Human serum albumin (HSA) is the most prominent protein in plasma and has exceptional ligand binding capacity. Almost all pharmaceuticals released into the blood find themselves in the presence of a high concentration of HSA, which is known to have strong affinity for various chemical compounds. Formoterol (FRM) and salbutamol (SAL) are potent b2-adrenergic receptor agonists widely used in the treatment of asthma disease. Here, Interaction of FRM and SAL with HSA was investigated by using molecular modeling, circular dichroism (CD) and isothermal titration calorimetry (ITC) measurements. The REDUCE software was utilized to add missing hydrogens to the X-ray crystal structure of HSA, and Autodock VINA was used to perform docking simulations. Different clefts in HSA structure were searched for potential high-affinity binding sites. The results were compared with known drug-HSA complexes. Experimental measurements show that interaction of FRM and SAL with HSA does not induce considerable structural changes in protein. Docking results show that, in order of increasing affinity, FRM binds to drug site 1, FA site 6 and HEM binding site of HSA. The same analysis for SAL indicates that the highest affinity corresponds to HEM binding site followed by FA site 6 and drug site 1. Results of current study make a better understanding of the extent of distribution and side effects of anti asthma drugs in the blood and the interaction with the Human serum albumin (HSA) as a model protein.