

Exploitation of BIACORE S51 data for compound development from hit selection to pre-clinical studies

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INTRODUCTION

The Drug Discovery compound selection process starts classically by subjecting a given target to a library of hundreds of thousands of compounds using a technology amenable to HTS. Although discriminating, the HTS often yields hundreds or thousands of positives that should be sorted in order to select out the most promising compounds. This step is carried out using a secondary screening assay. A way to speed-up this process is to eliminate the false positive and the least interesting molecules based on their binding properties on the target protein before engaging more efforts. Since BIACORE S51 allows the monitoring of small molecule - macromolecule interaction, it may therefore be used to assess the strength and the specificity of the binding of selected compounds toward their targets. Such an approach was undertaken at NOVEXEL for the compound selection in an antifungal program where 572 molecules were ranked using BIACORE S51 data. Later in the compound development process, after chemical improvement, candidate molecules must be carefully characterized. In addition to their specificity and activity against the target, they must be proven safe and their ADME characteristics must be determined. Once again, BIACORE binding data are complementary to those obtained by other methods. Interestingly, BIACORE may also provide data where other techniques fail. It is exemplified here with our candidate molecule NXL103, currently in phase I clinical studies, for which binding to plasma proteins data of one of its components (component P I) could not be determined due to non specific binding in equilibrium dialysis experiments. BIACORE data allowed the full characterization of this molecule.

METHODS

System and reagents

All assays were carried out with Biacore S51, using Series S Sensor Chip CM5 and Series S Sensor Chip L1. Running buffer was 10mM phosphate buffered saline (PBS), pH 7.4 containing 0.5% or 5% DMSO as indicated below. Stock solutions of NXL101, NXL103 PI and PII were 100mM in DMSO. A mixture of 30% PI 70% PII was prepared in DMSO to evaluate the binding of the formulated NXL103. Stock solutions for hit identification were 10 mM in DMSO. Phospholipids for liposomes preparation were from Avanti Polar Lipids.

Immobilization

HSA and AGP

HSA was immobilized on spot 1 using a standard amine coupling procedure. PDEA-modified AGP was immobilized on spot 2 using a standard surface thiol coupling procedure. The immobilization level used was 1550 Resonance Unit (RU) for HSA and 2600 RU for AGP. Running buffer without DMSO was used during the immobilization procedure.

Lipoproteins: HDL and LDL

Commercially available HDL and LDL (Sigma L8039 and L7914 respectively) were immobilized by amine coupling on spot1 and spot2 (respectively) of a Series S Sensor Chip CM5. A 10 mM acetate buffer pH 5 was used for immobilization. 14800 RU and 39000 RU were captured for HDL and LDL respectively.

Liposomes

POPC and a mixture of POPE/POPS/POPC (5:3:2 w/w) were used to prepare LUVETs. Liposomes suspension (1.3 mM) were injected over the surface of Series S Sensor Chip L1 and resulted in the capture of 6000-8000 RU.

Run conditions

HSA and AGP binding characterization

A running buffer consisting of PBS containing 5% DMSO was used. Compounds were serially diluted from 500 μ M down to 977 nM and DMSO content was adjusted at 5% for each sample. Pre-conditioning of the sensor surface and flow system was performed using three injections of 10 mM Glycine pH1.5, followed by three start-up cycles of running buffer injections at the start of each run. Sample cycles consisted successively of a 60 s sample injection, a 30 s buffer flow (= dissociation phase), a needle & tube wash with 50% DMSO, a 30 s regeneration pulse of 10 mM glycine-HCl, pH 1.5, a 30 s stabilization period and finally, a 60 s buffer injection to check for carry-over. All injections within the sample cycle were made at a flow rate of 90 μ l/min. Solvent correction cycles using eight correction points (4.5 to 5.8% DMSO) were also run at intervals during the assay. Assays were carried out at 25°C.

Binding to Lipoproteins: HDL and LDL

All samples were adjusted to 5 % DMSO in PBS. Each run started by three start-up cycles of running buffer injections. Sample cycles consisted successively of a 60 s sample injection, a 30 s buffer flow (= dissociation phase), a needle & tube wash with 50% DMSO, a maximum of 3 X 30 s regeneration pulse of 5 mM NaOH (adaptive regeneration if the relative response after dissociation exceeded 10 resonance units), a 30 s stabilization period and finally, a 60 s buffer injection to check for carry-over. All injections within the sample cycle were made at a flow rate of 30 μ l/min. Solvent correction cycles using eight correction points (4.5 to 5.8% DMSO) were also run at intervals during the assay. Assays were carried out at 25°C.

Binding to liposomes

Pre-conditioning of the sensor surface and flow system was performed using one injection of 20 mM CHAPS. Each sample cycle consisted of a liposome capture where a 1:7.5 dilution (PBS) of the stock liposome solution (10 mM) was injected over the surface at 10 μ l/min for 180 s. This was successively followed by a 60 s stabilization period, a 60 s sample injection, a 60 s buffer flow (= dissociation phase), a needle & tube wash with 50% DMSO, a 30 s regeneration by a 40% isopropanol / 60% NaOH v/v solution to remove the liposomes from the L1 surface. Solvent correction cycles using eight correction points were also run at intervals during the assay. Assays were carried out at 25°C.

Data Evaluation

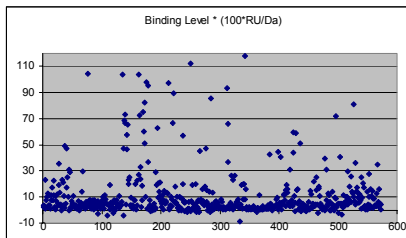
Data were evaluated using Biacore S51 Evaluation software, version 1.2.1. For experiments using DMSO containing solutions, solvent correction was applied to compensate for small differences that may arise in the bulk contribution of DMSO to the SPR response between the reference and sample surfaces, due to mismatches between sample and running buffer composition and the excluded volume effect of immobilized ligand on the sample surface. To allow a comparison of the binding levels of different molecules, the response (RU) was divided by the molecular weight of the molecule and multiplied by 100 (100*RU/Da). Concerning binding to liposomes, since the capture level was not identical for all the cycles, binding levels were normalized by dividing the response by the capture level and multiplying the result by a constant (8000) in an Excel worksheet. Scatter plots were generated using Spotfire.

RESULTS

Post-HTS Hit selection

In complement to other biochemical assays, an antifungal protein target was immobilized to a Series S Sensor Chip CM5 by conventional amine coupling. Activity of the surface was assessed by including in all the injection series, several injections of the protein natural ligand. This approach allowed to eliminate non desired compounds and to select the most interesting ones.

Figure 1 Ranking of 572 molecules against an antifungal protein target



HIT IDENTIFICATION STATISTICS

1104 cycles with 15 immobilisations were needed

17 promiscuous binders found (3%)

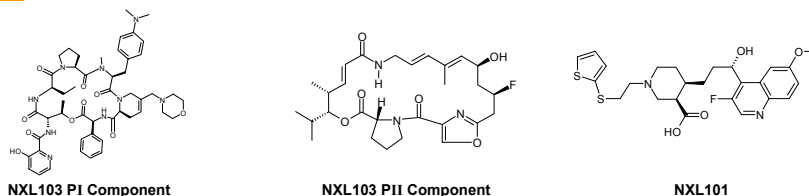
80 high value hits

1 series selected by Biacore is currently under further evaluation

Plasma protein binding evaluation of Development molecules

NXL103 and NXL101 are two antibacterial molecules currently under development. NXL103 (figure 2) consists of a 30:70 ratio of RPR202868 (PI) and RPR132552 (PII) which exhibit bactericidal synergy against common respiratory tract infection (RTI) organisms. NXL101 (figure 2) is a completely novel molecule active against Gram positive bacteria.

Figure 2 Structures of NXL103 and NXL101



As shown in Table 1 below, NXL103 PI, PII, and their combination exhibit moderate binding to HSA and AGP. It is worth mentioning that no protein binding data could be obtained with component P I using conventional equilibrium dialysis experiments. In addition, the binding of component P II correlates well with previously obtained results (49±4%). It is also the case for NXL101 for which a 87.5±0.4% binding value was obtained by equilibrium dialysis using [¹⁴C]-NXL101.

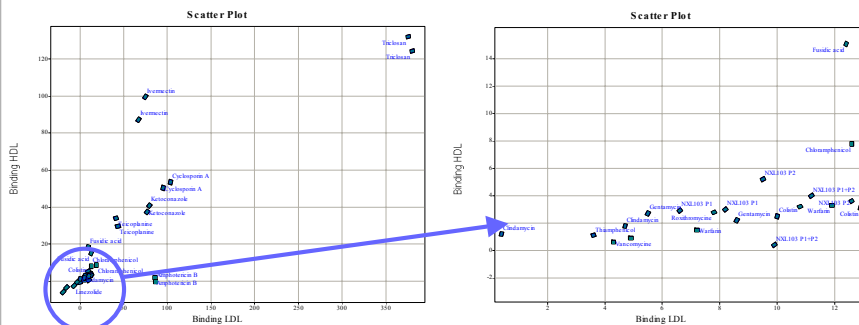
TABLE 1 Protein binding results

	Evaluation Method	KD HSA (M)	KD AGP (M)	Calculated % bound (10 μ M compound conc.)
NXL103 PI	Steady State	3.6 10 ⁻³	3.15 10 ⁻⁴	20.6
NXL103 PII	Steady State	1.05 10 ⁻³	1.05 10 ⁻⁴	46.3
NXL 103 30:70	Kinetics	1.26 10 ⁻³	3.25 10 ⁻⁴	37.9
NXL101	Steady State	1.09 10 ⁻⁴	7.61 10 ⁻⁵	86.6

NXL103 lipoprotein binding evaluation

NXL103 P I, PII, and their combination were compared to reference compounds. High binders to lipoproteins such as amphotericin B, cyclosporin A, or ivermectin, as well as low binders were used in order to validate this approach. The obtained data (figure 3) show that the binding of reference molecules is consistent with published data and that NXL103 is a low binder and behaves similarly to other antibiotics.

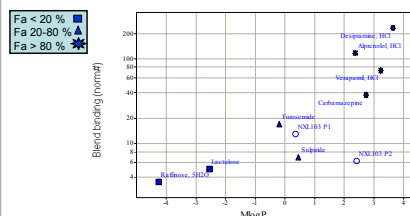
Figure 3 Comparison of the binding to HDL and LDL between NXL103 and reference molecules. Compounds were injected in duplicate. Binding responses are expressed in 100*RU/Da.



NXL103 liposomes binding evaluation

Absorption of orally administrated molecules may be predicted through their binding to charged liposomes (Frostell-Karlsson *et al*, 2005). In our hands the best correlation of the Fa% and the liposome binding was obtained with compounds at 500 μ M in PBS 0.5% DMSO at 25°C. In these conditions NXL103 displays an intermediate absorption profile, comparable to sulpiride and furosemide (44 and 61% absorption respectively). In addition the PI component is predicted to be better absorbed than PII (figure 4).

Figure 4 Comparison of the binding to charged liposomes of NXL103 and reference molecules. Binding responses are expressed in 100*RU/Da.



CONCLUSION

BIACORE S51 is a useful tool to progress molecules from hit identification to the development phase in pharmaceutical R&D. In particular, the absence of labelling needs and the possibility to obtain binding data with almost any molecules make this technology unique.

REFERENCES

Frostell-Karlsson A *et al*, Biosensor analysis of the interaction between drug compounds and liposomes of different properties: a two-dimensional characterization tool for estimation of membrane absorption. J. Pharm. Sci. 94: 25-37 (2005)