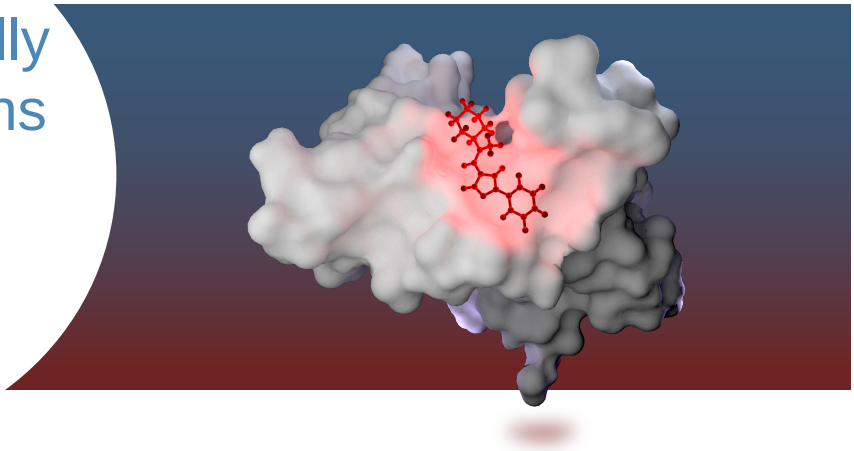
CYCLICA

PROBE$_X$ uncovers potentially fatal drug-protein interactions



Ligand Express™ reveals the proteins that interact with BIA 10-2474 and could explain its fatal neurotoxicity

PROBLEM
There is a need to discover the cause of the previously uncharacterized and potentially fatal toxic-effect suffered by recipients of experimental drug BIA 10-2474.

TECHNOLOGY
Ligand Express™ tools:
PROBE$_X$ Proteome Docking,
SWITCH$_X$ Ligand Effect Prediction,
DIVE$_X$ Systems Biology Discovery Platform.

SOLUTION
Drug-protein interactions predicted by PROBE$_X$ docking were filtered using DIVE$_X$ and enriched using SWITCH$_X$ to identify proteins that could explain the observed toxicity.

INTRODUCTION

Scientists are constantly improving the safety of drugs by designing therapies targeted to very specific disease-causing proteins. By maximizing a drug's ability to bind a particular protein, it is hoped that the drug will preferentially interact with its target protein over other proteins. Unfortunately, most drugs will interact with many other proteins in the body, even drugs that are considered safe[1]. In some cases, these additional interactions may complement the intended pharmaceutical end-goal, as is the case with the multi-targeted salicylate drugs[2]. In other cases, unintended drug interactions can have an adverse or toxic effect on the body. All drugs have a therapeutic window, outside of which high drug concentrations are toxic and low concentrations have no effect. The adage, "the dose makes the poison", accredited to Swiss-German scientist Paracelsus, addresses the link between drug and toxin, with chemicals regarded as safe – even something as innocuous as water – becoming lethal if overconsumed. In the case of the fatty acid amine hydrolase (FAAH) inhibitor BIA 10-2474 (**Figure 1**), it was clear that the safe therapeutic window was exceeded in clinical investigations. Consequently, one patient died and five others were hospitalized due to severe neurological trauma[3].

FAAH facilitates the breakdown of anandamide, a neurotransmitter linked to cannabinoid receptors in the CNS, which can both mediate pain and lead to feelings of bliss[4]. An inhibitor of FAAH would lead to an increase in anandamide which has shown therapeutic relevance for pain or anxiety management. Given this promising and novel treatment strategy, many pharmaceutical agents were developed to target FAAH, including BIA 10-2474. It was during the dose-escalation phase of BIA 10-2474's safety study that participants suffered intracranial hemorrhaging. An official investigation found that it was likely the drug or one of its metabolites that caused the toxic side effects[5], possibly through an off-target interaction[3]. Intracranial bleeding is an adverse effect that has yet to be encountered in the clinics, suggesting that a novel protein target may be responsible. Cyclica's Ligand Express™ platform is capable of identifying such novel ligand-protein interactions that may explain the adverse effects of BIA 10-2474.

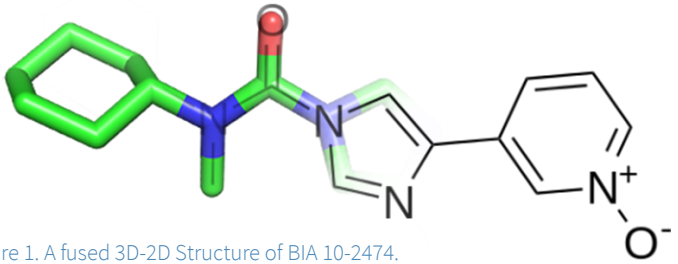


Figure 1. A fused 3D-2D Structure of BIA 10-2474.

METHODOLOGY

Cyclica scientists used the Ligand Express™ toolkit to discover the underlying biological activity of BIA 10-2474 that led to brain hemorrhage. BIA 10-2474 was screened using the PROBE$_X$ proteome docking tool to generate an exhaustive list of proteins likely to interact with BIA 10-2474. We then used DIVE$_X$ to search for proteins with links to both biological function and disease associations linked to the brain and bleeding, and visualized these proteins on an interconnected, biological function annotation network. Specifically, potential protein targets were categorized as either (1) modulators of blood coagulation, (2) brain tissue remodelling, or (3) other intracellular processes. The protein-ligand interactions identified by PROBE$_X$ were evaluated using SWITCH$_X$ for their modulatory effect and associated biological consequences. The result from these sequential analyses is a short list of proteins putatively involved in the observed toxicity.

RESULTS

Using PROBE$_X$ proteome docking, we generated a complete list of proteins that are likely to interact with BIA 10-2474 (the desired target, FAAH, was identified as the 104th hit, placing it in the top 99.9 percentile of proteins examined). This list was restricted to proteins that were related to the blood and brain using DIVE$_X$, and was visualized in a biological function annotation network shown in **Figure 2**. These top results were then examined using SWITCH$_X$, which determined that BIA 10-2474 likely inhibited most of the related proteins (**Table 1**).

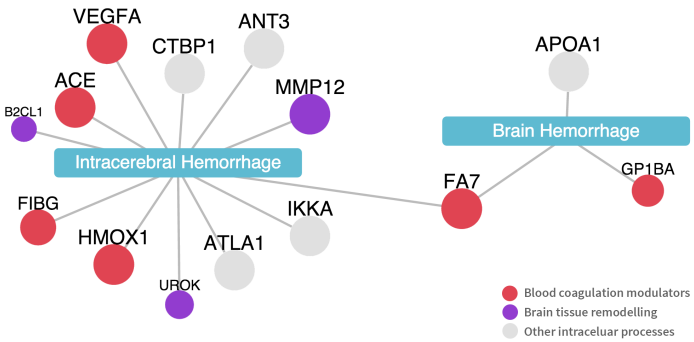


Figure 2. Proteins predicted to interact with BIA 10-2474 to cause neurotoxicity (brain hemorrhage/bleeding). Neurotoxic processes, blood coagulation proteins, brain tissue remodeling proteins, and proteins related to other intracellular processes are colored accordingly. Radii of circles and font size represent the likelihood of the predicted interaction.

Inhibition of these targets could explain the acute and rapid onset of the adverse effects and are related to the symptoms (e.g. hemorrhaging) observed in the clinical trial. Due to its greater degree of interconnectivity with intracranial bleeding, protein coagulation factor VII (FA7) is particularly suspect and should be a leading candidate for further investigation. Importantly, PROBE$_X$ also provides a detailed binding pose for all ligand-protein interactions, providing information on intermolecular contacts that can be used to design a safer version of the drug (**Figure 3**).

SUMMARY

To help explain the serious, potentially-fatal side effects exhibited by the drug candidate BIA 10-2474 in clinical trials, Cyclica employed Ligand Express™ to help understand the mechanism of this toxicity. Initial proteome docking results were systematically filtered down, leading to the emergence of protein coagulation factor VII (FA7) as a top candidate for causing the observed intracranial hemorrhaging. Further *in vitro* investigations by other researchers can validate factor VII, or one of the other candidates, and thus explain the cause of the problem. Cyclica's technology can provide valuable insight into anticipated toxicity and its mechanisms early in the drug discovery process, leading to safer drugs and reducing the cost and risks of drug development.

Table 1: Predicted effects and consequences of BIA 10-2474 interaction with neurotoxicity-associated proteins. Blood coagulation proteins and brain tissue remodeling proteins are colored accordingly. *, predictions were verified by manual examination of binding sites; #, number of instances in the scientific literature.

Target Protein	Effect Needed for Brain Hemorrhage	% Negative Regulators (#)	% Positive / Other Regulators (#)	Predicted Effect	Likely to Cause Brain Hemorrhage
FA7	Inhibition	67.3 (202)	16.0 (48)	Inhibition*	Yes
ACE	Inhibition	98.0 (99)	2.0 (2)	Inhibition	Yes
VEGFA	Activation	25.0 (5)	75.0 (15)	Activation*	Yes
FIBG	Inhibition	50.0 (1)	50.0 (1)	Inhibition*	Yes
HMOX1	Inhibition	50.0 (1)	50.0 (1)	Inhibition*	Yes
GP1BA	Inhibition	0.0 (0)	0.0 (0)	Unclear	Unclear
UROK	Inhibition	84.9 (326)	15.1 (58)	Inhibition*	Yes
MMP12	Inhibition	98.3 (227)	1.7 (4)	Inhibition	Yes
B2CL1	Inhibition	0.0 (0)	0.0 (0)	Unclear	Unclear

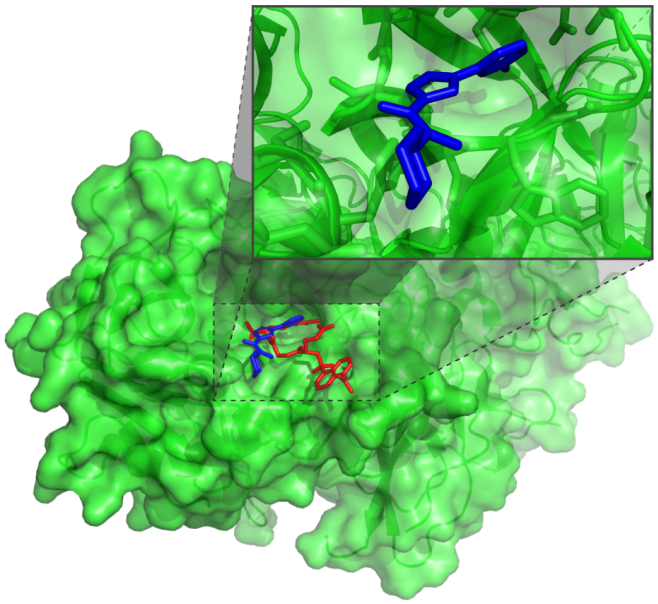


Figure 3. Ligand Express™ predicts BIA 10-2474 to bind coagulation factor VII (FA7) (PDB: 3TH3) in the same region as a known inhibitor. Inhibition of coagulation factor VII by BIA 10-2474 may cause deadly neurotoxicity (bleeding within the brain) as observed in the French clinical trial.

RESOURCES

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