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A Combinatorial Scaffold Approach toward Kinase-Directed Heterocycle Libraries

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With the large number of novel proteins being identified from genomics, proteomics, and traditional biochemical approaches there is a tremendous need to develop more efficient methods for the discovery and optimization of small molecule ligands that can be used to elucidate the function of these proteins. Not surprisingly, many of these new targets come from protein families that have received considerable attention[1] in the past such as GPCRs, proteases, and kinases. This presents the combinatorial chemists with the opportunity to take scaffolds developed against a particular protein family member and develop generalized synthetic schemes that allow other family members to be selectively targeted.

A survey of the literature[2] reveals that the vast majority of kinase inhibitor scaffolds consist of planar heterocycles that present both key hydrogen bond donating/accepting functionality and proper hydrophobicity (Figure 1). A particularly impressive example is the development of a wide array of scaffolds including substituted pyridinylimidazoles, indoles, pyridinyloxazoles, and pyridinylpyrazoles that all inhibit a key mitogen activated kinase, p38.[2] While a number of combinatorial methods for the construction of heterocyclic scaffolds have appeared, most have focused on a single scaffold. Here we demonstrate a simple synthetic approach whereby the heterocyclic scaffold is treated as a diversity element thereby enabling the synthesis of diverse heterocycle libraries, many members of which have been demonstrated to inhibit a wide variety of kinases.

Our general strategy involves the capture of a dichloroheterocyclic scaffold (including substituted purines **S1**, pyrimidines **S2**, quinazolines **S3**, pyrazines **S4**, phthalazines **S5**, pyridazines **S6**, and quinoxalines **S7**) where one chloro group is susceptible to nucleophilic aromatic substitution with a resin-bound amine nucleophile. Depending on the type of heterocycle being captured, an initial alkylation, acylation, or coupling reaction can be performed prior to the capture step to introduce one diversity element.[3] The remaining chloro substituent is then available for nucleophilic displacement or a palladium-mediated coupling reaction with anilines, phenols, and boronic acids (Scheme 1).

We initially chose to explore this heterocycle capture strategy using 2,6-dichloropurine because the 6-chloro can be selectively displaced by amines and the 2-chloro has been demonstrated to function in palladium-mediated coupling reactions in solution.[4] To obtain a resin-bound nucleophilic amino group, primary amines were coupled to a (4-formyl-3,5-dimethoxyphenoxy)methylpolystyrene resin (PAL-resin) by reductive amination using sodium triacetoxyborohydride with 1% acetic acid to afford the PAL-amine resin.[5] The PAL linkage offers the advantage that functionalized amines can readily be installed and cleavage results in an NH group

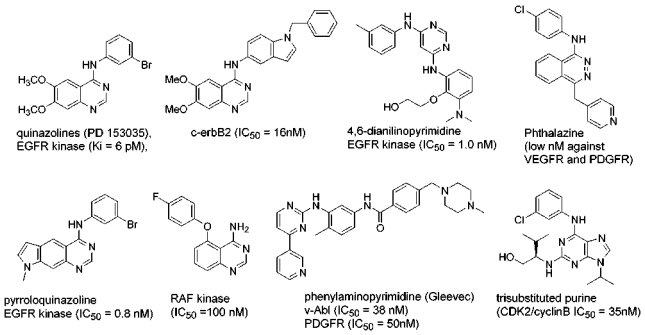

Figure 1. Diverse kinase inhibitor scaffolds.

that serves as a key hydrogen bond donor to many kinase active sites. The sequence starts by loading 2,6-dichloropurine onto the PAL-amine at the more reactive C6 position in butanol at 80 °C with exclusive regioselectivity. Modification of the N9 position of purine can be achieved by either Mitsunobu alkylation of N9 on solid support (Path B) or by capturing the product of a solution phase Mitsunobu alkylation of 2,6-dichloropurine (Path A, Scheme 2). The later approach offers the advantages of using less reagents and ease of handling. However, alkylation on solid support provides improved regioselectivity (N9/N7), presumably because N7 is more sterically hindered due to the presence of a large substituent at C6. Having the flexibility to perform the alkylation either on support or in solution offers different operational advantages when making large combinatorial libraries. Most primary and secondary alcohols lacking additional acidic hydrogens have previously been shown to afford high yields in the Mitsunobu reaction at N9.[6]

As illustrated in Scheme 2, a palladium-catalyzed cross coupling reaction was performed as the final derivatization step with use of 5 equiv of the coupling partner (arylboronic acids, anilines, or phenols), 7 mol % of $Pd_2(dba)_3$, 14 mol % of the corresponding ligand,[7] and 6 equiv of the base for 12 h at 80 °C with 1,4-dioxane for C−C and C−N bond formation or toluene for C−O bond formation as solvent. To explore the scope of the palladium-catalyzed cross-coupling reaction, resin-bound purine **3** (X = Cl, Y = H, Scheme 2) was reacted with a variety of arylboronic acids, anilines/amines, and phenols (Table 1). Analysis of the products following TFA-mediated cleavage by LC-MS revealed greater than 95% conversion with a variety of electron-rich or -poor aromatic ring systems. The amination reaction proved to be the most versatile with diverse substrates ranging from primary and secondary anilines to a sterically hindered primary amine (2-amino-3-methylbutanol) and cyclic/acyclic secondary amines (Table 1).

Encouraged by the efficiency of palladium-catalyzed C2-couplings on solid support, we next sought to extend the reaction

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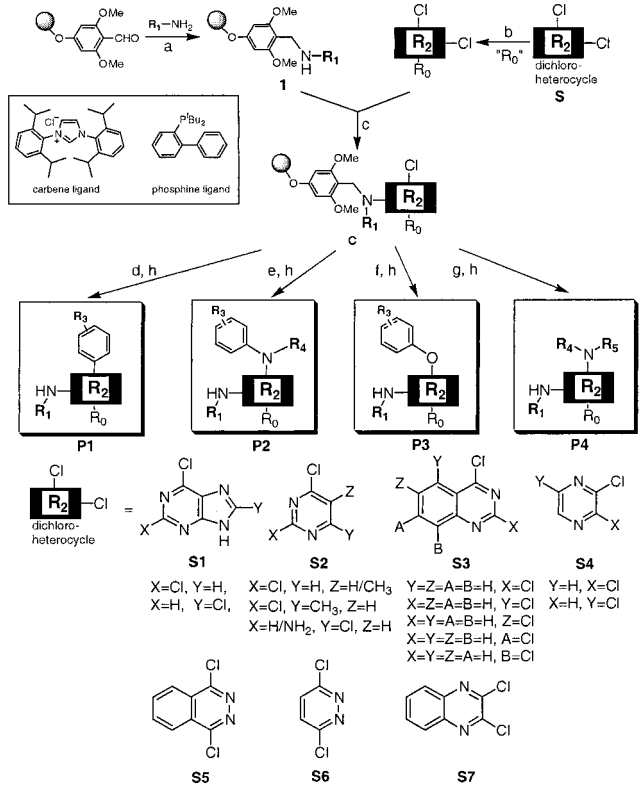
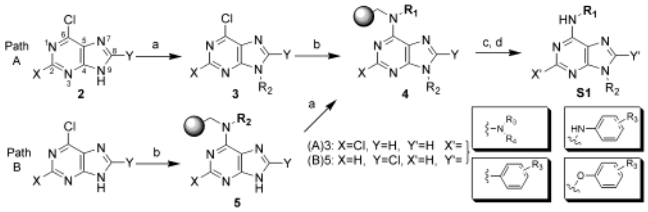
Scheme 1



[a] $NaBH(OAc)_3$, 1% HOAc, THF. [b] Solution phase heterocycle modification: alkylation or acylation. [c] DiEA, BuOH, 90 °C, 24 h. [d] 5 equiv of boronic acids, 7% of $Pd_2(dba)_3$, 14% of carbene ligand, 6 equiv of Cs_2CO_3, 1,4-dioxane, 90 °C, 12 h. [e] 5 equiv of anilines, 7% of $Pd_2(dba)_3$, 14% of carbene ligand, 6 equiv of KOtBu, 1,4-dioxane, 90 °C, 12 h. [f] 5 equiv of phenols, 7% of $Pd_2(dba)_3$, 28% of phosphine ligand, 7 equiv of K_3PO_4, toluene, 90 °C, 12 h. [g] 5 equiv of 1° or 2° amines, 90 °C, 12 h. [h] CH_2Cl_2:TFA:Me_2S:H_2O/45:45:5:5.

Scheme 2

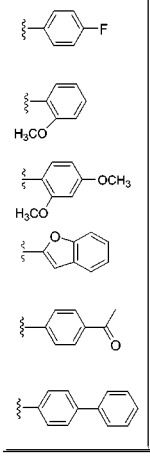
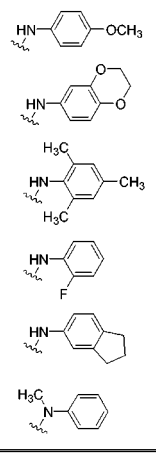
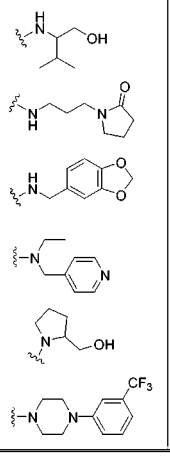
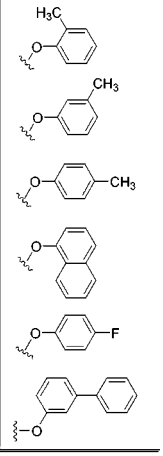


[a] 1.5 equiv of R_2OH, 2 equiv of PPh_3, 1.3 equiv of DiAD, THF, room temperature. [b] 0.5 equiv of 1, 1.5 equiv of DiEA, BuOH, 80 °C. [c] Please refer to conditions *d*, *e*, *f*, and *g* detailed in Scheme 1. [d] CH_2Cl_2:TFA:Me_2S:H_2O/45:45:5:5.

to C-8 bromo- or chloro-substituted purines. The C-8 bromo/chloro-substituted purines can be prepared by lithiation of the C8 position of 6-chloro-9-tetrahydropyranyl or 2,6-dichloro-9-tetrahydropyranyl purine with LDA followed by quenching with appropriate halogen donors.[8] The tetrahydropyranyl protecting group can be removed by treatment with 10% acetic acid in methanol. After Mitsunobu alkylation of N9 and resin capture at C6, the support-bound purines can be modified at C8 or C2 and C8 simultaneously by using palladium-mediated cross coupling reactions as described above (Scheme 2). This chemistry provides access to generic 6−5−6 triaryl systems that represent a large class of bioactive pharmacophores.[9]

To assess the versatility of this heterocycle resin-capture strategy, a collection of dichloro-heterocycles were tested for their ability to be coupled to PAL-amine resin. The general resin capture condition involves reacting 2 equiv of dichloro-heterocycles with

Table 1. Validations of Purine C2 Substitution via Palladium-Catalyzed Cross Coupling Reactions

Boronic Acids	Anilines	Amines	Phenols
			

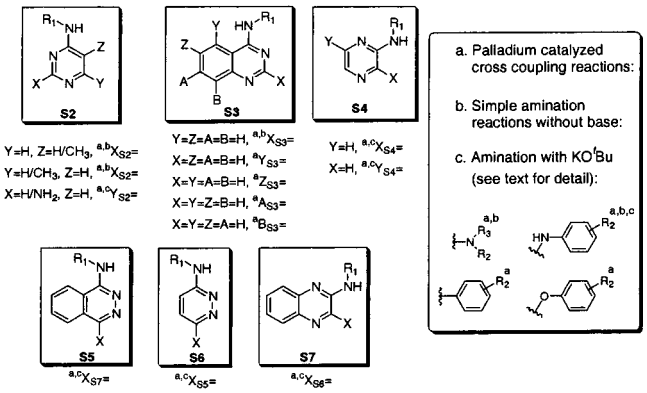
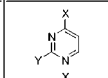
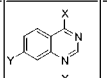
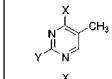
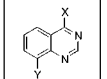
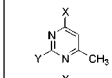
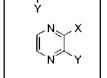
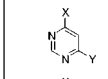
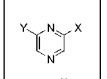
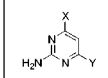
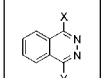
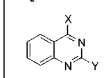
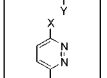
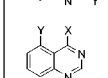
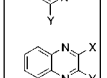
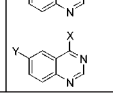


Figure 2. Diverse heterocycles constructed by the combinatorial scaffold approach.

PAL-amine in the presence of 3 equiv of diisopropylethylamine at 90 °C in butanol for 24 h. Because the resin-capture presumably proceeds through a nucleophilic aromatic substitution mechanism, only certain electron-poor dichloro-heterocycles could be loaded on solid support quantitatively with use of the reaction condition described above. These include all the heterocycles shown in Scheme 1. Relatively less electron poor dichloro-heterocycles, such as pyridine and indole analogues, did not react with PAL-amine resin effectively under these conditions. While heterocycles such as 2,4-dichloropyrimidine can be captured at room temperature with high efficiency, other heterocycles including S4 to S7 required more forcing conditions (90 °C, *n*-butanol, 24 h, quantitative loading). For scaffolds where regioselectivity is an issue only 2,4-dichloropyrimidine resulted in a regioisomeric mixture with some resin-bound amines. For example, PAL-resin-bound primary amines capture 2,4-dichloropyrimidine exclusively at the C4 position, while PAL-anilines resulted in an approximately 1:1 mixture of C2/C4 capture products.

Having ascertained the types of dichloro-heterocycles that can be captured on solid support we next explored substitution of the remaining chloro substituent (Scheme 1 and Figure 2). While palladium-catalyzed cross-coupling reactions offer the most versatility in terms of substrate structure, such reactions usually require inert conditions, limiting their use in spatially separate 96-well reaction format. Therefore nucleophilic aromatic substitution conditions were investigated first. The less reactive C2-chloro group of pyrimidine (S2) and quinazoline (S3) was found to react with various amines quantitatively at high concentration (>2 M) for 12

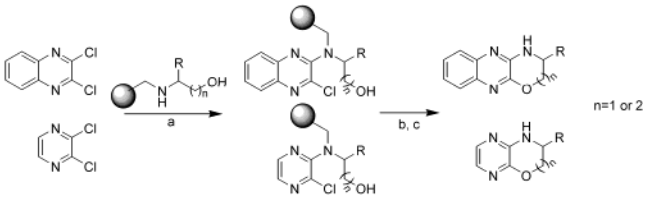
Table 2. Validation of Heterocyclic Scaffolds as Diversity Inputs

Entry	Scaffolds (R_2)	(Y_1) Purity & Yield (%)	(Y_2) Purity & Yield (%)	(Y_3) Purity & Yield (%)	Entry	Scaffolds (R_2)	(Y_1) Purity & Yield (%)	(Y_2) Purity & Yield (%)	(Y_3) Purity & Yield (%)
1		95 (90)	96 (90)	92 (86)	9		91 (82)	95 (89)	95 (89)
2		95 (91)	95 (91)	91 (85)	10		93 (83)	93 (85)	92 (88)
3		96 (91)	96 (90)	93 (88)	11		95 (85)	93 (86)	96 (88)
4		93 (89)	95 (90)	96 (88)	12		94 (84)	94 (89)	91 (84)
5		91 (85)	88 (82)	90 (83)	13		92 (85)	92 (84)	90 (83)
6		95 (90)	94 (89)	93 (89)	14		91 (84)	92 (87)	91 (86)
7		86 (79)	89 (78)	85 (78)	15		93 (84)	88 (81)	91 (83)
8		92 (85)	93 (86)	94 (85)					

h at 100 °C. It was also discovered that less nucleophilic anilines reacted with the C2 chloro group of pyrimidine scaffold (among all other scaffolds shown in Figure 2) more readily (0.2 M at 80 °C) than alkylamines, presumably proceeding through an auto and acid-catalyzed substitution reaction.[10] The C6-chloro group of pyrimidine and the second chloro group of scaffolds S4 through S7 can react quantitatively with anilines in the presence of a stoichiometric amount of potassium *tert*-butoxide as the base.

As only a small subset of the heterocycles could be modified under a standardized nucleophilic aromatic substitution condition, we next investigated the use of palladium-catalyzed cross-coupling reactions on solid support. With $\mathbf{R_1}$ fixed as *p*-methoxybenzylamine (Table 2), the remaining chloro group of the resin-captured heterocyclic scaffolds was subjected to a palladium-catalyzed cross coupling reaction as the final derivatization step. The reaction conditions are essentially the same as described for the purines. These conditions were found to be most general for modifying the resin-captured heterocycles (Table 2) and generally afforded quantitative conversion of the starting material (the remaining chloro group) with different substrates on the solid support. Exploration of the reaction scope revealed the same broad range of substrates can be used as demonstrated for the purine scaffold (Table 1). A survey of resin-bound amines also showed considerable diversity in primary amines that are effective substrates. Only β-branched resin-bound primary amines were found to have relatively slow capture rates. Interestingly, the use of resin-bound amino alcohols, such as ethanolamine derivatives, gave intramolecular cyclization products for 2,3-dichloroquinoxaline and 2,3-dichloropyrazine under the palladium-catalyzed C−C or C−O bond formation conditions to form six- or seven-membered ring systems[11] (Scheme 3).

Scheme 3



[a] DiEA, n-BuOH, 90 °C, 24 h. [b] 7% of $Pd_2(dba)_3$, 14% of carbene ligand, 7 equiv of Cs_2CO_3, 1,4-dioxane, 80 °C, 12 h. [c] CH_2Cl_2:TFA:Me_2S:H_2O/45:45:5:5.

In summary, a general method for the solid phase synthesis of various substituted heterocycles has been demonstrated. Alkylated purines chlorinated at the 6,8 or 2,6,8 positions and various dichloroheterocycles can be captured onto solid support and further elaborated by aromatic substitution with amines at elevated temperature or by anilines, boronic acids, and phenols via palladium-catalyzed cross-coupling reactions. Libraries consisting of 45 140 discrete and highly diverse heterocyclic small molecules constructed with use of these chemistries are currently being evaluated in a variety of cell and protein-based assays.

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Supporting Information Available: Detailed experimental procedures and spectra data of the compounds disclosed in this paper (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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