Benzotriazole ultraviolet stabilizers (BUVSs) are prominent chemicals widely used in industrial and consumer products to protect against ultraviolet radiation. They are becoming contaminants of emerging concern since their residues are frequently detected in multiple environmental matrices and their toxicological implications are increasingly reported. We herein investigated the antiandrogenic activities of eight BUVSs prior to and after human CYP3A4-mediated metabolic activation/deactivation by the two-hybrid recombinant human androgen receptor yeast bioassay and the in vitro metabolism assay. More potent antiandrogenic activity was observed for the metabolized UV-328 in comparison with UV-328 at 0.25 μM ((40.73 ± 4.90)% vs. (17.12 ± 3.00)%), showing a significant metabolic activation. In contrast, the metabolized UV-P at 0.25 μM resulted in a decreased antiandrogenic activity rate from (16.08 ± 0.95)% to (6.91 ± 2.64)%, indicating a metabolic deactivation. Three mono-hydroxylated (OH) and three di-OH metabolites of UV-328 were identified by ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-Q-TOF-MS/MS), which were not reported previously. We further surmised that the hydroxylation of UV-328 occurs mainly at the alicyclic hydrocarbon atoms based on the in silico prediction of the lowest activation energies of hydrogen abstraction from C-H bond. Our results for the first time relate antiandrogenic activity to human CYP3A4 enzyme-mediated hydroxylated metabolites of BUVSs. The biotransformation through hydroxylation should be fully considered during the health risk assessment of structurally similar analogs of BUVSs and other emerging contaminants.