SUPPORTING INFORMATION

Inhibition of Insulin Amyloid Fibrillization by Glyco-Acridines: *In vitro* and *in silico* study

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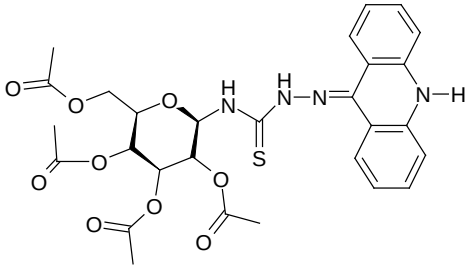
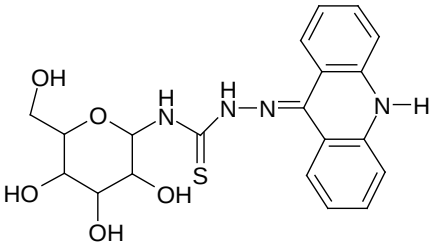
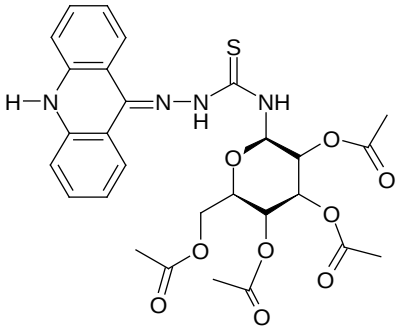
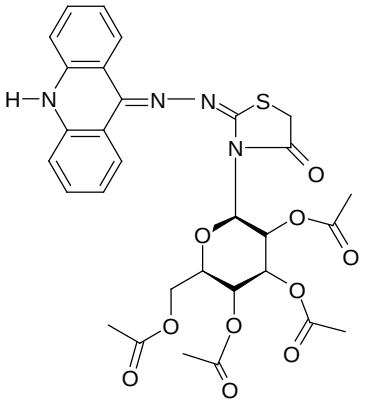
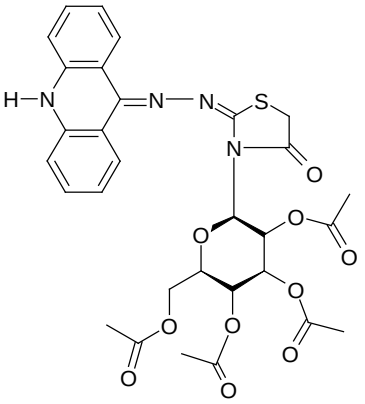
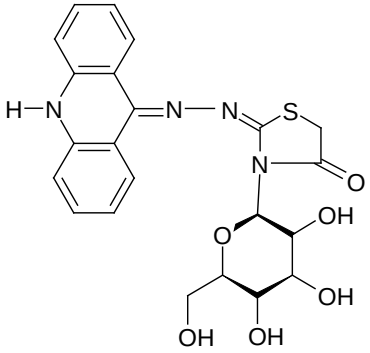
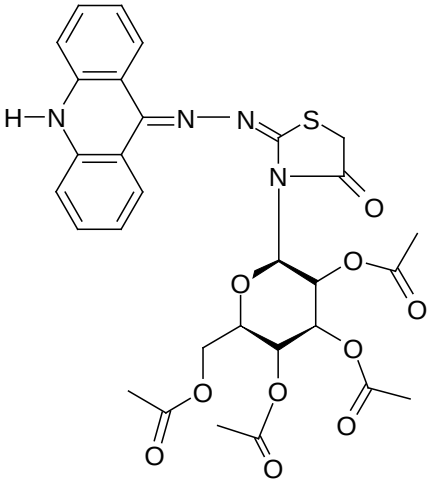
Table S1. Chemical composition and formula of studied glyco-acridines.

Thiosemicarbazides	
 **T1** 4-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranosyl)-1-(9,10-dihydroacridin-9-ylidene)thiosemicarbazide	 **T1OH** 4-(*β*-D-glucopyranos-1-yl)-1-(9,10-dihydroacridin-9 ylidene)thiosemicarbazide
 **T2** 4-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-1-(9,10-dihydroacridin-9-ylidene)thiosemicarbazide	
Cyclic 1,3-thiazolidinones	
 **C1** 2´-(9,10-Dihydroacridin-9-ylidene)hydrazono-3´-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-1´,3´-thiazolidin-4-one	 **C2** 2´-(9,10-Dihydroacridin-9-ylidene)hydrazono-3´-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-1´,3´-thiazolidin-4-one

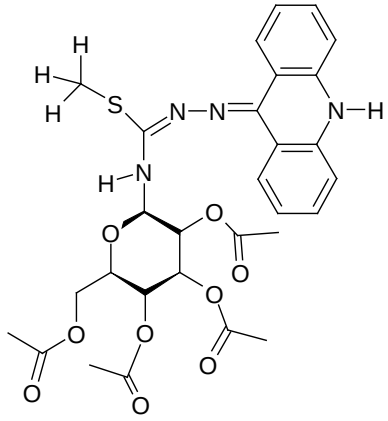


C2OH
2´-(9,10-Dihydroacridin-9-ylidene)hydrazono-3´-(*β*-D-galactopyranos-1-yl)-1´,3´-thiazolidin-4-one

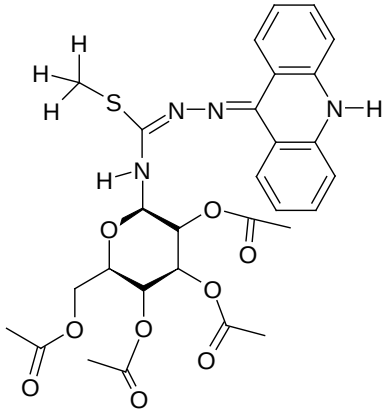


C3
2´-(9,10-Dihydroacridin-9-ylidene)hydrazono-3´-(2,3,4,6-tetra-*O*-acetyl-*β*-D-manopyranosyl)-1´,3´-thiazolidin-4-one

Isothiosemicarbazides



I1
S-Methyl-1-(9,10-dihydroacridin-9-ylidene)-4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)isothiosemicarbazide



I2
S-Methyl-1-(9,10-dihydroacridin-9-ylidene)-4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)isothiosemicarbazide

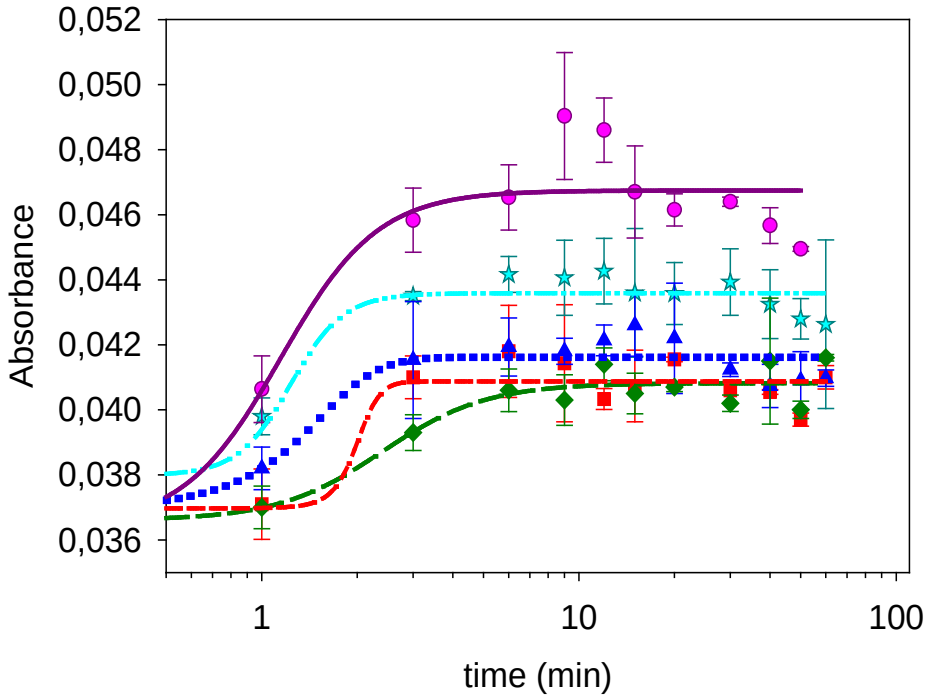


Figure S1. The kinetics of insulin fibrillization alone and in presence of glyco-acridine T1OH (cyan dash dot dot line), T2 (blue dot line), C1 (red dash line) or I1 (green long dash line) observed by changes in absorbance at 600 nm. Concentration of insulin was 10 μM, concentration of acridines was 60 μM. Measurements were performed using Synergy MX (BioTek) spectrometer in a 96-well plate. The experiments were performed in triplicates and the reported values are the averages of measured values with standard deviation.

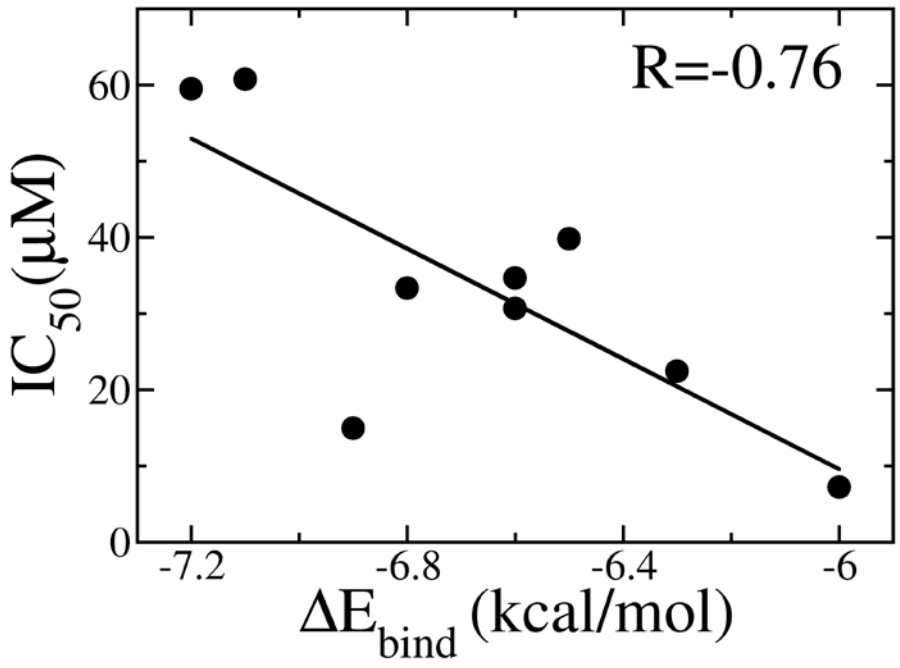


Figure S2. Correlation between experimentally determined IC_{50} values and binding energies obtained by the docking method. There are no error bars for ΔE_{bind} as it was calculated for one snapshot of the best docking mode.

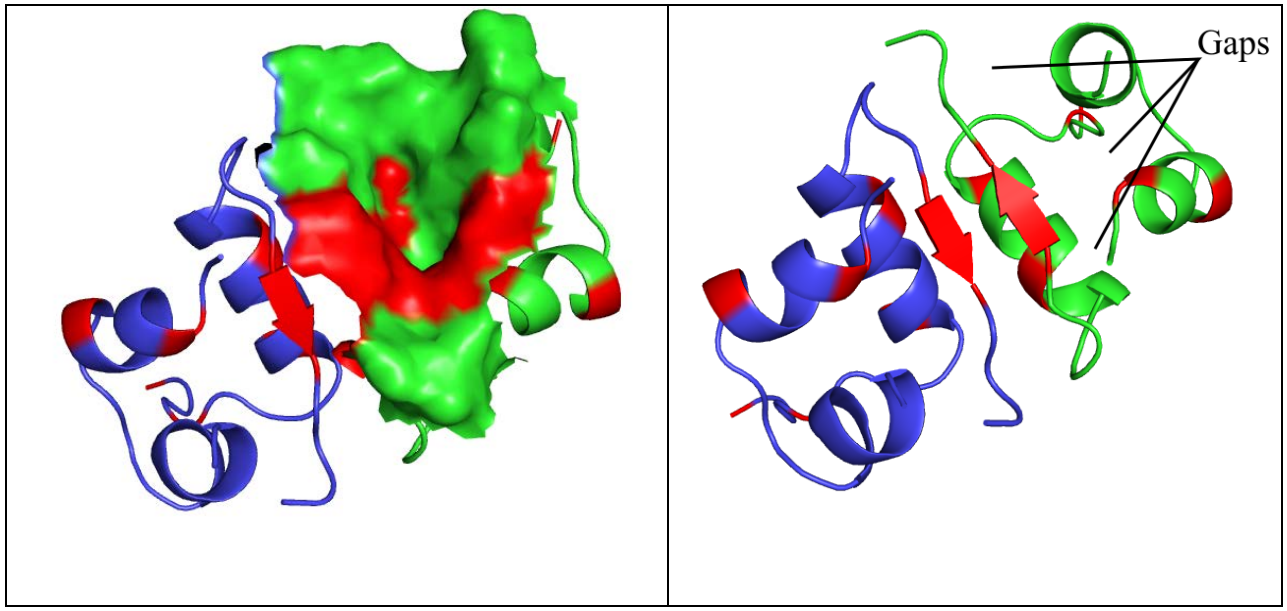


Figure S3. Binding pocket of insulin dimer, one monomer is in green, the other is in blue. HIS, PHE, TYR and TRP residues which have aromatic rings are in red. The flexible binding pocket including three gap points is located adjacently to the tangent of two monomers.

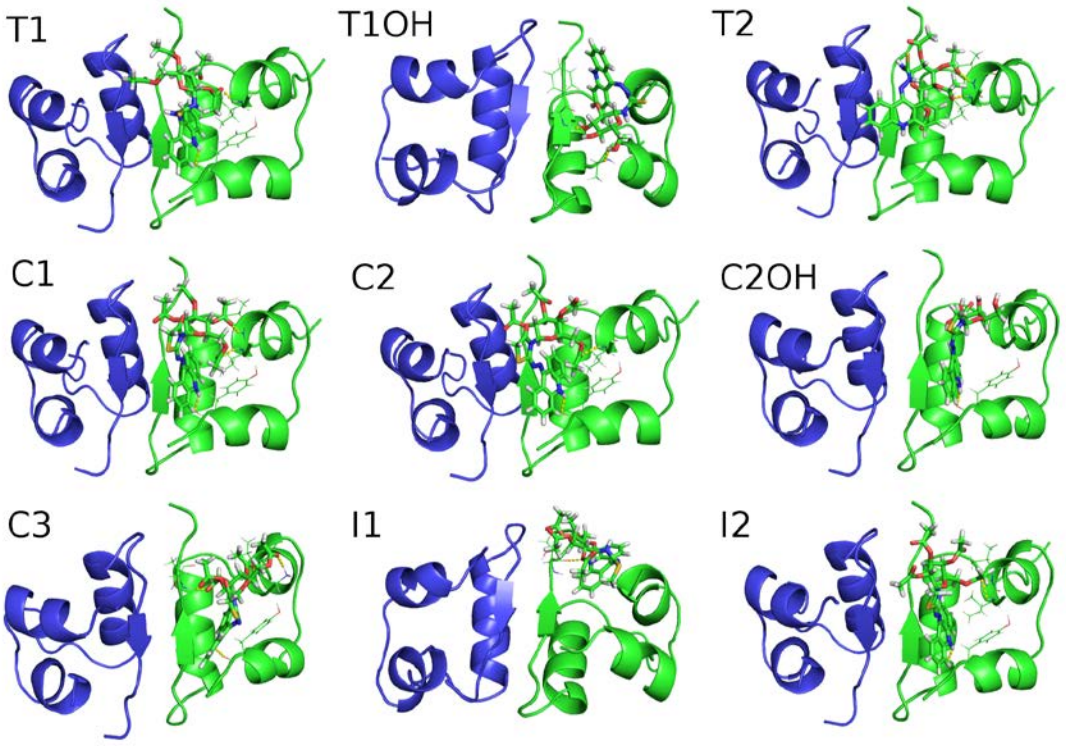
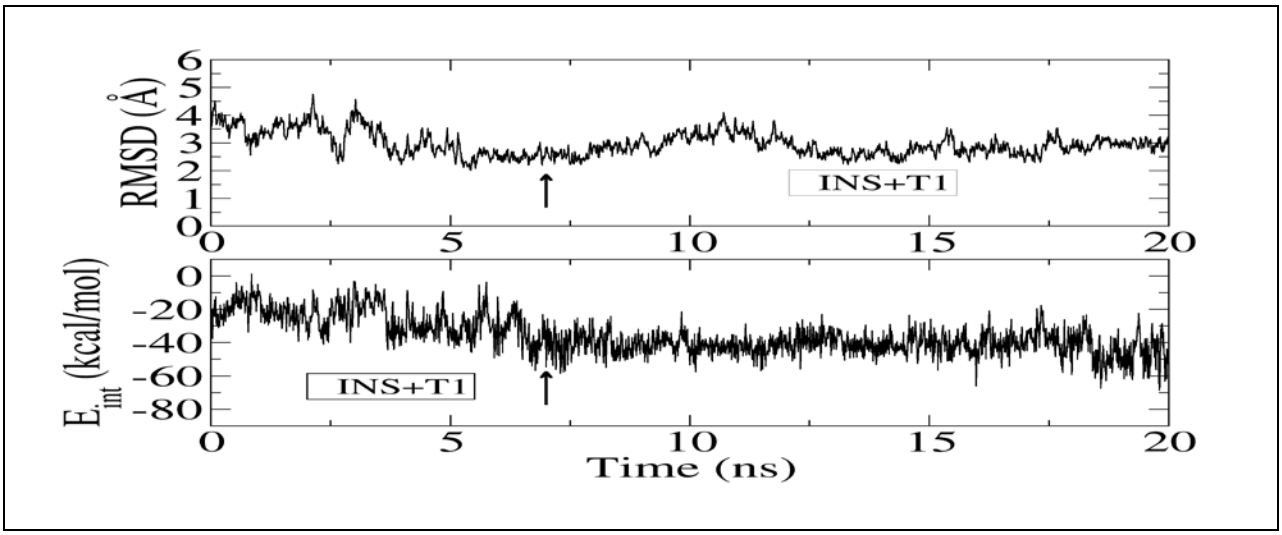
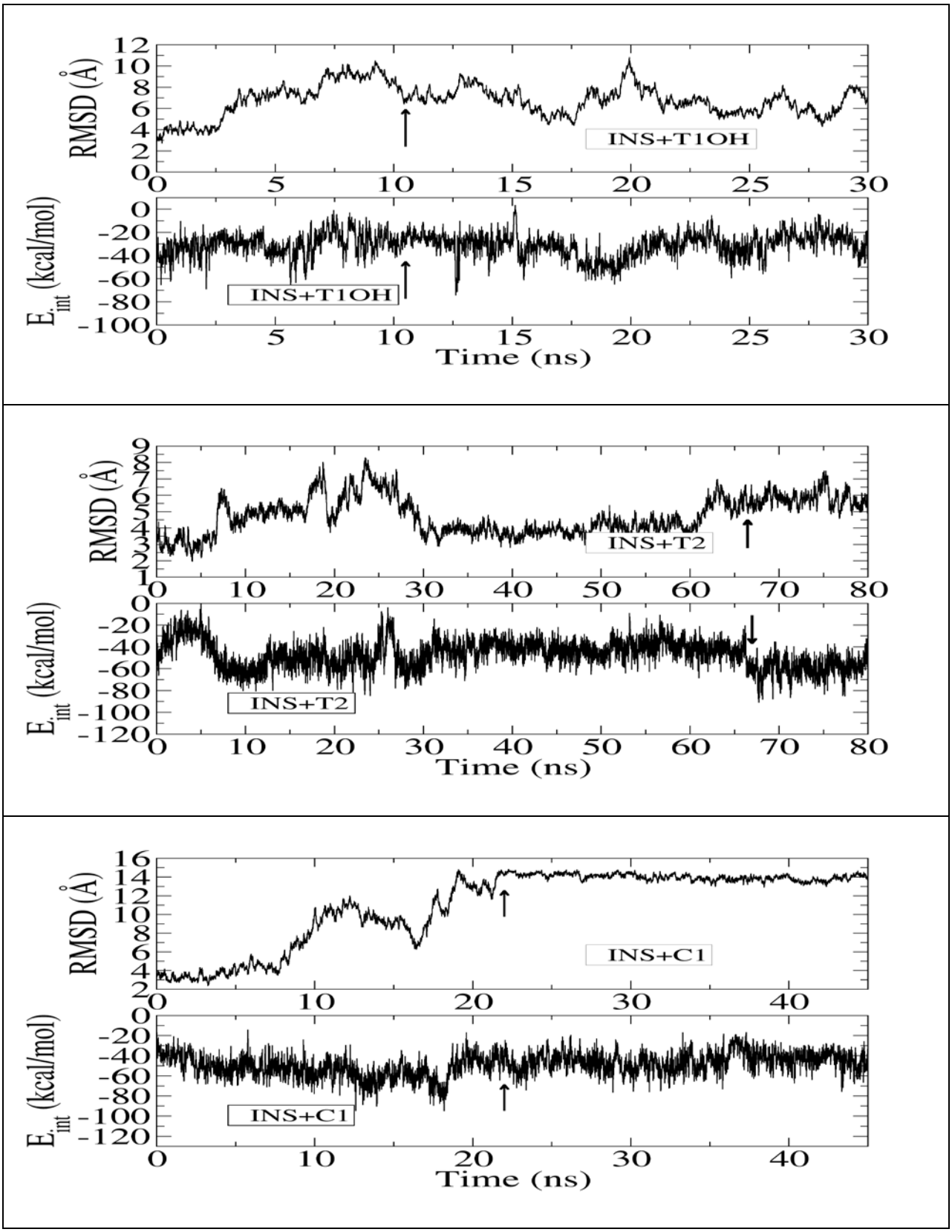
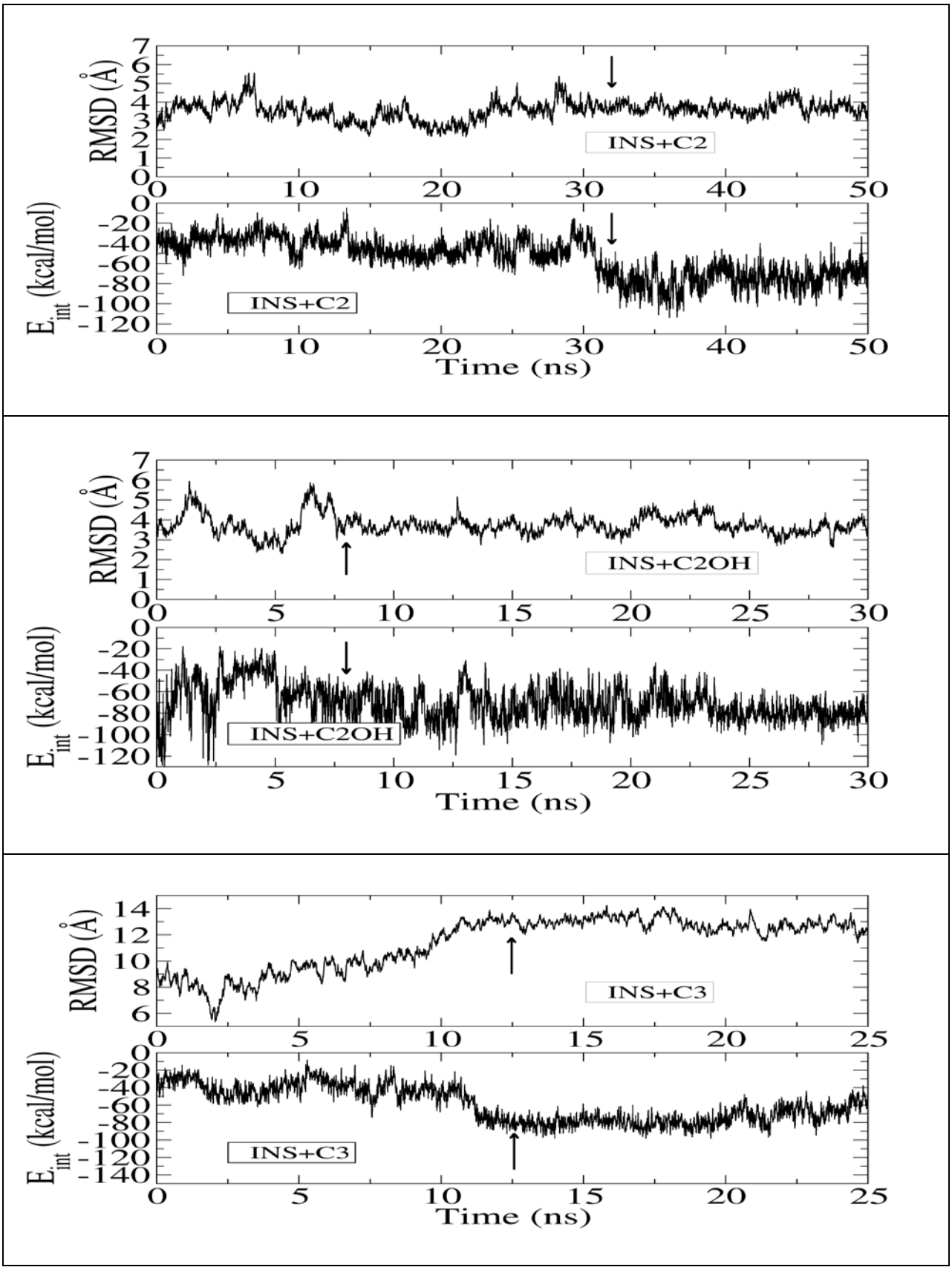


Figure S4. The docking poses of all glyco-acridine derivatives in complex with insulin dimer obtained in the best docking mode.




RMSD (Å)
INS+T1OH
E_int (kcal/mol)
INS+T1OH
Time (ns)
RMSD (Å)
INS+T2
E_int (kcal/mol)
INS+T2
Time (ns)
RMSD (Å)
INS+C1
E_int (kcal/mol)
INS+C1
Time (ns)


RMSD (Å)
INS+C2
E_int (kcal/mol)
INS+C2
Time (ns)
RMSD (Å)
INS+C2OH
E_int (kcal/mol)
INS+C2OH
Time (ns)
RMSD (Å)
INS+C3
E_int (kcal/mol)
INS+C3
Time (ns)

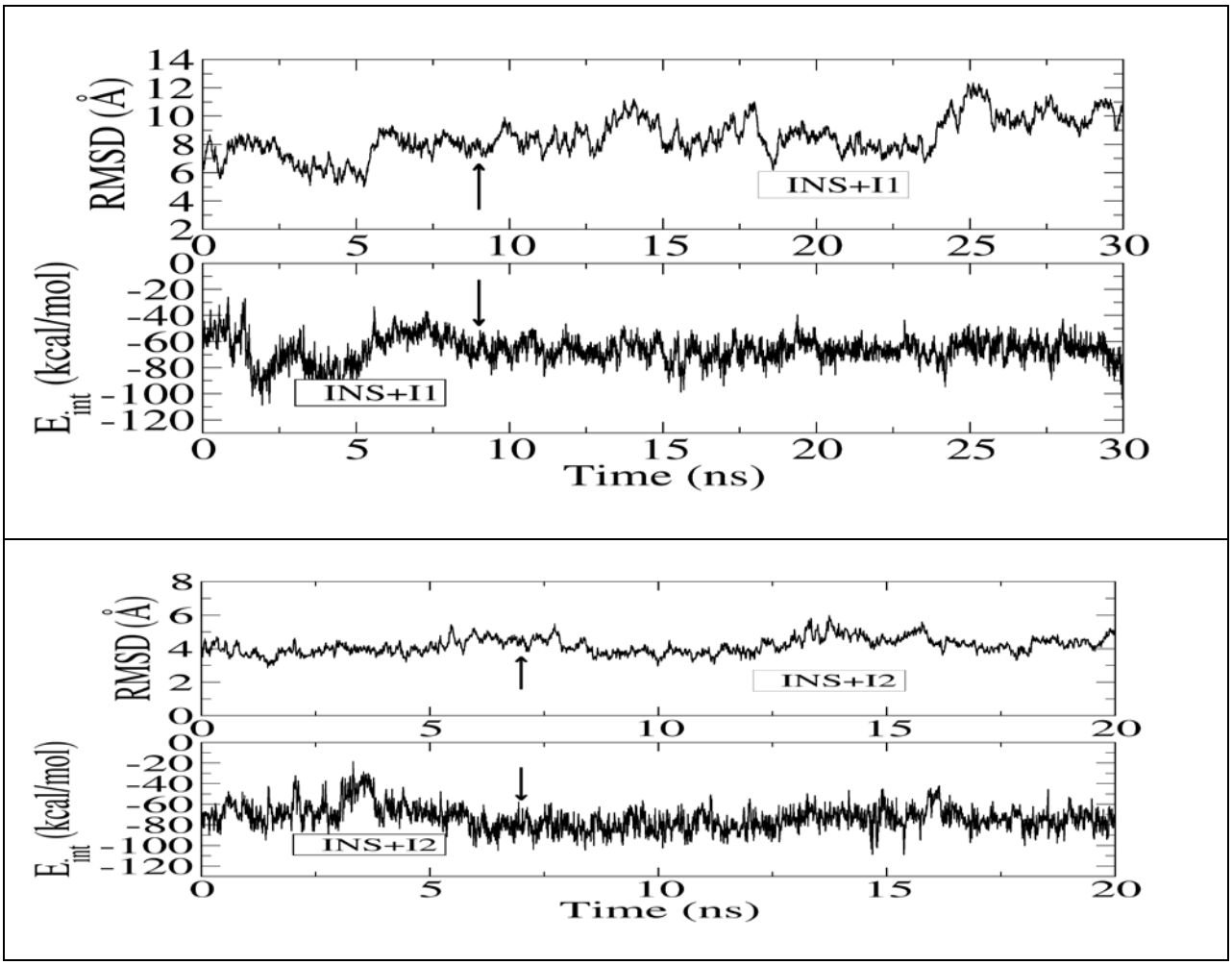


Figure S5. Time dependence of Cα RMSD of insulin with respect to the PDB structure and the interaction energy of glyco-acridine derivatives with insulin. Arrow refers to time when the system reaches equilibrium.

Table S2. Free binding energy ΔG_{bind} and hydrogen bond energy ΔE_{HB} obtained in equilibrium from MD simulation.

Ligands	ΔE_{HB} (kcal/mol)	ΔG_{bind} (kcal/mol)	IC_{50}[μM]
T1	-28.4	-8.2	39.79
T1OH	-12.6	-2.2	60.75
T2	-33.4	-14.4	22.44
C1	-2.8	-8.3	59.48
C2	-1.7	-12.1	34.64
C2OH	-1.8	-18.4	14.95
C3	-8.7	-23.0	30.67
I1	-1.6	-23.9	7.22
I2	-13.7	-16.2	33.31

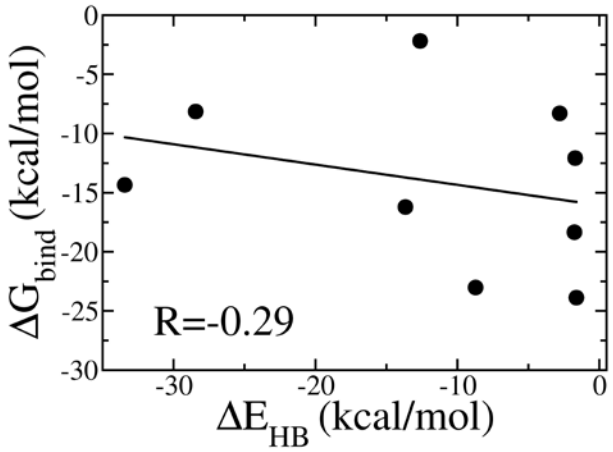


Figure S6. Correlation between ΔG_{bind} and the hydrogen bond energy (ΔE_{HB}).

Table S3. van der Waals interaction energies of tricyclic core, linker and side chain of acridine ligands with the receptor. The energy is measured in kcal/mol.

Ligands	Core	Linker	Side chain	ΔG_{bind}
T1	-16.5	-8.6	-11.2	-8.2
T1OH	-13.1	-5.7	-2.8	-2.2
T2	-24.1	-7.0	-13.8	-14.4
C1	-19.4	-9.9	-11.9	-8.3
C2	-18.0	-3.9	-24.6	-12.1
C2OH	-23.5	-11.8	-9.7	-18.4
C3	-23.8	-12.4	-19.8	-23.0
I1	-24.7	-9.6	-18.1	-23.9
I2	-21.0	-7.9	-19.7	-16.2

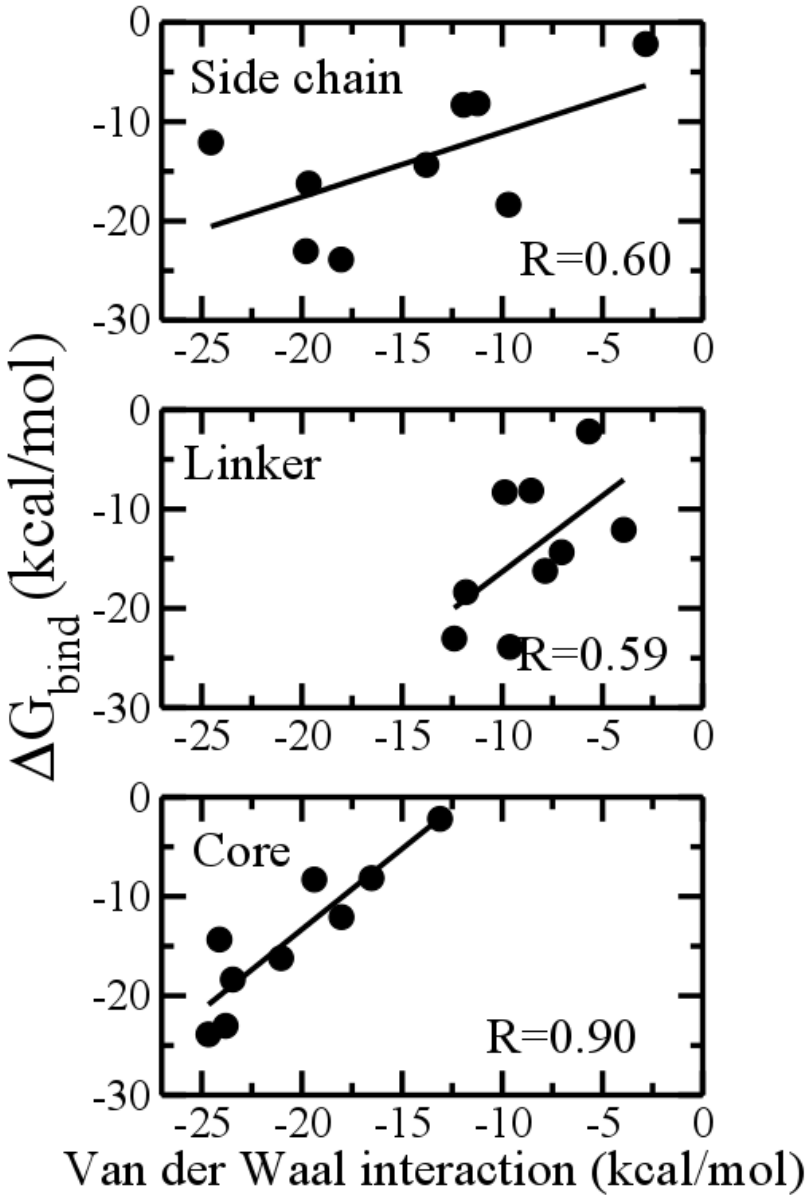


Figure S7. Correlation between the free binding energies ΔG_{bind} and van der Waals interaction energies of tricyclic core, linker and side chain of glyco-acridine derivatives.

Table S4. Interaction energies between aromatic core part of ligands and aromatic rings of receptor, $\Delta E_{ar\text{-}ar}$. ΔE_{vdw} and ΔE_{elec} are contribution of Van der Waal interaction and electrostatic interaction, respectively.

Ligands	ΔE_{vdw} (Kcal/mol)	ΔE_{elec} (Kcal/mol)	$\Delta E_{ar\text{-}ar}$ (Kcal/mol)	ΔG_{bind} (Kcal/mol)
T1	-0.9	27.4	26.5	-8.2
T1OH	-3.5	16.7	13.2	-2.2
T2	-8.4	26.5	18.1	-14.6
C1	-4.6	17.0	12.4	-8.3
C2	-7.2	17.1	9.9	-12.1
C2OH	-2.7	18.9	16.2	-18.4
C3	-4.7	12.5	7.8	-23.0
I1	-2.2	16.8	14.7	-23.9
I2	-4.2	13.0	8.8	-16.2

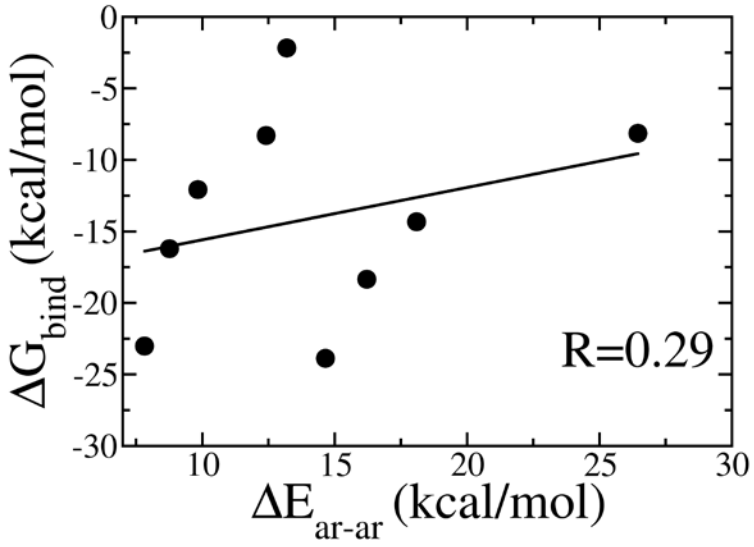


Figure S8. Correlation between the free binding energies and interaction energies between aromatic core part of ligands and aromatic rings of receptor, $\Delta E_{ar\text{-}ar}$.

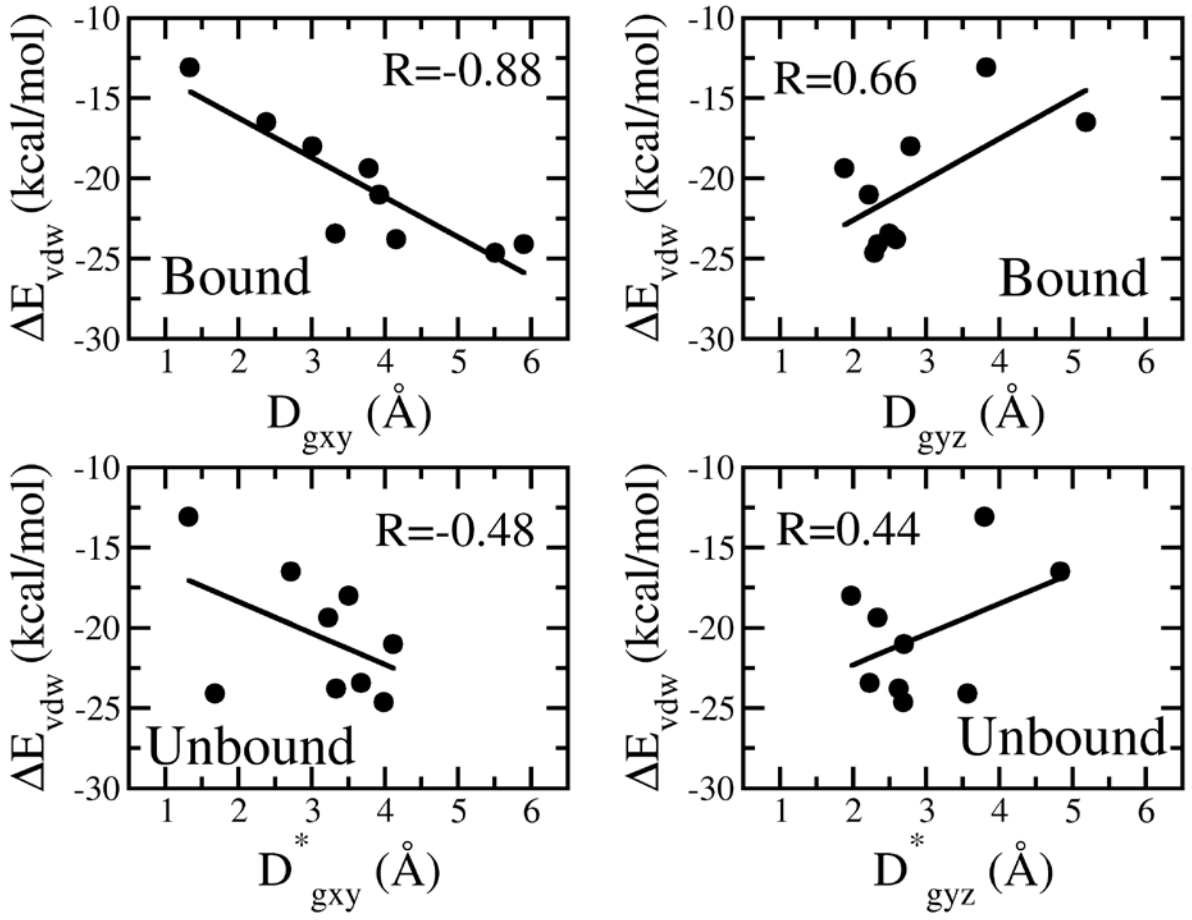
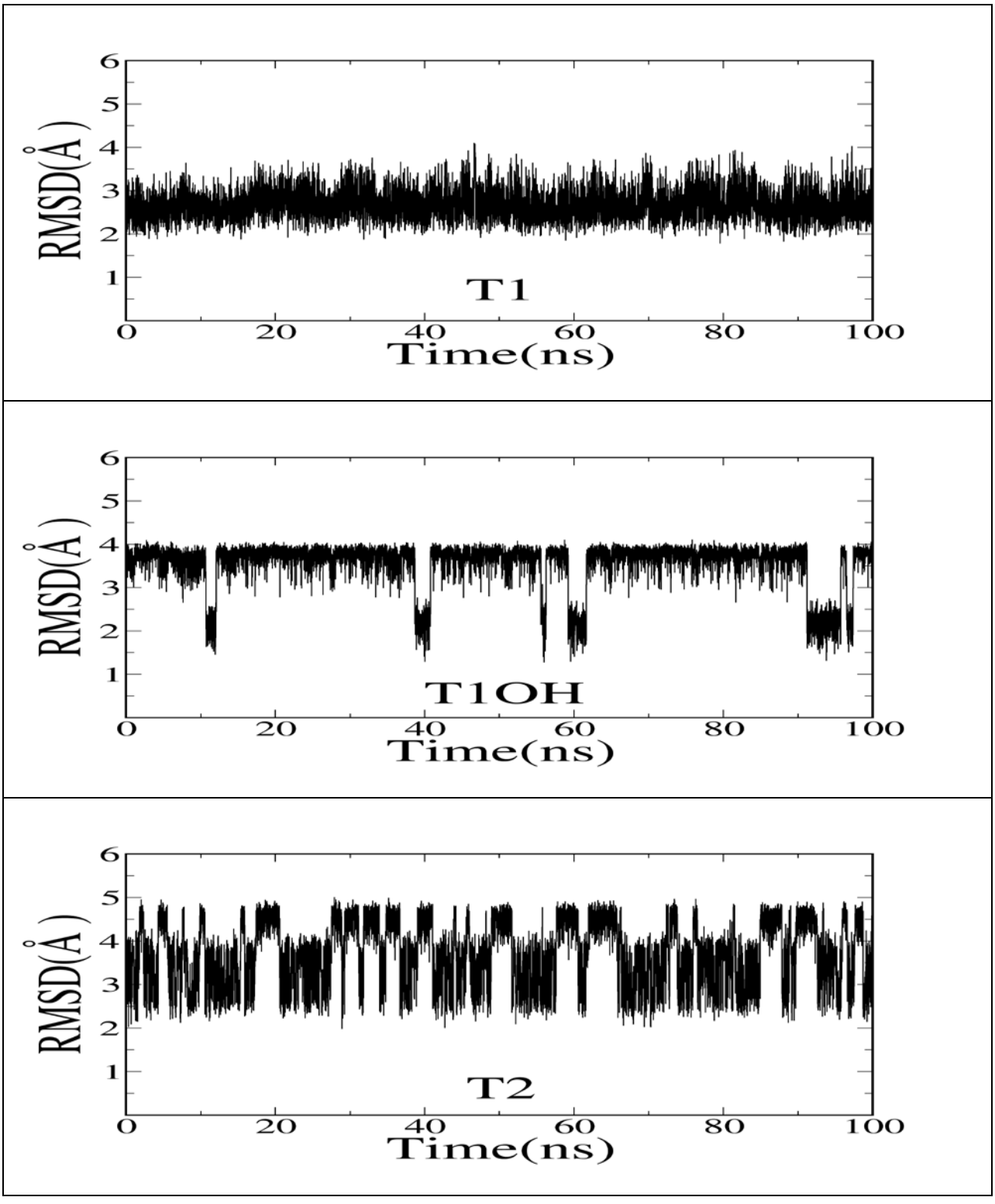
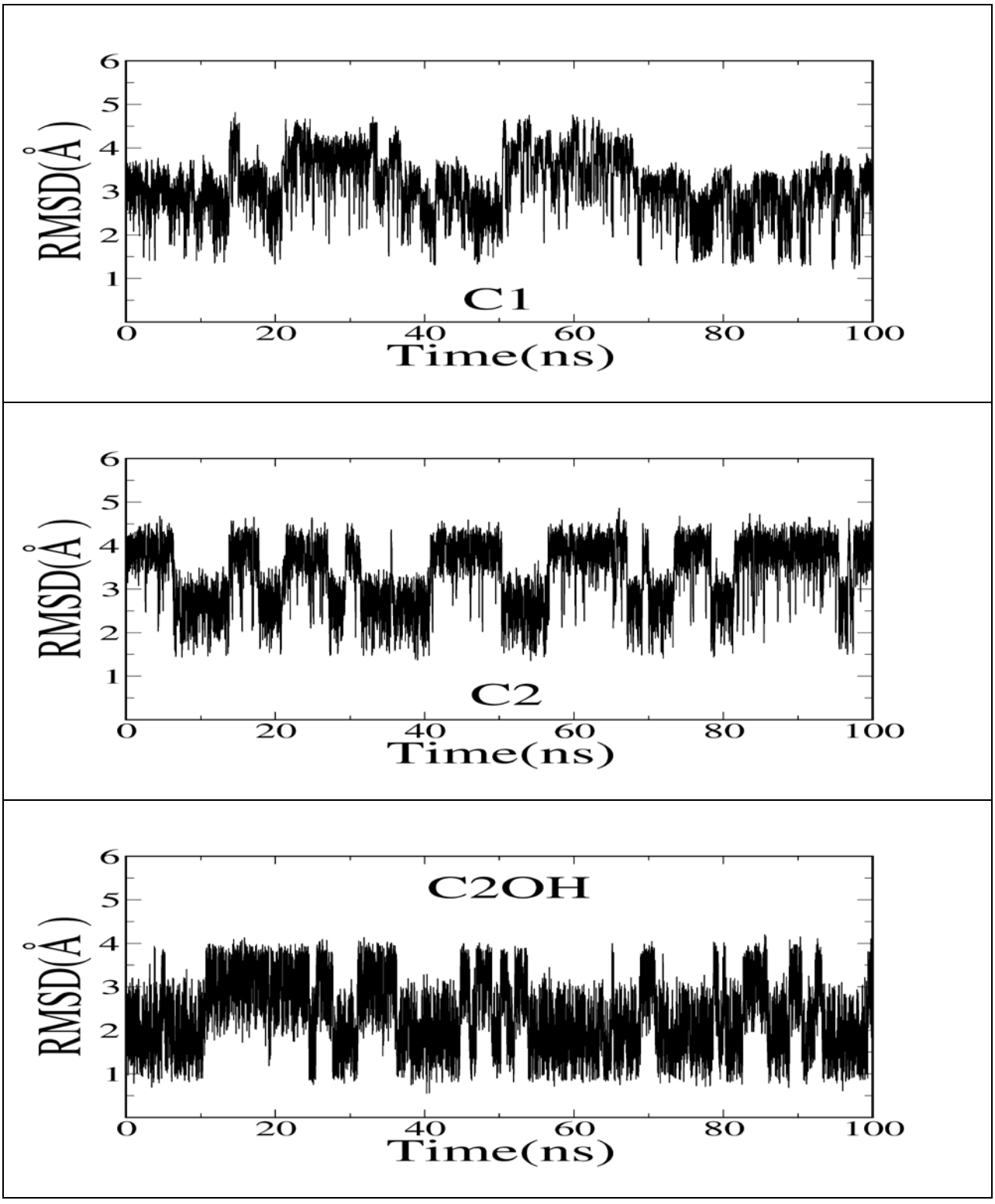


Figure S9. Correlation between the van der Waal interaction energy of tricyclic core and geometrical descriptors of glyco-acridine derivatives at bound and unbound states, * indicates the unbound state.


RMSD(Å)
Time(ns)
T1
T1OH
T2
0
20
40
60
80
100
1
2
3
4
5
6


RMSD(Å)
C1
Time(ns)
RMSD(Å)
C2
Time(ns)
C2OH
RMSD(Å)
Time(ns)

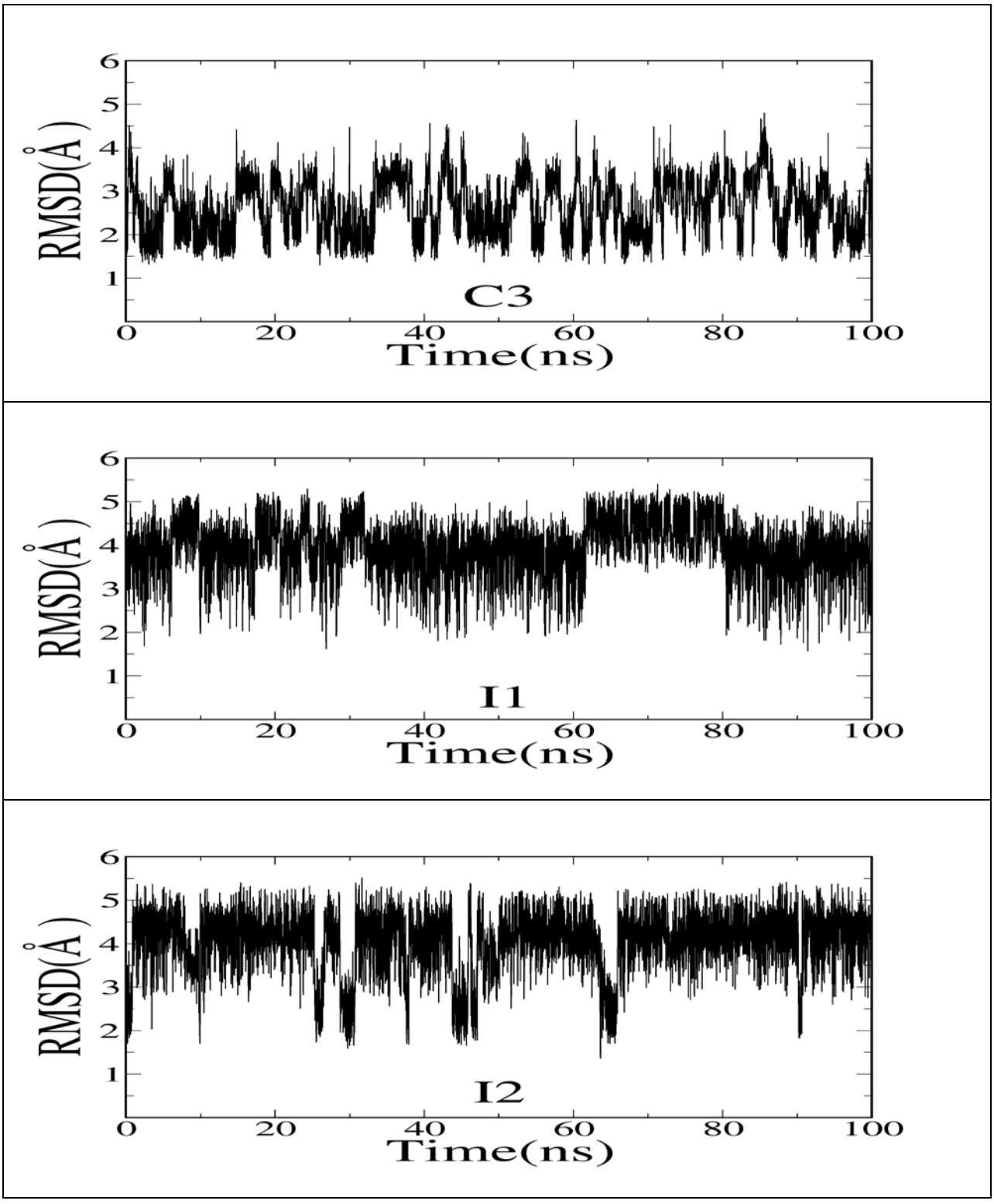


Figure S10. Time dependence of RMSD of glyco-acridine derivatives in unbound state.