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Solid-Phase Synthesis of Azidomethylene Inhibitors Targeting Cysteine Proteases

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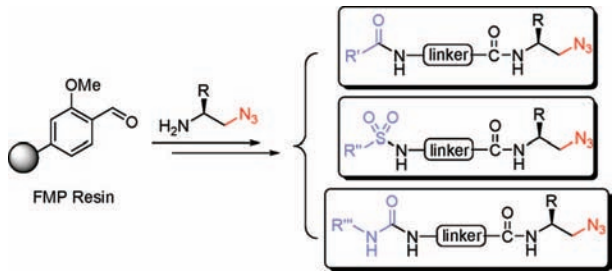
ABSTRACT



An efficient strategy for the solid-phase synthesis of azidomethylene inhibitors targeting cysteine proteases is described. The method is highlighted by its compatibility with readily available building blocks, as well as its ability to accommodate different functional groups. A 249-member library has thus far been successfully synthesized, characterized, and screened against Caspase-1, -3 and -7.

Recent advances in genomics and proteomics necesssiates the development of highly efficient chemical reactions capable of rapid synthesis of potential drug candidates.[1] Such reactions are exemplified by their high efficiency (e.g., quantitative product formation), good tolerance toward a variety of functional groups, and compatibility with screening (e.g., reaction is carried out in water; no product purification is necessary before biological assay). Thus far, the Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction, or better known as "click chemistry", between an azide and an alkyne, has been extensively studied,[2] and proven to be highly successful, especially for high-throughput synthesis and discovery of enzyme−inhibitors.[2b] The amide-forming reaction between an amine and an acid is one of the most efficient reactions known, and has recently been applied successfully in the rapid discovery of inhibitors against a number of enzymes, such as HIV proteases,[3a,b] β-aryl sulfotransferase,[3c] α-fucosidases,[3d,e] and SARS-3CL protease.[3f] This chemistry has some key characteristics of a high-throughput amenable reaction, in that quantitative product formation can be achieved using powerful acylating/coupling reagents. As such, in situ biological screening may be carried out directly without product purification. The method, however, is severely limited by the presence of coupling reagents and, more often than not, byproduct and excessive starting materials in the reaction, and subsequently during in situ

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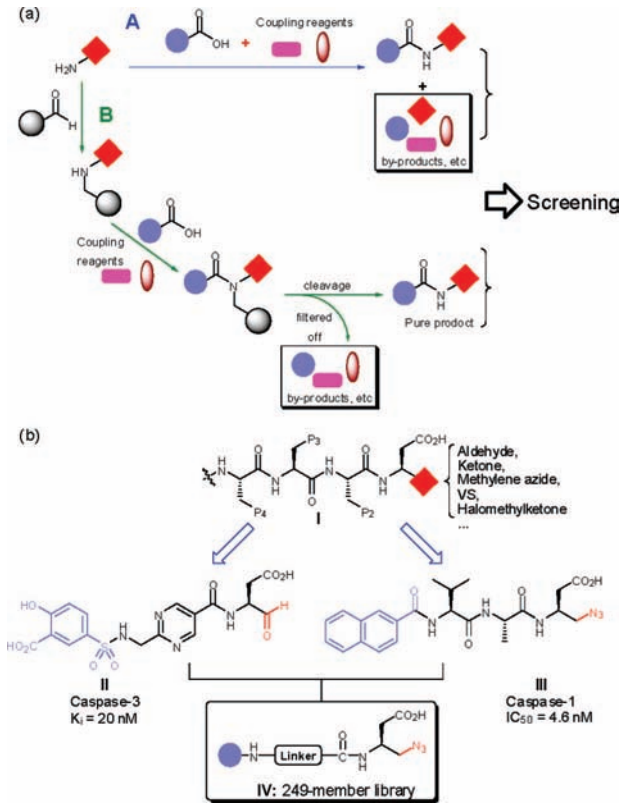

Figure 1. (a) Two strategies using amide-forming reactions; (b) structures of different known caspase inhibitors.

screening, which may lead to false results (Figure 1a; pathway A).[4] In fact, in a recent study by Wong et al., the authors unexpectedly discovered that it was the intermediate active ester formed between a carboxylic acid (a starting material) and HBTU (a coupling reagent) which gave rise to potent inhibition against the target, SARS-3CL protease.[3f] Herein, we report a traceless solid-phase method for rapid synthesis of novel small molecule inhibitors using amide bond-forming reactions (Figure 1a; pathway B); the strategy takes advantage of the 4-formyl-3-methoxyphenoxy (FMP) resin for (1) amine capturing using an aldehyde handle; (2) subsequent solid-phase amide-forming reaction with a linker and an incoming acid using suitable coupling reagents; and (3) cleavage/release of desired products which are of sufficient purity for direct in situ screening. The method is "traceless", allowing the use of the exact same sets of starting materials as in pathway A (e.g., same amine/acid building blocks) in solid phase without any modification. We have successfully implemented this strategy for the facile synthesis of 249-member azidomethylene inhibitors targeting cysteine proteases.

Among the numerous classes of cysteine proteases involved in human diseases, caspases are well-known to play key roles in the regulation of apoptosis and inflammatory responses.[5] With more than 15 different members identified so far, the hallmark of this family of enzymes is their absolute requirement for an aspartic acid residue at the P_1 site of their substrates. Over the past few years, a variety of caspase inhibitors, both reversible and irreversible, have been developed.[6] Most inhibitors contain an electrophilic "warhead" such as an aldehyde, ketone, halomethyl ketone, epoxide, or vinyl sulfone (Figure 1b, I), which reacts with the cysteine residue located in the active site of a caspase. To improve "druglike" properties, the P_2 and P_3 positions in a peptide-based caspase inhibitor may be replaced with suitable nonpeptide linkers (see II[7] in Figure 1b) with minimal effect on the inhibitor potency. The P_4 position in a caspase inhibitor is known to be the major determinant of both its binding and specificity toward different caspases.[5] For example, it has been shown that caspases 3 and 7 prefer charged/hydrophilic groups at this position, while caspase 1 prefers predominantly hydrophobic groups. Consequently, potent and (in some cases) specific inhibitors, such as II and III, were successfully developed.[7,8] We were particularly intrigued by inhibitors such as III, which were recently reported by Fairlie and co-workers,[8a] as they contain a relatively unreactive azidomethylene group as the electrophilic warhead. We thought since an azidomethylene group is chemically and metabolically inert, molecules possessing this "warhead" might offer special advantages as potential cysteine protease inhibitors.[1] We therefore decided to develop an efficient route capable of making azidomethylene inhibitors targeting different classes of cysteine proteases including caspases (Scheme 1).[9] It is noted that, while our manuscript was in preparation, Fairlie et al. reported that peptidic azidomethylene inhibitors were unexpectedly susceptible to photolytic degradation, giving rise to traces of aldehyde and monoacyl aminal products.[8b]

A 249-member small molecule library was constructed following Scheme 1, using the IRORI directed sorting technology.[10] As shown in Figure 1b (boxed), most members consist of an aspartic acid-derived azidomethylene warhead, a suitable linker located at the P_2 and P_3 positions to render the inhibitors more druglike, and a diverse P_4 group introduced from commercially available building blocks (acids, sulfonyl chlorides, chloroformates, and isocyanates). To make the central amino azide scaffold, **4**, an Fmoc-protected amino acid **1** was converted into the corresponding alcohol **2**, then subsequently into the corresponding azide **3** by the Mitsunobu reaction with hydrogen azide.[11] Following

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(5) Denault, J. B.; Salvesen, G. S. *Chem. Rev.* **2002**, *102*, 4489.

(6) (a) Otto, H.-H.; Schirmeister, T. *Chem. Rev.* **1997**, *97*, 133. (b) Powers, J. C.; Asgian, J. L.; Ekici, O. D.; James, K. E. *Chem. Rev.* **2002**, *102*, 4639.

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(9) Existing strategy for the azidomethylene inhibitors made use of solution-phase chemistry which is low throughout and extremely tedious, therefore not HT amenable. See ref 8 for details.

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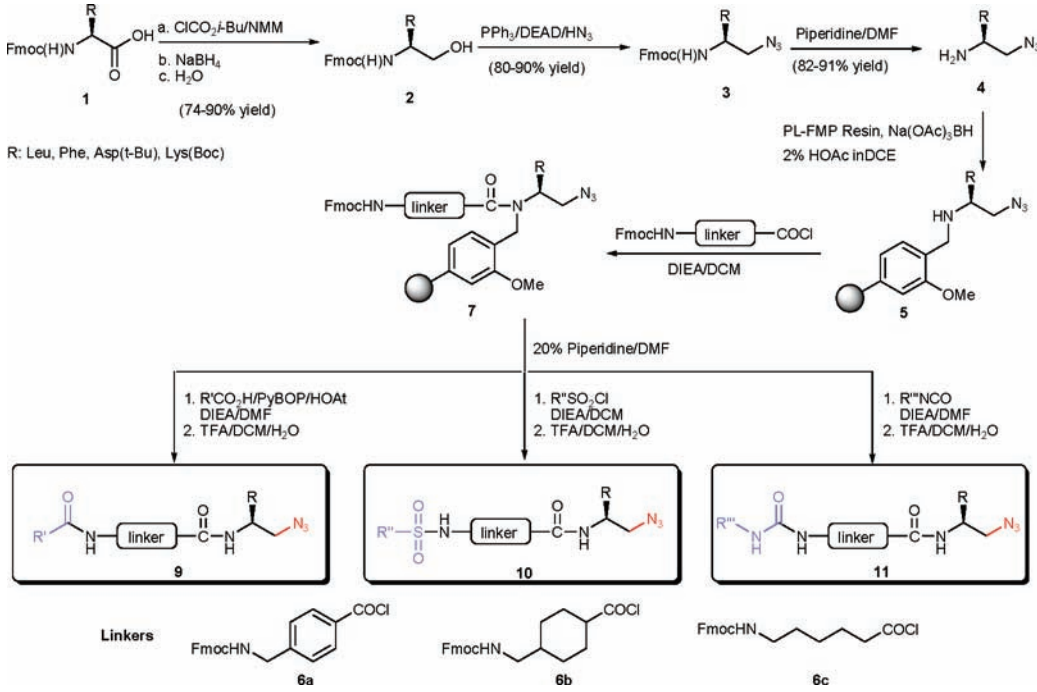
Scheme 1. Traceless Solid-Phase Synthesis of Azidomethylene Inhibitor



removal of Fmoc group with 20% piperidine in DMF, the desired amino azides, **4a**−**d**, were obtained in good yields. Next, **4** was attached to the FMP resin by reductive amination (6 equiv of $NaBH(OAc)_3$ in DCE with 1% AcOH) via its amino group, which was concurrently converted to the resin-bound, secondary amine **5**. Subsequently, acylation of the amines was efficiently carried out with the corresponding Fmoc-protected carboxylic acid chloride linker (**6a**−**c**, 5 equiv) with DIEA (10 equiv) in anhydrous CH_2Cl_2, giving **7**. Upon Fmoc removal (20% piperidine in DMF), the resulting resin was treated with a variety of commercially available building blocks (see Supporting Information). In general, acylation with acids (aromatic/aliphatic) was found to proceed cleanly with HBTU/HOBt, but for less reactive

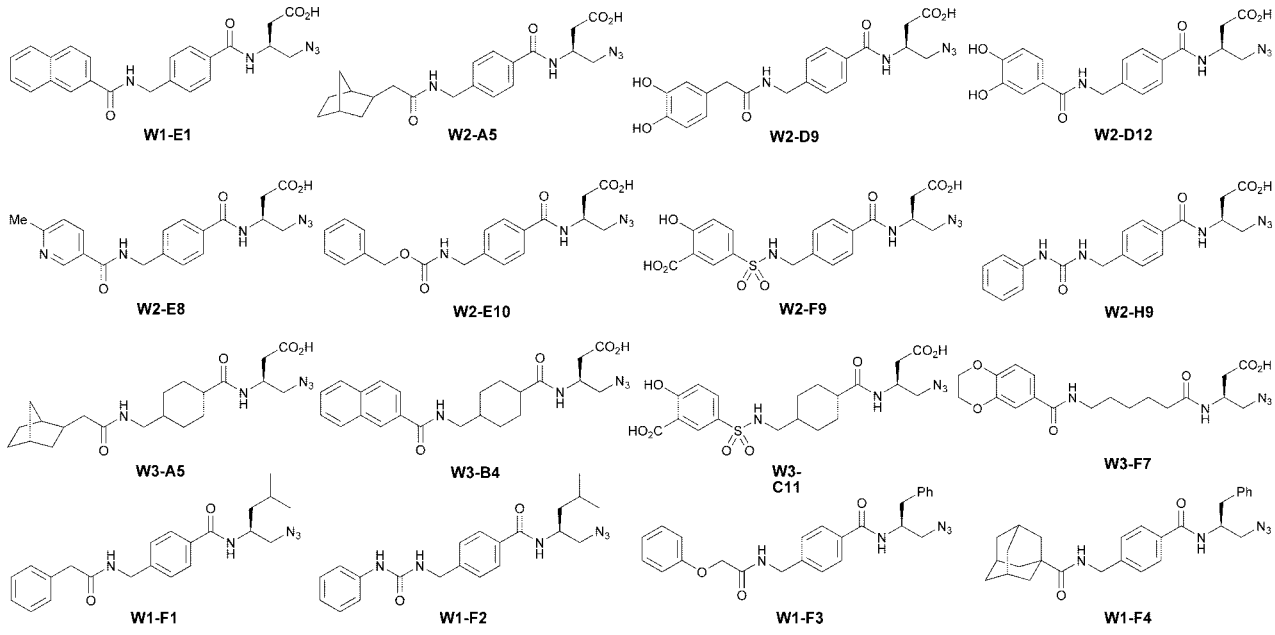


Figure 2. Representative structures of the 249-member azidomethylene library.

carboxylic acids, PyBOP/HOAt was shown to work better. Sulfonylation was found to proceed cleanly in DCM/DIEA with a catalytic amount of DMAP. Reactions with isocyanates proceeded smoothly in the presence of DIEA in DMF. Lastly, the cleavage was accomplished with a TFA/DCM/H_2O (5:4:1) mixture, affording the desired products as the corresponding amides, carbamates, sulfonamides, and ureas in good yields (based on a theoretical loading of 0.9 mmol/g). A variety of functional groups such as esters, nitriles, halogens, hydroxyl, nitro, and even salicylic acid, as shown in Figure 2, were easily tolerated.

To ensure crude products generated from our synthetic route were sufficiently pure for direct in situ screening, we characterized them by LCMS and NMR (Supporting Information); in most cases the desired produts were obtained with >90% purity and shown to be spectroscopically homogeneous (by NMR). It is also worth noting that, unlike what Fairlie et al. reported,[8b] we have NOT noticed any product degradation from selected compounds in our library even after 10-month storage (see Supporting Information).

We next screened the compounds for inhibiton against Caspase-1, -3, and -7 (Supporting Information); at 10 μM inhibitor concentration, while most compounds showed little or no inhibiton against Caspase-3 and -7, a few of them inhibited Caspase-1 fairely well (i.e., **W2-E1**, **W2-E2**, **W2−F6**, **W3-A2**). Therefore these compounds were further screened against Caspase-1 to obain their IC_{50} and K_i values. We were pleased to find that the most potent inhibitor, **W2-E1**, was able to inhibit Caspase-1 with IC_{50} and K_i values of 1.78 and 1.16 μM, respectively (Figure 3). The same compound showed much weaker inhibiton against Caspase-3 and -7 (IC_{50} > 200 μM and K_i values could not be accurately determined due to compound precipitation at higher concentrations). These values are moderate when compared to other potent Caspase-1 inhibitors.[6] It nevertheless indicates that methylene azides are indeed useful pharmacophores for future development of cysteine protease inhibitors.

In summary, we have developed an efficient and traceless solid-phase synthesis of azidomethylene derivatives as potential cysteine protease inhibitors. The strategy allows facile assembly of azidomethylene warheads with suitable nonpeptide linkers as well as a variety of readily available building blocks, all without the need of introducing extra functionalities in each of the components. Products generated were of uniformly high quality, enabling future direct in situ screening with a suitable biological target. Preliminary results indicate selected compounds are indeed moderate caspase inhibitors. This method should serve as a useful model for future development of other high-throughput amenable chemistry in the emerging field of catalomics.[1,12]

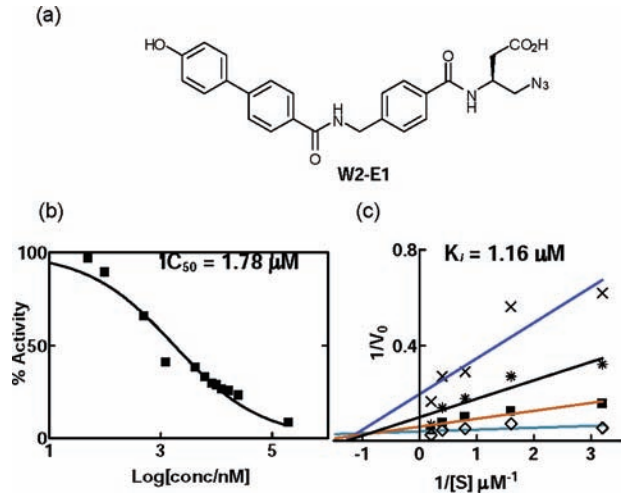


Figure 3. (a) Structure of **W2-E1**; (b and c) IC_{50} and K_i of **W2-E1** against Caspase-1, respectively. In panel c, the inhibitor concentrations are (×) 1250, (∗) 500, (■) 100, and (◊) 0 nM.

Acknowledgment. Funding support was provided by the National University of Singapore (NUS) and the Agency for Science, Technology and Research (A*STAR) of Singapore.

Supporting Information Available: Full characterizations of compounds and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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