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Small-Molecule Inhibitors of Protein Geranylgeranyltransferase Type I

Sabrina Castellano,† Hannah D. G. Fiji,† Sape S. Kinderman,† Masaru Watanabe,‡ Pablo de Leon,† Fuyuhiko Tamanoi,‡ and Ohyun Kwon*,†

Department of Chemistry and Biochemistry, Department of Microbiology, Immunology and Molecular Genetics, Jonsson Comprehensive Cancer Center and Molecular Biology Institute, University of California, Los Angeles, California 90095-1569

Received January 13, 2007; E-mail: ohyun@chem.ucla.edu

Although library approaches to the discovery of small-molecule enzyme inhibitors or receptor ligands are well-established,[1] many reactions continue to pose challenges when applied to solid-phase synthesis for the generation of compound libraries. From our development of phosphine catalysis of allenoates,[2] we envisioned that these reactions might be adaptable to solid-phase synthesis for the generation of heterocycle libraries using resin-bound allenoates. Before embarking on the potentially time-consuming development of solid-phase processes, however, we decided to screen our model compounds synthesized through solution-phase reactions. If we could identify a biologically important molecule from the preliminary screen, it would then be worthwhile pursuing a library generated through solid-phase split-pool synthesis. Herein, we report the first example of phosphine catalysis of polymer-bound allenoates and a combinatorial library approach to the development of potent inhibitors of protein geranylgeranyltransferase type I (GGTase-I).

Protein prenylation, a post-translational modification of nascent proteins by either the farnesyl or geranylgeranyl isoprenoid at the C-terminus cysteine residue, is a key event in the regulation of many protein functions.[3] Of particular interest is the farnesylation of the oncogenic forms of Ras proteins, which is required for their membrane association and cell transforming activity.[4] Constitutively activated mutant Ras proteins are found in ca. 30% of human tumors.[5] Consequently, the development of FTase inhibitors (FTIs) as anticancer agents has been the focus of much academic and industrial research.[6] Upon blocking FTase, however, the human oncogenic Ras isoform *K*-RasB is geranylgeranylated by protein GGTase-I.[7] Geranylgeranylation functionally substitutes the farnesylation of Ras proteins. This phenomenon suggests that to effectively block Ras processing, the development of selective inhibitors of GGTase-I (GGTIs) is required just as importantly as the development of FTIs.[8]

With this premise in mind, we screened a collection of 138 heterocycles[9] for their ability to inhibit the activity of human GGTase-I to geranylgeranylate K-Ras4B or RhoA. Purified GGTase-I was incubated with its substrate protein K-Ras4B or RhoA, [^{3}H]GGPP, and the 138 compounds. After 30 min, the degree of incorporation of tritiated geranylgeranyl groups was measured using a scintillation counter. We identified a number of compounds as GGTIs (Figure 1).

This discovery of promising lead GGTI compounds and their moderate activity warranted the development of efficient and rapid syntheses and evaluations of analogous structures in the search for better inhibitors; we envisioned a short, modular synthetic route (Scheme 1), using SynPhase lanterns as the solid support.[10] Validation of the synthetic route on the polymer support commenced with formation of resin-bound allenoates **5**. The loading of allenoic acids onto solid supports has not been reported previously. The allenoic acids **4** were coupled to the benzyl alcohol units of the SynPhase-PS lanterns grafted with Wang resin **3** using Mukaiyama's reagent and Hünig's base for **4a**/**b** or Et_3N for **4c**/**d**.[11] The direct use of an unmodified Wang resin minimizes the number of synthetic operations run on solid support. In addition, our strategy enabled simple trifluoroacetic acid (TFA)-mediated cleavage to release the carboxylic acid group, a key functional group in our GGTIs.

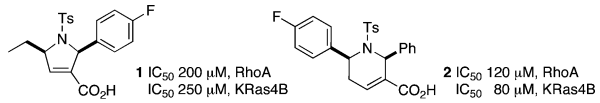
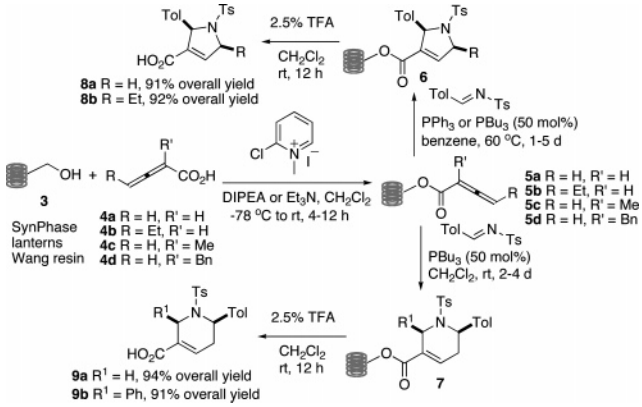


Figure 1. Protein GGTase-I inhibitors **1** and **2**.

Scheme 1. Solid-Phase Syntheses of Dihydropyrroles **8** and Tetrahydropyridines **9**

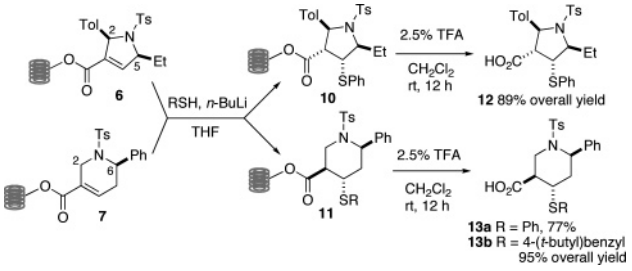


Because we were unaware of any previous examples of phosphine catalysis of solid-bound allenoates, we were pleased to discover that the phosphine-catalyzed annulation between polymer-supported allenoates **5** and *N*-tosylimines proceeded smoothly. The allenoates **5a** and **5b** were treated with *N*-tosyltolualdimine and 50 mol % of PPh_3 (for **5a**) or PBu_3 (for **5b**) in benzene at 60 °C to provide the polymer-bound dihydropyrroles **6**.[2b] Tetrahydropyridines **7** were formed from the reactions of **5c** and **5d** with *N*-tosyltolualdimine in the presence of 50 mol % of PBu_3 at room temperature for 2 and 4 days, respectively.[2a] Heterocycles **6** and **7** were cleaved from the resin using 2.5% TFA in DCM to provide the carboxylic acids **8** and **9** in 91−94% yield (based on a theoretical loading of 15 μmol/lantern) with high diastereoselectivities (dr = 99:1 for **8b**; 93:7 for **9b**) after chromatographic purification.

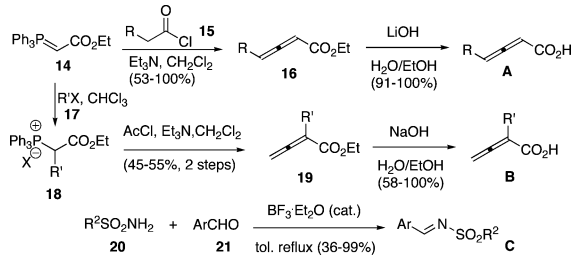
The α,β-unsaturated enoate functionalities in **6** and **7** were utilized to further increase the modularity and number of analogues. For example, the Michael additions of thiols to **6** and **7** using *n*-butyllithium as base[12] provided **10** and **11**, respectively, which

† Department of Chemistry and Biochemistry.
‡ Department of Microbiology, Immunology and Molecular Genetics.

Scheme 2. Solid-Phase Diastereoselective Michael Additions



Scheme 3. Building Block Preparation

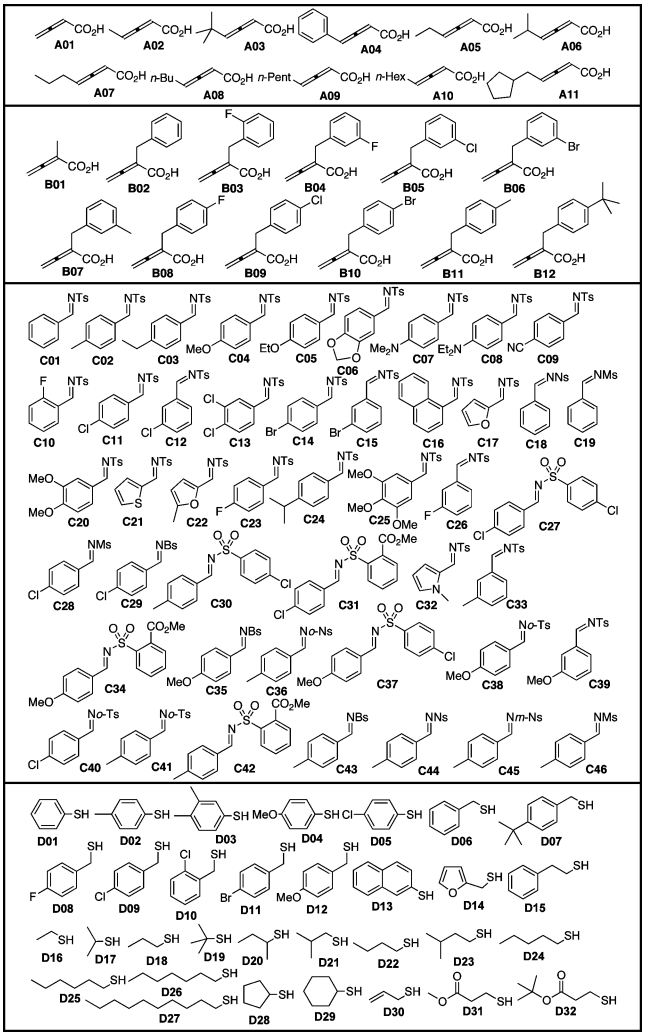


upon TFA-mediated cleavage yielded **12** and **13**, respectively, in 77−95% yield (Scheme 2). These two-step sequences occurred with high diastereoselectivities, providing the pentasubstituted pyrrolidine **12** and the tetrasubstituted piperidine **13** as single diastereoisomeric products.[13] Apparently, thiols added opposite to the pre-existing substituents in both the dihydropyrrole **6** and the tetrahydropyridine **7**.[15] Interestingly, however, the protonation of the resulting α-carbanions occurred anti (for **10**) and syn (for **11**) to the added *β*-mercapto groups.

Having successfully established the solid-phase reaction conditions, we next prepared the α- and *γ*-substituted allenoic acid building blocks (Scheme 3). The reactions between the phosphorane **14** and the acid chlorides **15** (1 equiv) in the presence of Et_3N (1 equiv) provided the allenoates **16**,[16] which were hydrolyzed into *γ*-substituted allenoic acids **A**.[17] Phosphorane **14** was treated with the alkyl halides **17** to give the phosphonium salts **18**, which we converted to the α-substituted allenoates **19** upon treatment with Et_3N (2 equiv) and acetyl chloride (1 equiv).[18] α-Substituted allenoic acids **B** were prepared through saponification of the esters **19**. The *N*-sulfonylimines **C** were formed simply through azeotropic removal of water from a mixture of the appropriate sulfonamide **20**, aldehyde **21**, and $BF_3{\cdot}OEt_2$ under reflux in toluene.[19] Chart 1 presents the building blocks synthesized as illustrated in Scheme 3 and the commercially available thiol building blocks **D**.

The building blocks were tested so that only the ones that provided high purity and stereoselectivity (as judged from ^{1}H NMR spectra and LCMS analyses) for their crude cleavage products would be used in the synthesis of the GGTI analogue library.[14] Eleven *γ*-substituted allenoic acids **A** and 12 α-substituted allenoic acids **B** were loaded and reacted with imine **C02** in the presence of a catalytic amount of phosphine, as indicated in Scheme 1. After cleavage with TFA and analysis (^{1}H NMR and LCMS spectra) of the purity and diastereoselectivity of the 23 annulation products **6** and **7**, we found that each of the allenoic acids, except for **A10**, yielded a single identifiable compound (dr ≥12:1; ^{1}H NMR) in high purity (72−100%; LCMS/UV210). The resin-bound allenoates derived from allenoic acids **A01**, **A05**, **B01**, and **B05** were selected to assess the reactivity and stereoselectivity of 46 imines in phosphine-catalyzed annulations (because **A01**, **A02**−**A11**, **B01**, and **B02**−**B12** required different annulation reaction conditions). Using the criteria of >70% purity and >9:1 dr, we selected 30 (of 46) imine building blocks for allenoate **A01**, 21 for **A05**, 25 for **B01**, and 31 for **B05**.

Chart 1. Eleven *γ*-Substituted Allenoic Acids **A**, 12 α-Substituted Allenoic Acids **B**, 46 *N*-Sulfonimines **C**, and 32 Thiols **D**



For Michael addition of the thiols, all three building blocks were tested as follows: To select suitable allenoic acids, the 23 annulation products synthesized above were subjected to Michael addition using benzenethiol, toluenethiol, or benzyl thiol. Analysis of the cleaved products indicated that eight (of 23) allenoic acids were suitable candidates. For imine selection, the annulation products of 46 imines with **A05** and **B01** were tested because the allenoic acids **A01**−**04** and **B02**−**B12** were excluded from the Michael addition sequence. The number of imines selected was 25 (of 46) for **A05** and 21 for **B01**. The annulation products **6** and **7** (Scheme 2) were used to select the thiols; the various thiols required different reaction times and temperatures. Upon extensive optimization, 19 (of 32) thiols for dihydropyrrole **6** and 17 for tetrahydropyridine **7** were selected. The combination of these selected building blocks resulted in the preparation of 4288 compounds.[14]

Using the chosen building blocks, we commenced the split-pool syntheses of the 4288 GGTI analogues on the SynPhase lanterns. Tagging was performed by inserting colored spindles and cogs into the lanterns prior to synthesis of the library.[14] Twenty-three allenoic acids **A** and **B** were loaded onto the Wang resin **3** using Mukaiyama's reagent (Scheme 1). The resulting allenoate-loaded lanterns **5** were pooled and split into a number of flasks corresponding to the number of imines for each group of allenoic acids

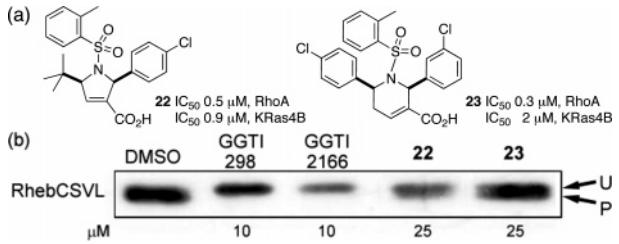


Figure 2. (a) GGTIs **22** and **23** that exhibited the highest potency. (b) Western blot of the cell (treated with **22** or **23** at 25 μM concentration) lysate qualitatively detecting the amount of geranylgeranylated Rheb. The descriptors P and U designate the processed and unprocessed Rheb, respectively.

(**A01**, **A02**−**A11**, **B01**, and **B02**−**B12**). Sets of 240 dihydropyrrole-loaded lanterns **6** and 366 tetrahydropyridine-loaded lanterns **7** were placed aside for cleavage. Sets of 3325 dihydropyrrole-bound lanterns **6** and 357 tetrahydropyridine-bound lanterns **7** were further divided into 19 and 17 flasks, respectively, and subjected to the thiol Michael reactions (Scheme 2).

The 4288 lanterns were inserted into 4288 vials and treated with 2.5% TFA in CH_2Cl_2 for 12 h; the lanterns were then removed and rinsed with CH_2Cl_2. The resulting solution was concentrated and further co-evaporated with $CHCl_3$ to effectively remove TFA. The cleaved compounds were weighed and redissolved in $CHCl_3$; a portion (2 μmol) of each compound was transferred into 54 96-well plates (80 compounds per well; two columns of wells in each plate were left empty to accommodate controls in subsequent assays), and the solvents were left to evaporate. The products were redissolved in DMSO and analyzed in the same assay for activity against GGTase-I.

In the in vitro assay, active compounds were sought for their ability to inhibit the geranylgeranylation of both RhoA and K-Ras4B. Figure 2a displays the two compounds (**22** and **23**) that exhibited the highest activities obtained so far. These compounds exhibit specific inhibition of GGTase-I; that is, they did not inhibit FTase at concentrations at which they inhibited GGTase-I by more than 90%.[14]

Finally, we investigated in vivo effects of **22** and **23**. Human embryonic kidney (HEK) 293 cells were transfected with the Rheb-CSVL construct that expresses the geranylgeranylated form of the Rheb protein.[20] Inhibition of the geranylgeranylation of this protein can be detected from a shift in its mobility on SDS polyacrylamide gel; the unprocessed form appears as a slowly migrating band. Figure 2b indicates that treatment of the cells with **22** or **23** resulted in the appearance of a slowly migrating band (cf. the DMSO lane with lanes **22** and **23**); known GGTIs (GGTI298, GGTI2166) were used as controls. These results suggest that compounds **22** and **23** inhibit geranylgeranylation within the cell. These dihydropyrrole and tetrahydropyridine-based GGTIs differ from the previously reported GGTIs, which, with the exception of Casey's GGTI-DU40, have been peptidomimetic compounds.[8]

In conclusion, small-molecule inhibitors of GGTase-I were identified through chemical genetic screens of the heterocycles produced through allene phosphine catalysis. This discovery instigated the development of the first solid-phase phosphine catalysis of resin-bound allenoates. To further improve the efficacy of the GGTIs and to explore their structure−activity relationships, 4288 GGTI analogues were synthesized on SynPhase lanterns in a split-pool fashion. Screening the 4288 analogues resulted in the identification of GGTIs **22** and **23** having submicromolar IC_{50} values. These powerful GGTIs should be useful for studies of the protein geranylgeranylation process and might ultimately lead to novel therapeutic leads.

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Supporting Information Available: Representative experimental procedures and spectral data for all new compounds (PDF). ^{1}H NMR spectroscopic and LCMS data for 251 library members (PDF). Crystallographic data for compounds **12** and **13a** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) The structures of 138 compounds are provided in the Supporting Information. For the synthesis of the compounds, see ref 2.
(10) The SynPhase lantern consists of a mobile surface polymer (e.g., polystyrene) grafted onto a rigid and unreactive base polymer of cylindrical shape. The rigid polymeric support beneath the grafted mobile phase makes weighing unnecessary and handling easier than that of resins. The SynPhase lanterns are available in three different sizes, with loadings of 15, 35, and 75 μmol, providing more material for a given compound than beads when used in a split-pool library synthesis. Nonchemical tagging methods, such as radiolabeling (similar to the IRORI system) or color-coding using colored spindles and cogs, are available, alleviating the need for chemical encoding.
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(13) The relative stereochemistry of **12** and **13a** was confirmed through X-ray crystallographic analyses.[14]
(14) See the Supporting Information for details.
(15) Dihydropyrroles **6** with C5-H, C5-Me, and C5-Ph substituents provided mixtures of diastereoisomers. On the other hand, dihydropyrroles with C5-*t*-Bu substituents were recalcitrant to the 1,4-addition conditions. For the tetrahydropyridines **7**, only those lacking a C2 substituent underwent the 1,4-addition of the thiol. Remarkably, the remote C6 substituent directed the Michael addition from the opposite side.
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