Benzotriazole UV stabilizers (BZTs) belong to one prominent group of ultraviolet (UV) stabilizers and are widely used in various plastics materials. Their large production volumes, frequent detections in the environment and potential toxicities have raised increasing public concern. BZTs can be transported in vivo by transport proteins in plasma and the binding association to transport proteins may serve as a significant parameter to evaluate the bioaccumulative potential. We utilized a novel HSA biosensor, circular dichroism spectroscopy, fluorescence spectroscopy to detect the dynamic interactions of six BZTs (UV-326, UV-327, UV-328, UV-329, UV-P, and BZT) with human serum albumin (HSA), and characterized the corresponding structure-activity relationships (SAR) by molecular dynamics simulations. All test BZTs potently bind at Sudlow site I of HSA with a binding constant of 10(4) L/mol at 298 K. Minor changes in the moieties of BZTs affect their interactions with HSA and differently induce conformations of HSA. Their binding reduced electrochemical impedance spectra and α-helix content of HSA, caused slight red-shifted emission, and changed fluorescence lifetime components of HSA in a concentration-dependent mode. UV-327 and UV-329 form hydrogen bonds with HSA, while UV-329, UV-P and BZT bind HSA with more favorable electrostatic interactions. Our in vitro and in silico study offered a significant framework toward the understanding of risk assessment of BZTs and provides guide for future design of environmental benign BZTs-related materials.