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SUPPLEMENTARY MATERIAL TO

Binding of *β*-casein with fluvastatin and pitavastatin

HAMID DEZHAMPANAH* and OMIDEH RAJABI MIANDEHI

Department of Chemistry, Faculty of Science, University of Guilan, P.O.B. 1914, Rasht 0098, Iran

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EXPERIMENTAL

Materials

Bovine *β*-CN (with purity 98 %, Sigma- Aldrich, Germany) was dissolved in phosphate buffer solution (PBS) with pH 7.2 and anionic strength of 0.1, which was stored in dark at 4 °C. Ethanol (99.6 %) and methanol (99 %) were obtained from Merck Company. As FLU and PIT were insoluble in water, stock solutions (1 mM) of FLU and PIT in ethanol and methanol were first prepared, respectively, and then diluted to final concentration with phosphate buffer solution (PBS). For the preparation of the *β*-CN solution, the protein solution was filtered through a porous membrane of 0.45 μm.[14] The stock solution of *β*-CN (1 mM) was prepared in PBS (pH 7.2). Above stock solutions were prepared in redistilled water and were used freshly after preparation. The concentration of ethanol and methanol in the final solutions was lower than 2 vol.%.

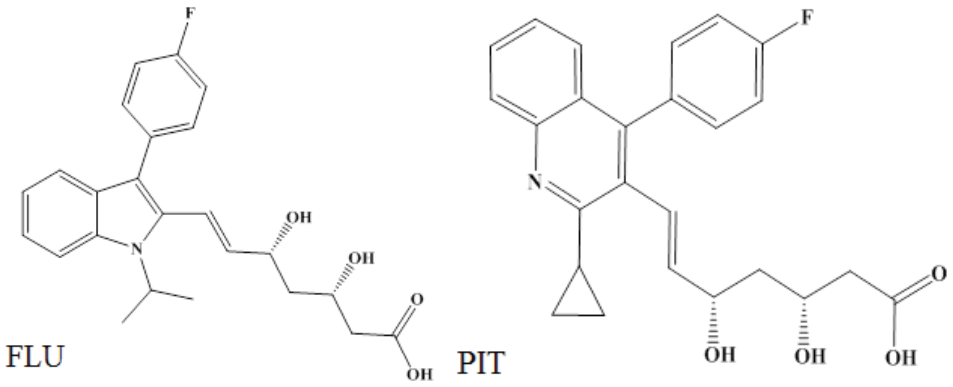

Fig. S-1. Chemical structure of FLU and PIT.

* Corresponding author. E-mail: h.dpanah@guilan.ac.ir

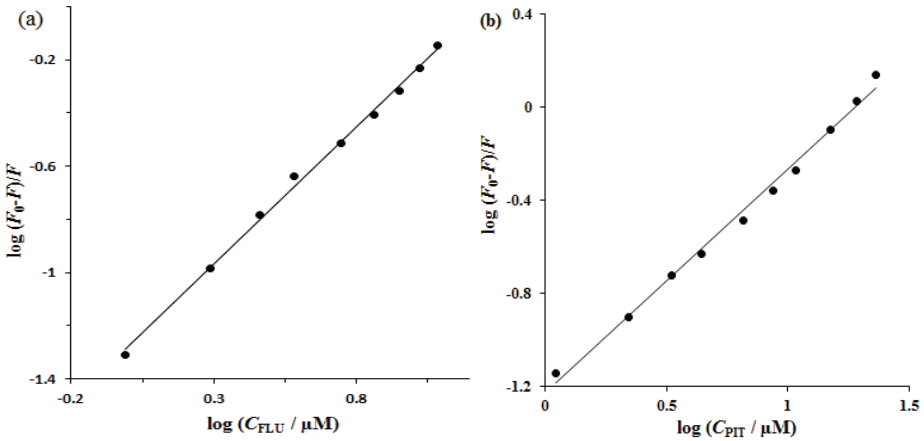

Fig. S-2. Double logarithmic plots of log $(F_0–F)/F$ against log C_Q derived from the fluorescence quenching of *β*-CN (10 μM) induced by the different concentration of FLU and PIT at 298 K, FLU-*β*-CN (a) and PIT-*β*-CN (b).

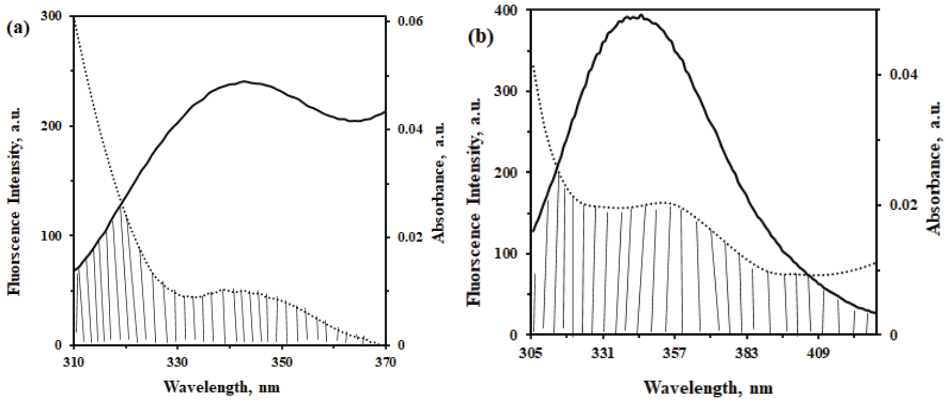

Fig. S-3. (a) *β*-CN fluorescence emission (solid lines) and UV-Vis absorption (dashed lines) of solutions containing FLU and PIT as ligand measured in PBS buffer pH 7.2 and $C_{α\text{-CN}} = C_{FLU} = 10$ μM. $C_{β\text{-CN}} = C_{PIT} = 10$ μM.

TABLE S-I. Secondary structure contents analysis of *β*-CN, FLU-*β*-CN and PIT-*β*-CN system

Structure components	Content, %		
	B-CN	FLU-*β*-CN	PIT-*β*-CN
α-Helix	28.73	31.16	24.97
β-Sheet	17.67	25.19	34.28
Random coil	24.43	27.4	19.39
β-Turn	22.0	14	18.71
β-Anti	7.13	2.24	2.65

TABLE S-II. Amino acid residues involved in interaction of FLU and PIT and *β*-CN with the free binding energy for the best selected docking positions

	Amino acids involved in casein protein bindings	ΔG / kJ mol^{-1}
FLU-*β*-CN	Leu-6, Leu-9, Val-10, Ala-13, Leu-14, Arg-40, Ile-41*, Leu-60, Lys-63, Ile-64, Phe-67	-27.58
PIT-*β*-CN	Met-159, His-160*, His-163, Gln-164, Pro-165, Leu-166, Ser-183, Lys-184*, Pro-187*, Pro-189, Pro-196*	-27.17

*Hydrogen bonding was observed with this amino acid

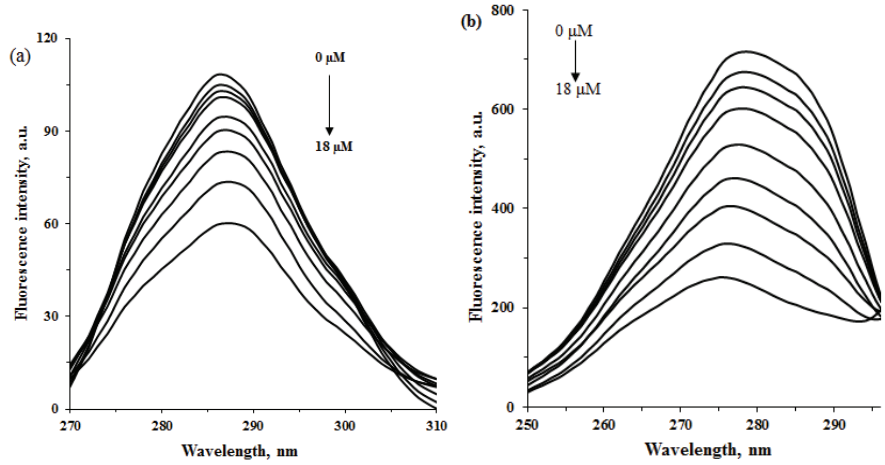

Fig. S-4. Synchronous fluorescence spectra of *β*-CN (10 μM) under physiological conditions (pH 7.2) in the absence and presence of FLU at room temperature when $\Delta\lambda$ values were set at 15 nm (a) and 60 nm (b), respectively. The concentrations of FLU were from 0 to 18 μM.

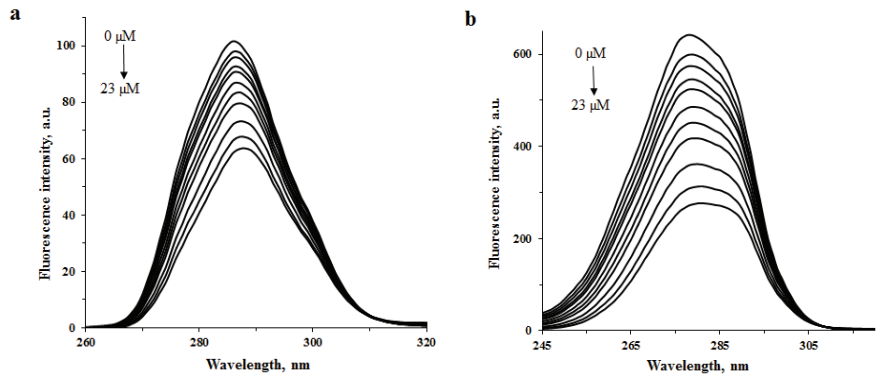

Fig. S-5. Synchronous fluorescence spectra of *β*-CN (10 μM) under physiological conditions (pH 7.2) in the absence and presence of PIT at room temperature when $\Delta\lambda$ values were set at 15 nm (a) and 60 nm (b), respectively. The concentrations of PIT were from 0 to 23 μM,

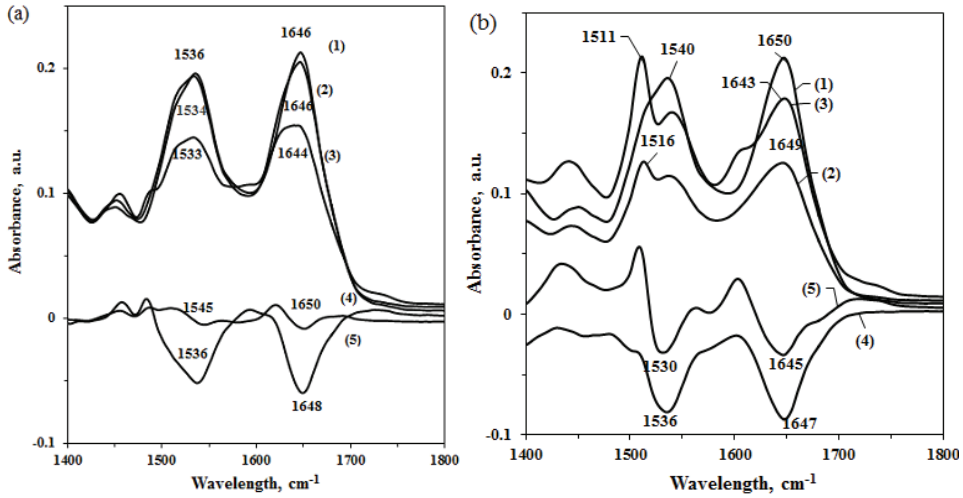

Fig. S-6. FTIR spectra in the region of 1800-1400 cm^{-1} of (a) FLU and (b) PIT for free *β*-CN 0.25 mM (1), *β*-CN -0.25 mM FLU or PIT (2), *β*-CN- 0.5 mM FLU or PIT (3), dif. *β*-CN-0.25 mM FLU or PIT (4) and dif. *β*-CN-0.5 mM FLU or PIT (5).

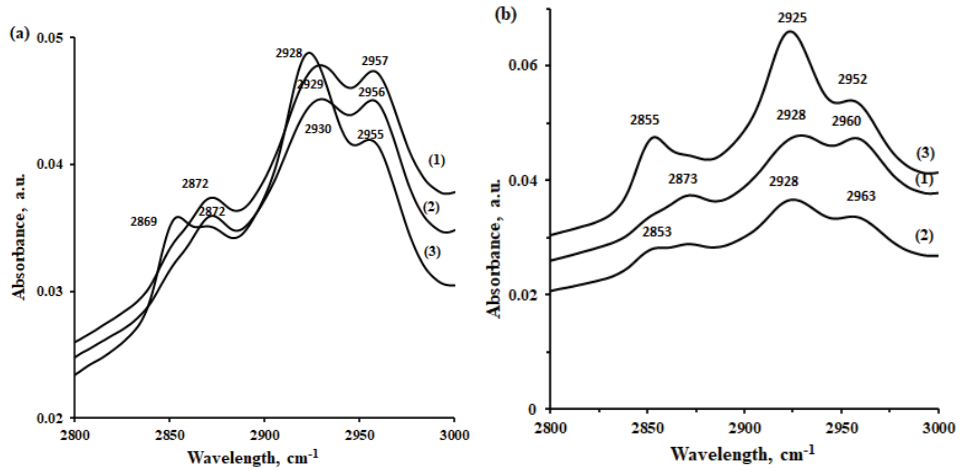

Fig. S-7. Spectral changes of casein CH_2 symmetric and anti-symmetric stretching vibrations *β*-CN complex with (a) FLU and (b) PIT for free *β*-CN 0.25 mM (1), *β*-CN-0.25 mM FLU or PIT (2), *β*-CN-0.5 mM FLU or PIT (3).

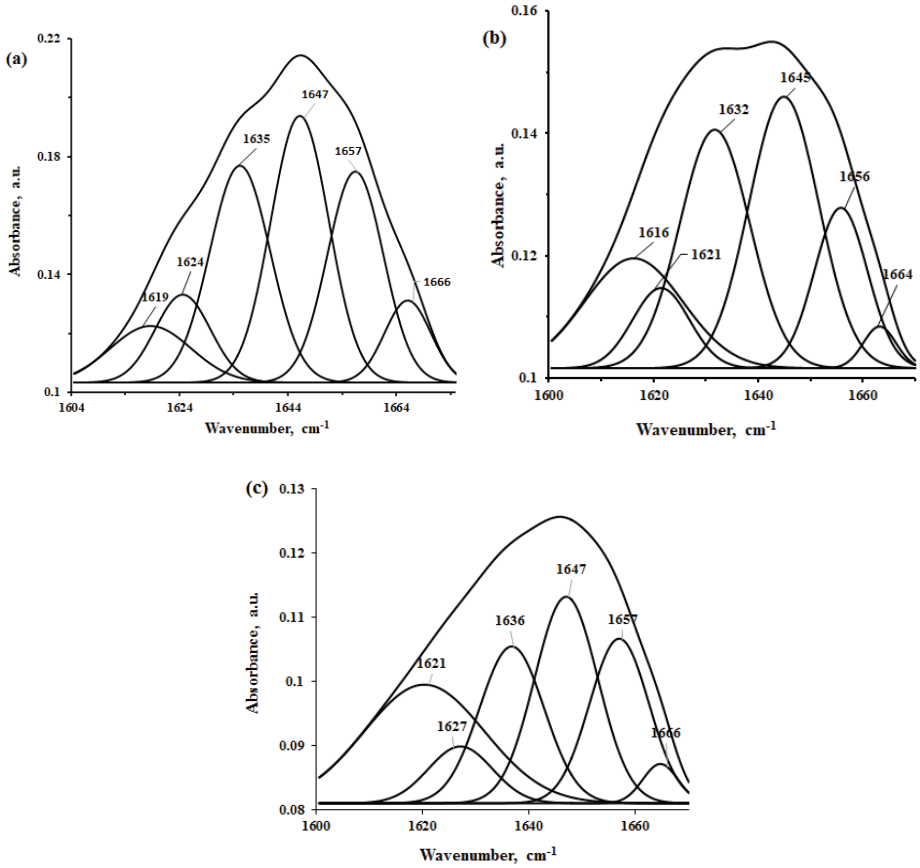

Fig. S-8. The curve-fit for *β*-CN (a), FLU-*β*-CN (b), and PIT-*β*-CN (c) in the amide I region from 1700 to 1600 cm^{-1}.