Uncovering biochemical assay's false positives by counter-screening a fragment library with affinity capillary electrophoresis

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Introduction

Biochemical assays are commonly used as screening technology in medicinal chemistry programs. They require a low amount of protein, are high throughput and straightforward to establish. This screening methodology is one of the most popular approaches to screen fragments (i.e., small molecules that have low affinities for the target and that will be grown into a larger compound upon optimization). However, biochemical assays are burdened with various interferences such as aggregators, fluorescent compounds or impurities. Therefore, orthogonal methods are needed to confirm fragment hits.[1]

Method

We developed and evaluated a partial-filling affinity capillary electrophoresis methodology with factor XIIa (FXIIa) as target for fragment counter-screen (Fig. 1). The method was applied to the screening of a small focused library and the results were compared to those obtained with the biochemical assay. The origin of some discrepant results was also investigated.

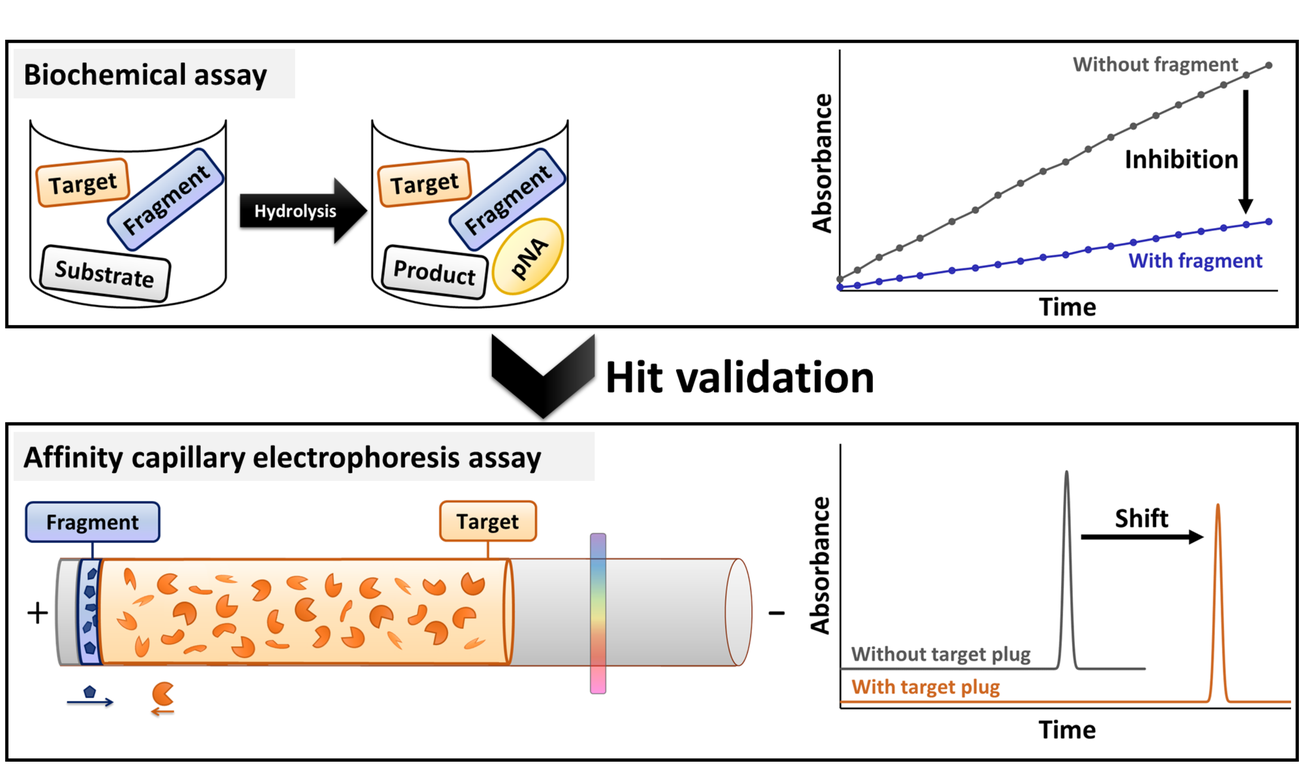

***Fig. 1**: Counter-screening process.*

Results [2]

The method follows interactions accurately

The complex dissociation constants of p-aminobenzamidine (PABZM) and pentamidine were evaluated. Similar results were obtained compared to biochemical assay (see Table 1).

***Table 1**: Complex dissociation constants of reference compounds*

Method	PABZM	Pentamidine
Biochemical assay	163.2 ± 0.5	12.4 ± 0.1
Affinity capillary electrophoresis	149 ± 14	6.3 ± 0.1

The method counter-screened a fragment library

A library of 24 fragments was screened by the biochemical assay and by the affinity capillary electrophoresis method (Fig. 2). A good correlation was observed between the two methodologies. Major differences were noticed with the compounds 1, 2, and 3. They exhibit a high ranking in the biochemical assay but are under or near the threshold in affinity capillary electrophoresis.

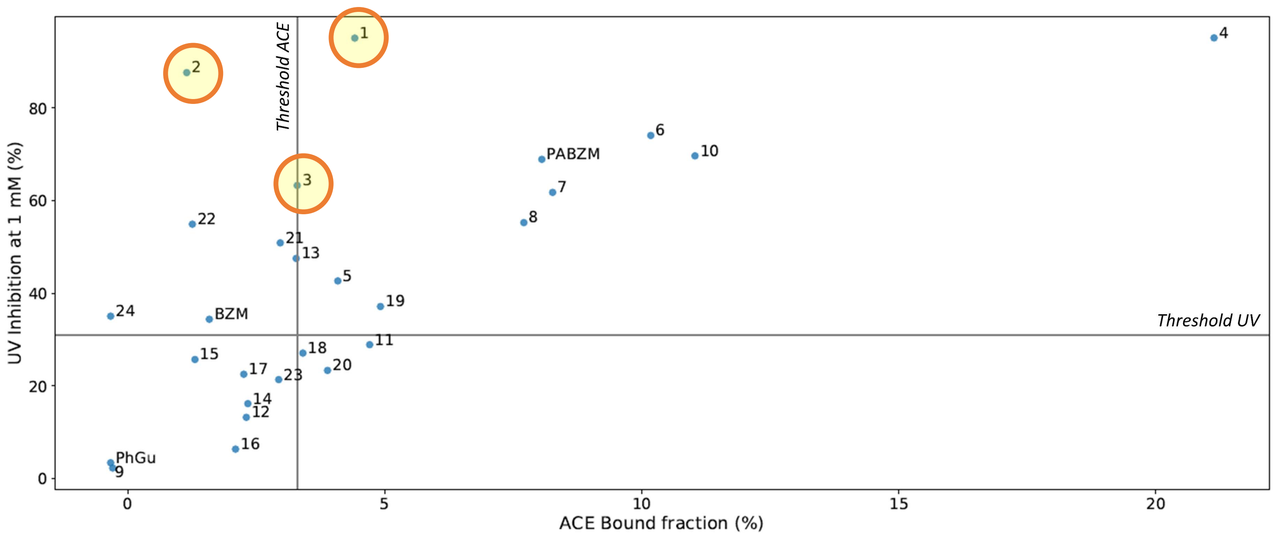

***Fig. 2**: Screening results from affinity capillary electrophoresis (x value) in comparison with biochemical FXIIa assay (y value).*

The method is unaffected by metallic contamination

To explain the discrepancy noticed with compounds 1, 2, and 3, we suspected their contamination by divalent metallic cations. First, we challenged the two methodologies with reference compounds spiked with metallic impurity. Unlike biochemical assay, the affinity capillary electrophoresis assay was unaffected by the metallic contamination of the samples. Regarding compounds 1, 2, and 3, the presence of zinc in the samples was identified by capillary electrophoresis using pre-capillary CDTA complexation (Fig. 3).

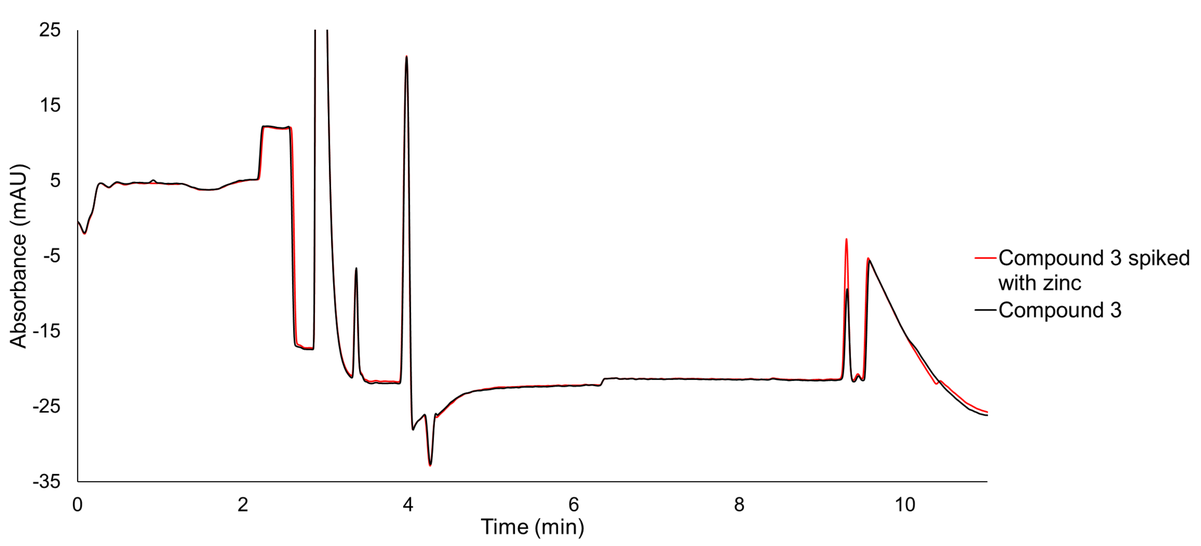

***Fig. 3**: Electropherograms of compound 3 (in black) and compound 3 spiked with 1.5 mM Zn^{2+} (in red).*

Conclusion

Biochemical FXIIa assay is sensitive to the presence of low-level metallic impurity. Zinc contamination led to three false positives, generating misleading structure-activity relationships. Hence, cross-validation by orthogonal techniques is essential to avoid wasting time and resources. Our study showed that affinity capillary electrophoresis can be powerful for fragment counter-screening thanks to its separative character.

Bibliography

[1] a) W. Jahnke, D. Erlanson, I. de Esch, C. Johnson, P. Mortenson, Y. Ochi, T. Urushima, J. Med. Chem. (2020), 63, 15494-15507 b) B. Davis, D. Erlanson, Bioorg. Med. Chem. Lett. (2013), 23, 2844-2852.
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