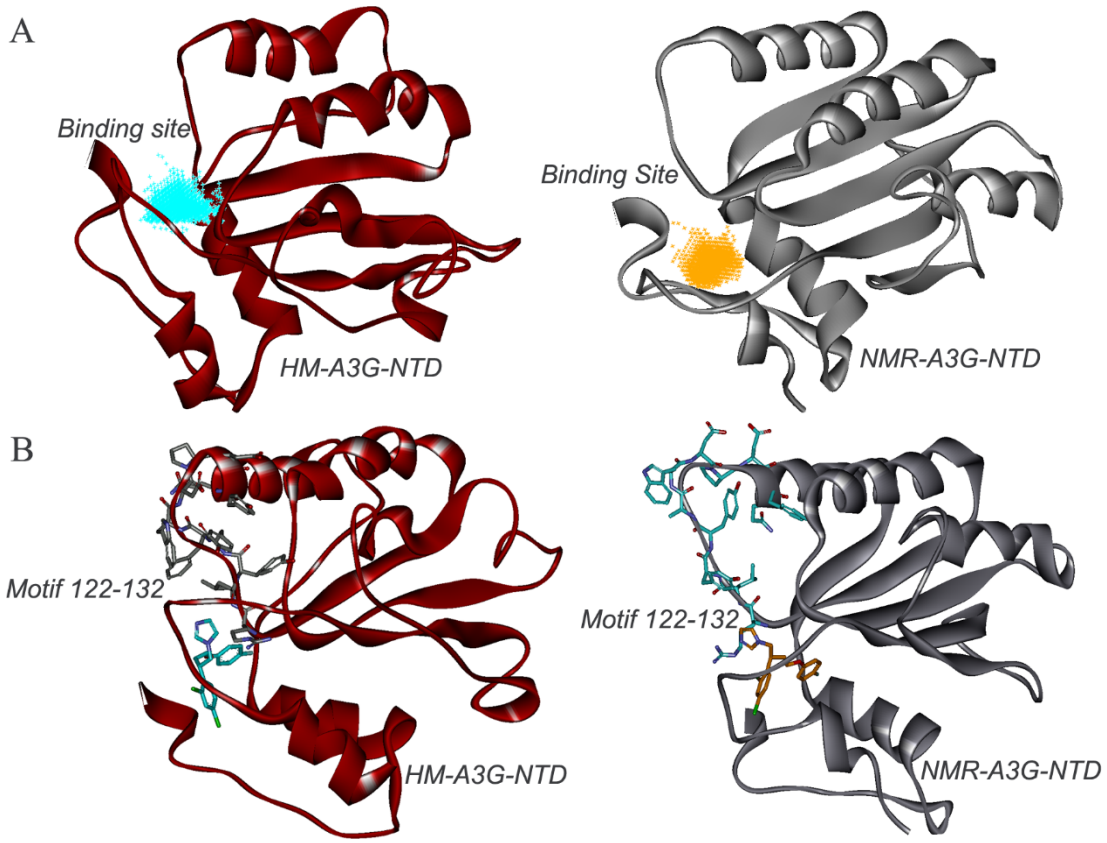


Figure S5. The predicted binding sites in A) the homology structure of A3G-NTD (HM-A3G-NTD) and the NMR structure of A3G-NTD (NMR-A3G-NTD) is closed to A3G/Vif interface (Motif 122-132); B) The similar predicted binding mode of IMB-301 in the binding site for the homology structure of A3G-NTD (HM-A3G-NTD) and the NMR structure of A3G-NTD (NMR-A3G-NTD), respectively.

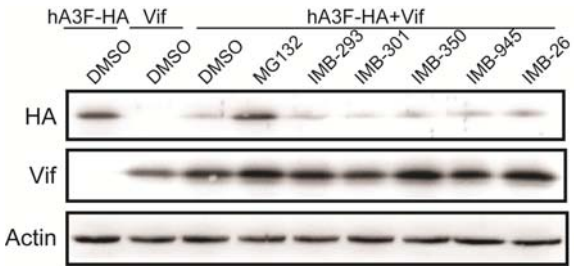
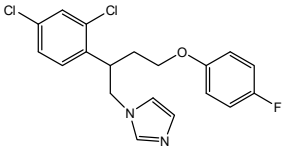
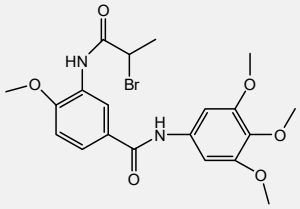
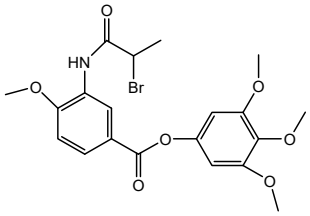
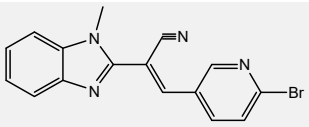
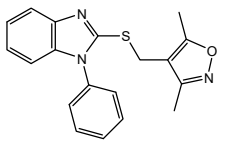
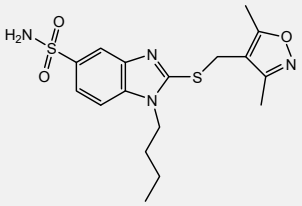
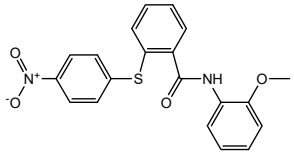
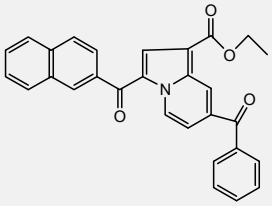


Figure S6. IMB-293, IMB-301, IMB-350, and IMB-945 specifically rescue hA3G degradation rather than hA3F in presence of Vif. 293T cells were co-transfected with hA3F-HA and Vif, 12 hours post-transfection, and the compounds were added at the concentration of 10μM. After 48 hours, cell lysates were harvested and proteins were detected by WB.

Table S1. The similarity analysis between IMB-301 and other active compounds. The similarity was calculated using Open Babel.

Name	Structure	Similarity
IMB-301		/
IMB-26		0.22
IMB-35		0.23
ZB-MA-1		0.20
MM-1		0.24
MM-2		0.23
RN-18		0.20
VEC-5		0.20