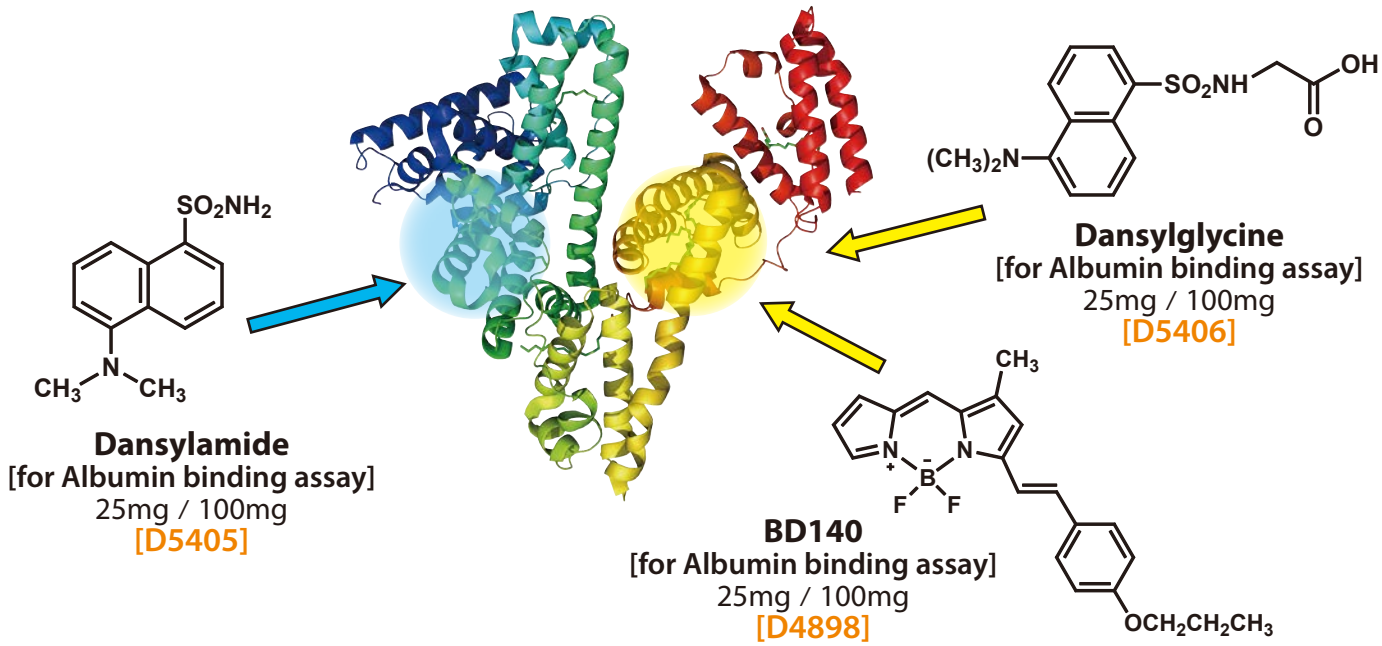
LIFE SCIENCE

Fluorescent Probes for the Characterization of Two Specific Drug Binding Sites on HSA

Powerful tool for drug discovery, R&D and metabolic toxicity assay



Dansylamide
[for Albumin binding assay]
25mg / 100mg
[D5405]

Dansylglycine
[for Albumin binding assay]
25mg / 100mg
[D5406]

BD140
[for Albumin binding assay]
25mg / 100mg
[D4898]

Advantages

- **Detectable drug binding to HSA at site I and II.**
- **Simple and quick assay can be performed.**

Human Serum Albumin (HSA) binds to a wide variety of drugs at two primary binding sites (I: blue and II: yellow), and can have a significant impact on their pharmacokinetics and pharmacological effects. The fluorescent probes dansylamide **[D5405]** and dansylglycine **[D5406]** selectively interact with sites I and II, respectively.[1,2] BD140 **[D4898]** also selectively binds to drug binding site II.[3] Changes in probe fluorescence are analyzed by fluorescence titrations as a result of competitive displacement by drugs and pharmaceuticals. The pattern of fluorescent probe displacement by these reagents enabled the identification of two specific drug binding sites, which illustrates their usefulness for the characterization of the binding sites in HSA.

References

1) G. Sudlow, D. J. Birkett, D. N. Wade, *Mol Pharmacol.* **1975**, *11*, 824. https://molpharm.aspetjournals.org/content/11/6/824
2) G. Sudlow, D. J. Birkett, D. N. Wade, *Mol. Pharmacol.* **1976**, *12*, 1052. https://molpharm.aspetjournals.org/content/12/6/1052
3) J. C. Er, M. Vendrell, M. K. Tang, D. Zhai, Y.-T. Chang, *ACS Comb. Sci.* **2013**, *15*, 452. https://doi.org/10.1021/co400060b

Typical Usage

[Reagents]

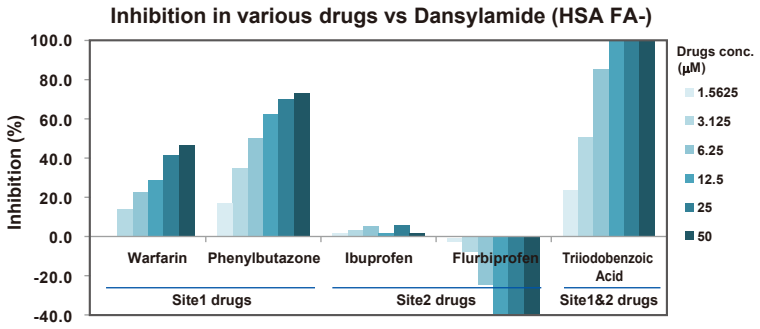
- 1% DMSO in 10mM phosphate buffer (pH 7.2-7.5)
- Human Serum Albumin (HSA) (fatty acid free)
- Various sample compounds
- HSA binding fluorescent probe : dansylamide **[D5405]**, dansylglycine **[D5406]**, BD140 **[D4898]**

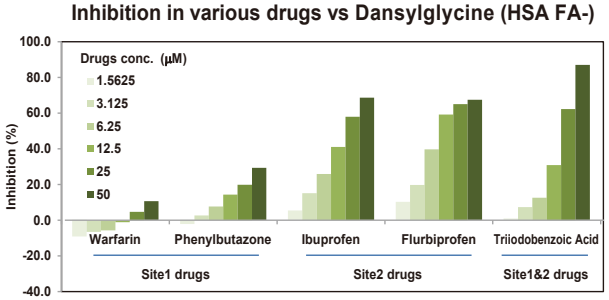
[Procedure]

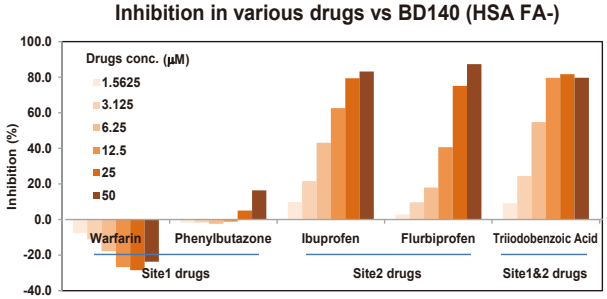
1. Prepare HSA, each fluorescent probe, and various sample compounds.
2. Apply each solution to black 96-well plate for fluorescent measurement. (Ex. 50 µL each)
3. Stir gently and let stand for 30 minutes with shading at room temperature (20-25°C).
4. Measure the fluorescence with a plate reader.

* As positive control, warfarin is often used for drug binding site I and ibuprofen is often used for drug binding site II.
* Detergents sometimes effects the reaction. Consideration of the application conditions are recommended.
* For the application of dansylglycine and BD140, fatty acid free HSA is recommended for use. (Please refer to data below.)

Application







<Assay condition>

Buffer: 1% DMSO in phosphate buffer (pH7.2-7.5), HSA (fatty acid free): 5 µM (50 µL/well), Drugs: each concentration (50 µL/well), Each fluorescent probe: 80 µM (50 µL/well), Incubation: 20-25°C for 30 minutes, Measurement: plate-reader; excitation=365 nm, emission=480 nm (Dansylamide and Dansylglycine) or 585 nm (BD140).

[Our testing results]

- The binding of dansylamide **[D5405]** to HSA (fatty acid free)
  Inhibition was achieved by warfarin, phenylbutazone and triiodobenzoic acid bound to drug binding site I. Alternatively, it was not inhibited by ibuprofen and flurbiprofen bound to drug binding site II, completely or partially.
- Binding of dansylglycine **[D5406]** and BD140 **[D4898]** to HSA (fatty acid free)
  It was not partially inhibited by warfarin and phenylbutazone bound to drug binding site I. However, it was inhibited by ibuprofen, flurbiprofen and triiodzendoic acid bound to drug binding site II.

Dansylamide makes it possible to confirm whether a drug binds to drug binding site I on HSA. Dansylglycine and BD140 makes it possible to confirm whether the drug binds to drug binding site II on HSA.

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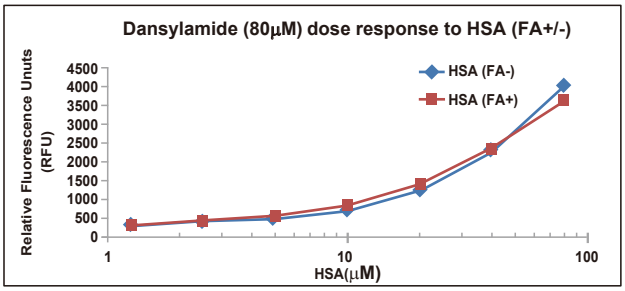
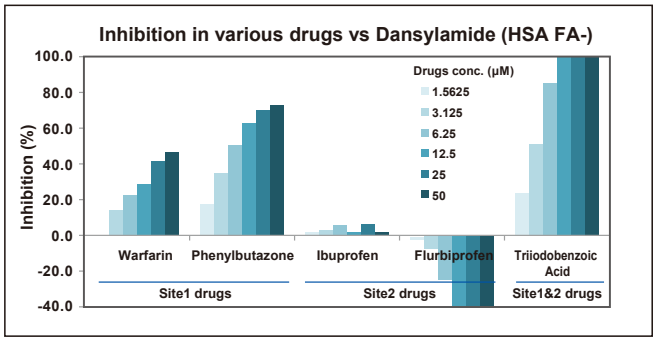
Reference information : Activity between each fluorescent probe and HSA

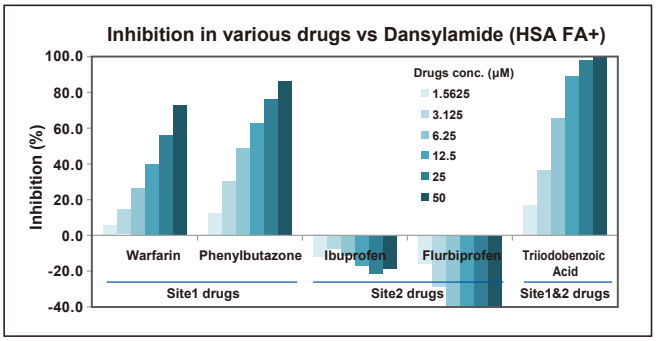
Activity between each fluorescent probe and HSA can vary considerably depending on grade of HSA.



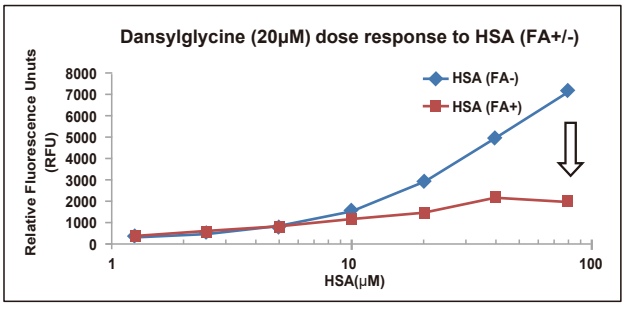
Dansylamide **[D5405]**, fluorescent probe for drug binding site I, did not show a significant difference in its activity regardless of whether fatty acid free (FA-) HSA (blue) or biochemical grade (FA+) HSA (red) was used.

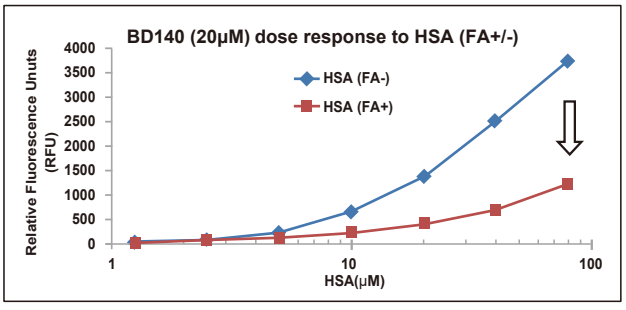
Dansylcamide did not show a large difference between the result of assay using FA+/FA- HSA, which allowed for the proper drug biding site to be confirmed.
Binding to drug binding site I can be confirmed regardless of HSA type.





The activity of dansylglycine **[D5406]** and BD140 **[D4898]** against biochemical grade (FA+) HSA (red) was markedly lower than the activity using fatty acid free (FA-) HSA (blue).
Therefore, when confirming the binding to drug binding site II with dansylglycine or BD140, biochemical grade (FA+) HSA is not recommended for use.





<Assay condition>
Buffer: 1% DMSO in phosphate buffer (pH7.2-7.5), HSA (Human Serum Albumin): 50 μL/well, each fluorescent probe : 50 μL/well,
Incubation: 20-25°C for 30 minutes.
Measurement: plate-reader; excitation=365 nm, emission=480 nm (Dansylamide and Dansylglycine) or 585 nm (BD140).

Fluorescent Probes for the Characterization of Two Specific Drug Binding Sites on HSA

Related Products : Compounds with reported binding activity to albumin [4-6)]

Drug binding site I compounds

Warfarin Sodium	5g / 25g [W0005]
Phenylbutazone	25g / 500g [P1686]
Indomethacin	25g / 100g / 500g [I0655]
3,5-Diiodosalicylic Acid	25g / 250g [D1677]
Furosemide	5g / 25g [F0182]
Bilirubin	100mg / 1g [B0460]
Oxaprozin	5g / 25g [O0377]

Drug binding site II compounds

Ibuprofen	25g / 100g / 500g [I0415]
Flurbiprofen	5g / 25g [F0371]
Propofol	25g / 500g [D0617]
Flufenamic Acid	25g / 500g [T2354]
Ketoprofen	25g [K0038]
L-Thyroxine Sodium Salt Pentahydrate	100mg / 1g [T0245]
L-Tryptophan	25g / 100g [T0541]

Drug binding site I and II compounds

TIBA	5g / 25g [T0451]

*Each product is not guaranteed for its binding activity.

References

4) J. Ghuman, P. A. Zunszain, I. Petitpas, A. A. Bhattacharya, M. Otagiri, S. Curry, *J. Mol. Biol.* **2005**, *353*, 38. https://doi.org/10.1016/j.jmb.2005.07.075
5) G. Sudlow, D. J. Birkett, D. N. Wade, *Mol. Pharmacol.* **1976**, *12*, 1052. https://molpharm.aspetjournals.org/content/12/6/1052
6) Y. Otagiri, *YAKUGAKU ZASSHI* **2009**, *129*, 413. https://doi.org/10.1248/yakushi.129.413
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