

# Thiol-reactive Clenbuterol Analogues Conjugated to Bovine Serum Albumin

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Several novel thiol-reactive clenbuterol analogues were coupled in high yield with bovine serum albumin (BSA). After labelling of unreacted cysteines with maleimide spin label (MiSL), the yield of the coupling reaction was determined by electron paramagnetic resonance (EPR) spectroscopy and spectral analysis. Two spin-probe populations with different mobility states were quantitatively determined. Molecular dynamics was used to model the structure of clenbuterol analogues and spin label conjugated to BSA and recognition of conjugates by anti-clenbuterol antibodies was demonstrated. The recognition of BSA-A, BSA-C and BSA-S conjugates with monoclonal and polyclonal anti-clenbuterol (mCLB-Ab and rCLB-Ab) antibodies was an indication, that chlorine substituents on the aromatic ring of clenbuterol derivatives are not necessary for the binding of antibodies to the conjugates. These results confirmed the importance of the *tert*-butylamino group as a part of the epitope and contribute to the understanding of the recognition process with anti-clenbuterol antibodies.

*Key words:* Clenbuterol, Electron Paramagnetic Resonance, Thiol Spin Labelling