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Triazoyl–phenyl linker system enhancing the aqueous solubility of a molecular probe and its efficiency in affinity labeling of a target protein for jasmonate glucoside

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ABSTRACT

In methods employing molecular probes to explore the targets of bioactive small molecules, long or rigid linker moieties are thought to be critical factors for efficient tagging of target protein. We previously reported the synthesis of a jasmonate glucoside probe with a highly rigid linker consisting of a triazoyl–phenyl (TAzP) moiety, and this probe demonstrated effective target tagging. Here we compare the TAzP probe with other rigid or flexible probes with respect to target tagging efficiency, hydrophobic parameters, aqueous solubility, and dihedral angles around the biaryl linkage by a combination of empirical and calculation methods. The rigid biaryl linkage of the TAzP probe has a skewed conformation that influences its aqueous solubility. Such features that include rigidness and good aqueous solubility resulted in highly efficient target tagging. These findings provide a promising guideline toward designing of better linkers for improving molecular probe performance.

Molecular probes are recognized as powerful tools for exploring the targets of bioactive small molecules.[1–3] An appropriately designed molecular probe affords highly efficient chemical tagging of target proteins. A molecular probe consists of four essential components: a pharmacophore, reactive functionality, a molecular tag, and a linker moiety (Fig. 1). Among these, the selection of a linker of the appropriate length and structure strongly affects the efficiency of target tagging (Fig. 1).[4–7] In general, it is considered that the role of the linker is to cast the molecular tag away from pharmacophore, which is an essential unit for binding with a specific target.[8] It is generally accepted that the longer or more rigid the linker is, the more effective the casting process.[9] To date, substantial attention has been paid to the design of the linker. Polymethylene,[9] oligopeptides, including polyglycine[10] or polyproline-rod,[5] and polyethyleneglycol (PEG)[11,12] are widely used as standard linkers (Fig. 1). However, a long linker causes serious issues associated with its hydrophobic nature. An increase in hydrophobicity is accompanied by a decrease in aqueous solubility as well as an increase in nonspecific binding.[6,13] Thus, a linker that combines rigidness for effective casting of the molecular tag with high aqueous solubility is strongly desired.

Recently, we reported the potency of a molecular probe (**1**) having a highly rigid triazoyl–phenyl (TAzP)[14] linker designed for efficient affinity tagging of the membrane target of jasmonate glucoside (**2**[16] in Fig. 2) (MTJG).[10,15] Surprisingly, even a short TAzP linker functioned well and provided useful target tagging. The TAzP probe can be easily constructed by copper-catalyzed azide alkyne cycloaddition (CuAAC)[17–19] between an alkyl azide and aryl acetylene, and it provided better results for tagging the corresponding target protein than the conventional molecular probe. In this study, we will discuss the chemical basis for the high efficiency of the TAzP linker and report our findings that the TAzP linker confers rigidness as well as good aqueous solubility to a molecular probe because of its skewed conformation around the biaryl linkage.

TAzP molecular probe **1** possesses a biaryl structure substituted by a pharmacophore and a large tag at each end of the unit (Fig. 2). In a previous study, we hypothesized that the reason for the favorable performance of the TAzP probe might be attributed to the rigidness of the biaryl structure, which functions to cast the tag away from the pharmacophore. Novel insight regarding the proficiency of the TAzP linker can contribute to the development of other high-performance linkers. However, our hypothesis has remained unproven because of a lack of appropriate control experiments. To resolve this deficiency, we designed and synthesized alkyne units **9**–**12** (Schemes S1–S4, Supplementary data), which provide 'CuAAC'-prepared probes **3**, **4**, **6**, and **7** (Figs. 2 and 3). Each alkyne unit (**9**–**12**)

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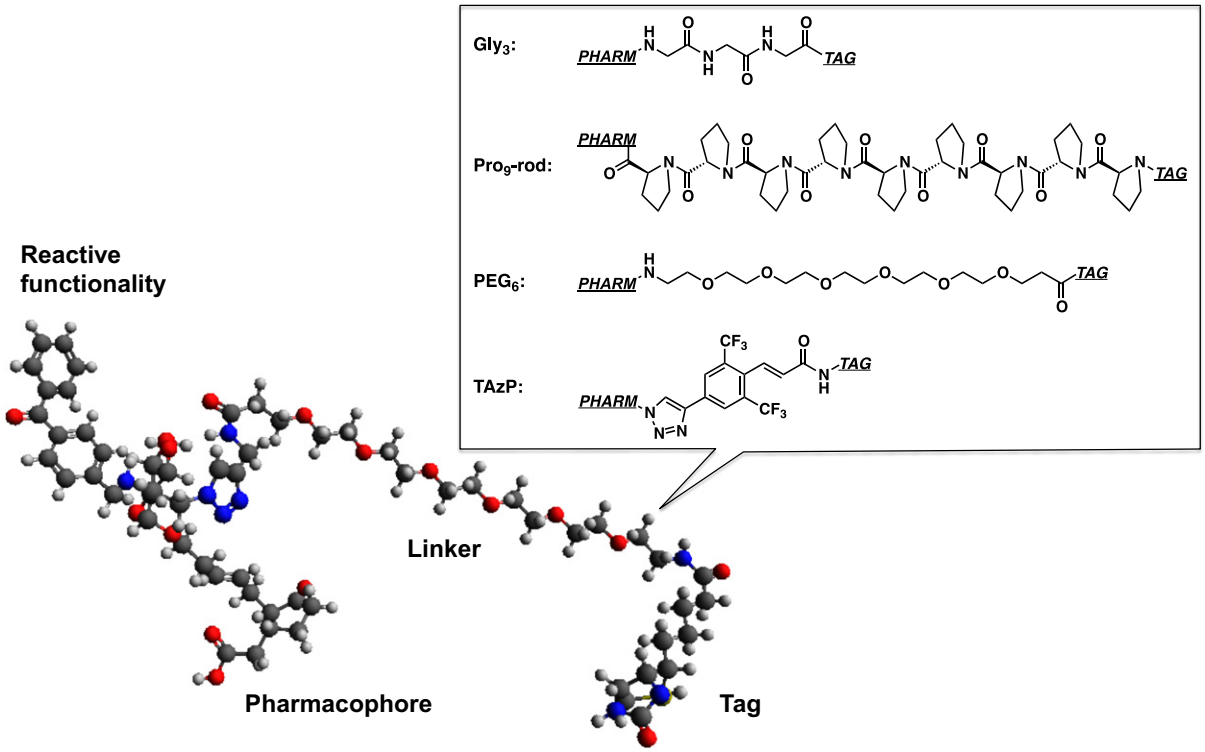

Figure 1. A ball-and-stick model of **5** consisting of four essential components: a pharmacophore, reactive functionality, a molecular tag, and a linker moiety. Structures of conventional linkers are also shown (framed rectangle).

was coupled with azide-JAG (**13**)[14,20] under CuAAC conditions using tris(3-hydroxypropyltriazolylmethyl)amine (THPTA)[21,22] as a catalyst. The resulting probes were used as appropriate controls for TAzP probe **1** in the following experiments.

According to our hypothesis,[14] the advantage of the TAzP linker is lost when its planarity is disrupted by the introduction of methylene units between the triazole and aryl rings (Fig. 2). Thus, we designed ethyl-tethered probe **3**, in which the C2-unit in **1**, encompassing the α,β-positions of the carbonyl group, was shifted to the position between the triazole and aryl groups. Probe **3** has the same linker length (14 atoms) as **1** (Fig. 2) and is a useful control for comparison with **1**. We also synthesized alkyne units **11** and **12**, which provided the triazoylquinolyl (TAzQ) probes **6** and **7**, respectively. Higher planarity can be anticipated for the biaryl group of **6** and **7** than for the corresponding moiety of **1**. The nitrogen atom in the quinoline ring in **6** and **7** serves to lower the hydrophobicity of the corresponding probe despite the large size of this aromatic ring.[23] In addition, alkyne unit **10** with a hexa-PEG linker provided probe **4** by possessing the long PEG fragment, which added flexibility as compared to the rigid TAzP probe (Fig. 2).

First, we compared the efficiencies in target tagging among ethyl-tethered probe **3**, TAzQ probes (**6** and **7**), PEG-substituted probe **4**, conventional triglycyl probe **5**[10], and TAzP probe **1**. The efficiency of each probe was assessed by the photoaffinity-based target tagging experiment with MTJG.[15] MTJG has been identified as a target protein for the leaf-closing factor of *Samanea saman* (**2**).[16,24,25] Photoaffinity labeling by each probe was performed using the motor cell of *S. saman*,[10,14] and the prepared membrane fractions were subjected to SDS–PAGE analysis with labeled MTJG. The preparation and subsequent utilization of *Samanea* protoplasts in photoaffinity labeling experiments were performed according to the procedures described in our previous study.[14] Unexpectedly, TAzP probe **1** provided the highest tagging efficiency among all the probes (**1** and **3**–**7**) followed by triglycyl-linked probe **5** (Fig. 4). Conversely, all of the remaining probes, including TAzQ, did not provide any band corresponding to biotinylated MTJG (Fig. 4).

Because ethyl-tethered probe **3** with the same linker length (14 atoms) as **1** does not show a band for tagged MTJG, it can be concluded that the efficient photoaffinity tagging by **1** is not related to the length of the linker but depends on the chemical nature of the biaryl structure in the TAzP linker. This result suggests that the advantage of the rigid TAzP linker might lie in its ability to effectively cast the large tag away from the pharmacophore.

This advantage of the rigid TAzP linker was also supported by the fact that hexa-PEG probe **4** equipped with a linker extending 27 atoms in length does not show a band for tagged MTJG. Hydrophilic and flexible PEG linkers are expected to adopt an extended conformation, in which the tag is effectively cast away from the pharmacophore, whereas experimental and theoretical studies suggest that low molecular weight PEG oligomers/polymers predominantly adopt a helical conformation in an aqueous environment.[26–28] A helical conformation of the PEG linker would confine the large tag close to the pharmacophore and sometimes cause aggregation with the attached pharmacophore.[27] Thus, this drawback of the PEG linker could be responsible for the disappointing results for hexa-PEG probe **4**. It is also important to determine whether nonspecific binding to proteins other than MTJG occurred using PEG-linked **4** (data not shown). It is reported that the use of a long PEG linker in affinity-based protein purification cannot suppress the incorporation of nonspecific proteins.[6,13] These results suggest that increasing the length of the linker would not be an effective strategy.

Interestingly, no biotinylated MTJG was found in photoaffinity labeling experiments using TAzQ probe **6** or **7**. As it is expected that the TAzQ linker affords planarity and rigidness equal to or greater than the TAzP linker, we suspected that an unknown chemical property of TAzP might be responsible for its notable target affinity.