

# Probing the Binding Site of Abl Tyrosine Kinase Using in Situ Click Chemistry

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## Supporting Information

**ABSTRACT:** Modern combinatorial chemistry is used to discover compounds with desired function by an alternative strategy, in which the biological target is directly involved in the choice of ligands assembled from a pool of smaller fragments. Herein, we present the first experimental result where the use of in situ click chemistry has been successfully applied to probe the ligand-binding site of Abl and the ability of this enzyme to form its inhibitor. Docking studies show that Abl is able to allow the in situ click chemistry between specific azide and alkyne fragments by binding to Abl-active sites. This report allows medicinal chemists to use protein-directed in situ click chemistry for exploring the conformational space of a ligand-binding pocket and the ability of the protein to guide its inhibitor. This approach can be a novel, valuable tool to guide drug design synthesis in the field of tyrosine kinases.

**KEYWORDS:** ligand-binding site, Abl tyrosine kinase, click chemistry, drug design synthesis



The findings that Bcr-Abl (cytoplasmic tyrosine kinase) is the cause of the leukemic phenotype and that the tyrosine kinase activity of Abl is fundamental for Bcr-Abl-mediated transformation have made this kinase an important target for the development of specific therapies. In the recent past, advances in the selective inhibition of Bcr-Abl kinase activity led to the development of several active compounds, and in particular, imatinib mesylate (Gleevec) is the one that currently represents the front-line therapy of CML. Considering our interest in the discovery of new inhibitors of tyrosine kinases,<sup>1–5</sup> we have recently reported the design and preparation of a small collection of quality *N*-[2-methyl-5-(triazol-1-yl)phenyl]pyrimidin-2-amine derivatives.<sup>6</sup> The anti-proliferative activity in the micromolar range pointed out the efficacy of the compound named FA030 (Figure 1,  $IC_{50} = 0.89 \mu\text{M}$  on the K-562 cell line). This one showed a potent antienzymatic activity against recombinant Abl kinase ( $IC_{50} = 0.9 \pm 0.1 \mu\text{M}$ ). The ADME prediction suggested no significant difference between the behavior exhibited by FA030 and that of imatinib that differs for an amide group in the place of the triazole ring.<sup>6</sup> Moreover, the binding mode was very similar to that of imatinib. FA030 seems to have six hydrogen bonds with the protein, and the majority of contacts are mediated by van der Waals interactions. The triazole ring is involved in two hydrogen bonds with the carbonyl groups of Asp 381 and His 361.

Over the last years, target-guided in situ synthesis<sup>7</sup> has attracted our attention because it proved to be a captivating and efficient approach to drug discovery. We applied this strategy to the formation of bivalent compounds that are able to target the tubulin/microtubules dynamic system.<sup>8</sup>

Thus, we successfully used tubulin as a target to influence the composition of the mixture of a dynamic combinatorial library by exploiting the disulfide bond exchange reaction.<sup>9</sup> In this scenario, we turned our attention to in situ click chemistry<sup>10</sup> based on Huisgen reaction,<sup>11–33</sup> which we considered attractive and stimulating for constructing inhibitors of tyrosine kinases—strategic for fighting cancer. We were driven by the antienzymatic activity and docking records of FA030. Thus, we took into consideration the possible use of Abl as a target for in situ click chemistry.

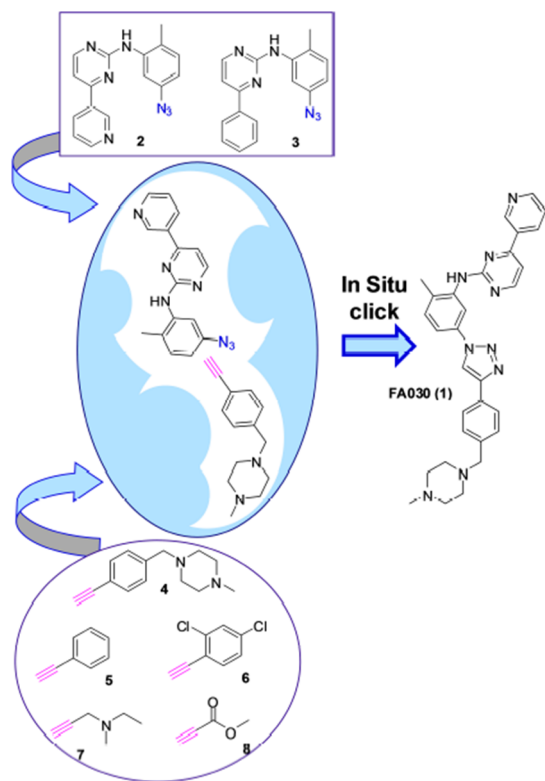
To investigate the ability of the azide **2**<sup>6</sup> and alkyne **4**<sup>6</sup> fragments to bind to the active site in a way that allows the click reaction to occur, docking simulations were performed using the docking program Glide.<sup>34</sup> At first, the azide was docked in the ATP-binding site of Abl. The result of docking simulation is shown in the Supporting Information. As we expected, the fragment forms the same hydrogen bonds of FA030 with

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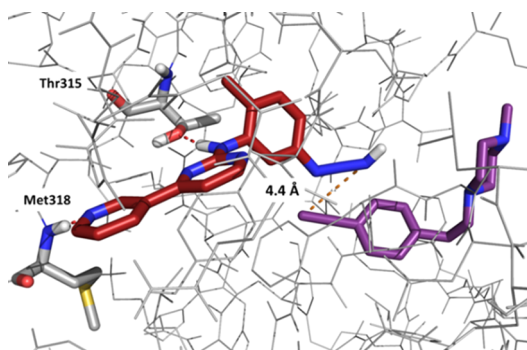
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**Figure 1.** Kinetic Abl-guided synthesis of FA030 via Huisgen 1,3-dipolar cycloaddition.

Met318 and Thr315, and the azide group is located in a profitable way near the triazole binding region of FA030. Then, the alkyne fragment was docked considering the azide as part of the receptor. The result of docking is shown in Figure 2. The



**Figure 2.** Azide 2 and alkyne 4 docked in the Abl binding site.

alkyne group is placed not far from the azide reactive group; the distance is less than 5 Å. On the basis of our theoretical studies, it is possible to assume that the two fragments can bind to the active site of Abl, allowing the click reaction to take place.

First, we evaluated the ability of Abl (~135 KDa, purity >70%) to favor the formation of FA030 through click reaction of azide 2 and alkyne 4. Two different concentrations for the reagents (0.25 and 0.370  $\mu\text{M}$ ) as well as for Abl tyrosine kinase (0.0185 and 0.370  $\mu\text{M}$ ) were used in phosphate buffer (Table 1) and compared with the respective control solutions lacking of Abl.

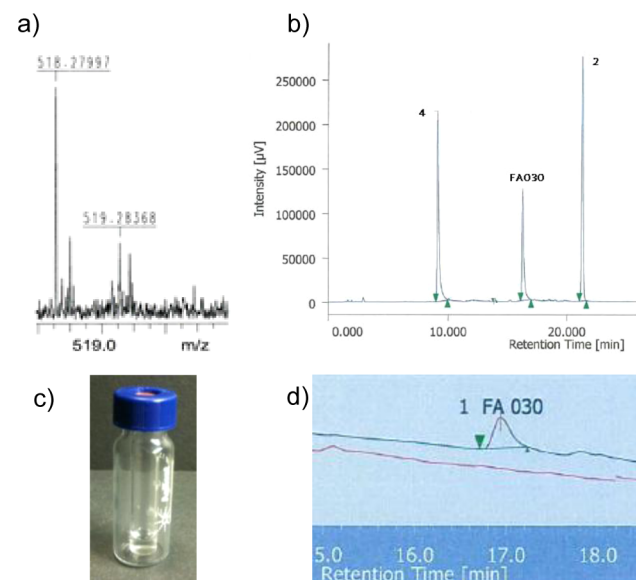
The presence of FA030 was checked with an ICP-FT-MS instrument that permits the isotopic mass measurement with

**Table 1.** Concentration of Azide 2, Alkyne 4, and Abl in the Reaction Mixture Maintained at 37 °C for 4 Days and Detection of FA030 by Mass Spectroscopy

entry	[azide 2] ( $\mu\text{M}$ )	[alkyne 4] ( $\mu\text{M}$ )	[Abl] ( $\mu\text{M}$ )	HR-ESI
1	0.25	0.25	0.0185	518.27997 <sup>a</sup>
2	0.25	0.25	0	no detection
3	370	370	0.37	518.27868 <sup>b</sup>
4	370	370	0	no detection

<sup>a</sup>Error (ppm), 4.7. <sup>b</sup>Error (ppm), 2.2.

little ppm error and has a resolution within the range of 15,000–5,000. We were fascinated with detecting the peak of the target compound FA030, after 4 days, in both solutions containing Abl (Figure 3a). The presence of FA030 was also confirmed by HPLC analysis (Figure 3b,d), using a RP column.



**Figure 3.** (a) FT-HRESI with the peak of FA030 (error, 4.7 ppm). (b) HPLC method (X Bridge Shield RP18; 5  $\mu\text{m}$ ; 4.6 mm  $\times$  150 mm; gradient  $\text{CH}_3\text{CN}$ ; from 20 to 50% in 20 min; 0.1 M phosphate buffer at pH 7; column  $T = 40$  °C; flow, 1 mL/min;  $\lambda = 250$  nm). (c) Experimental vessel containing Abl and the building blocks. (d) HPLC profiles of the solution with Abl (0.37  $\mu\text{M}$ , blue line) and control (red line).

To exclude any possible catalytic activity due to the unexpected presence of copper (buffer solutions), we analyzed, using ICP-MS, the control and test solutions, and we compared the values with the amount of Cu(I) generally used in the Sharpless conditions<sup>6</sup> (Table 2). The preparation of FA030 according to Sharpless conditions requires 1–5% (mol) of Cu(II) that is reduced in situ to Cu(I) by sodium ascorbate. We detected a low amount of copper in the control and test solutions (Table 2, 0.1 and 0.04%, respectively). Besides, it is

**Table 2.** Detection by ICP-MS of Cu (Molar Ratio) in Reaction Mixtures

	azides	alkynes	Cu	$T$ (°C)
Sharpless cond.	1	1	0.01–0.05	25–120
Abl in situ	1	1	0.0017	37
control	1	1	0.00044	37

reasonably in the oxidized form and thus not able to act as a catalyst. In the test solution, we also detected Mn (0.007%), Co (0.006%), Ni (0.27%), Zn (0.38%), and Pb (0.006%).

Having obtained this challenging result, we proved that Abl has the capacity to favor click reaction between azide **2** and alkyne **4**. Subsequently, we moved toward the investigation of Abl efficacy in the selected target-catalyzed formation of FA030 from a library of building blocks that were simultaneously present in a single reaction mixture. To that effect, a solution of azides **2** and **3** and alkynes **4**–**8** (370  $\mu$ M) was incubated for 4 days, at 37 °C, in the presence of Abl (0.37  $\mu$ M), in buffer solution (Figure 3c). In addition to that, a control solution was prepared without the tyrosine kinase. The use of HPLC and HRESI MS evidenced only the peak generated by FA030, without any trace of the other possible compounds that we know to be ineffective in the inhibition of Abl activity. The control solution did not show any detectable peak. We can confirm that Abl is able to selectively favor the formation of the best inhibitor.

The results obtained confirm the relevance of in situ click reaction and allow the possible use of this strategy for the discovery of new scaffolds useful for the inhibition of the large family of tyrosine kinases.<sup>35–38</sup> The selection of the coupling partners that have to be incubated with the enzyme can not be random and must be based on the structure of “hits” originating from docking studies and/or high-throughput screens. For example, many of the known inhibitors of TKs could be dissected in frameworks and properly functionalized with azide/alkyne moieties to be used as libraries of building blocks for the in situ click reaction, in the presence of any available tyrosine kinase. The detection of the favored compounds by HR-ESI MS will open the way for the preparation and in-depth biological evaluation of selective molecules as lead structures. They can be further modified by substituting the triazole ring with amide or carbamate groups to modulate the bioavailability and the biological performance.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Experimental details for the synthesis, docking studies, and instruments details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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