Synthesis and Phenotypic Screening of a Guanine-Mimetic Library

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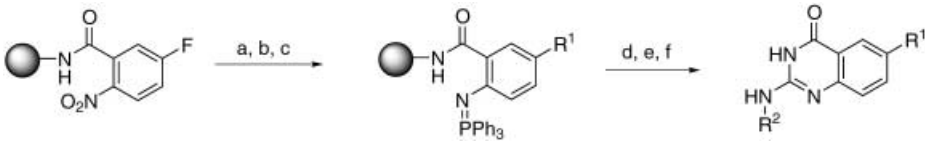
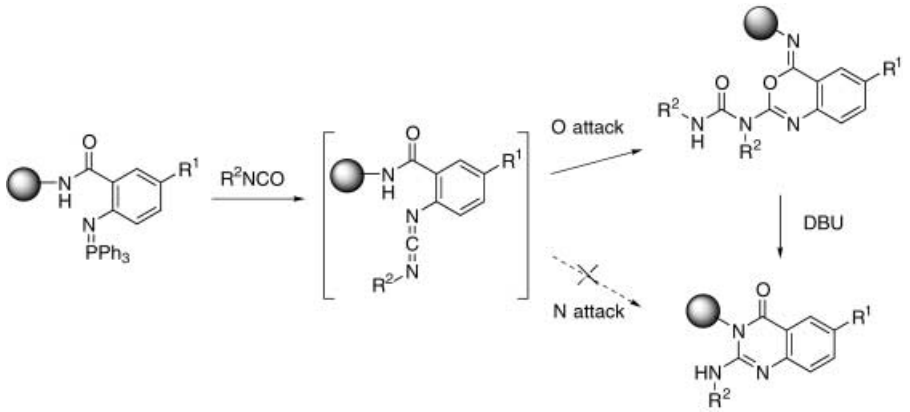
There has been considerable recent success in the specific inhibition of cellular kinases with adenine analogues,[1] but little attention has been paid to proteins that bind guanine nucleotides.[2] Guanine-derived small molecules play important roles in many aspects of cellular function. In particular, guanine, guanosine and its phosphorylated forms (cGMP, GMP, GDP, GTP), and the guanine-derived pteridine cofactors, such as folate and tetrahydrobiopterin, are all important endogenous small molecules. Proteins that bind guanine and its derivatives control a wide variety of cellular processes, and compounds that disrupt this binding would be valuable research tools as well as potential pharmaceuticals.

As a first step to generate cell-permeable guanine-mimetics, we have synthesized a split-pool library of 270 6-substituted-2-amino-4(3*H*)-quinazolinones (Scheme 1). Three key features of our aza-Wittig-mediated solid-phase synthesis distinguish it from all others.[3,4] First, we synthesized iminophosphoranes from aryl amines rather than azides; this dramatically increased the number and variety of accessible compounds. Second, our key ring-forming step involves intramolecular attack of a carbodiimide by an amide rather than cyclocleavage of a guanidinium ester. This avoids the potential formation of two isomeric products, and allows us to employ conditions that would cause premature cleavage of an ester linkage. Finally, all library members lack 3-substitution, and thus display the distinctive hydrogen-bond pattern of guanine. Most proteins that bind guanine nucleotides or the guanine-derived pteridine cofactors specifically recognize this moiety through bidentate hydrogen bonds to an Asp or Glu residue in a hydrophobic pocket.

Diversity in the scaffold was first introduced by nucleophilic aromatic substitution of a resin-bound 5-fluoro-2-nitrobenzamide with a variety of thiols, phenols, and primary alcohols. Following nitro reduction, all attempts to generate the iminophosphorane from the aryl amine under conditions reported in the literature were unsatisfactory. Finally, we discovered that we could selectively and efficiently form the iminophosphorane with Ph_3P/Cl_3CCCl_3/imidazole (3 h, 4 °C). Aza-Wittig reaction of the iminophosphoranes with one of 15 isocyanates yielded the carbodiimide, which was attacked intramolecularly by the amide (Scheme 2). Instead of the desired *N* attack to form the aminoquinazolinone, we observed *O* attack with formation of a 4-imino-4*H*-3,1-benzoxazine and the incorporation of a second isocyanate equivalent.[5] Attempted isomerization of the benzoxazine to the desired product by heating under reflux in toluene, as reported by Molina et al.,[5] worked in solution but gave very poor yields on solid support. After much experimentation, we found that the addition of DBU to the benzoxazine at room temperature promoted rapid isomerization to the desired product.[6] Cleavage from the 500μ Rink amide resin with TFA completed the "traceless" synthesis of these heterocycles and provided a copious amount of each compound for screening.



Scheme 1. *Synthesis of the guanine-mimetic library. Reagents and conditions: a) R^1OH, KOtBu, DMA or R^1SH, DBU, CH_2Cl_2, 12 h; b) 2 M $SnCl_2 \cdot 2H_2O$ in NMP, 12 h; c) Ph_3P, Cl_3CCCl_3, imidazole, CH_2Cl_2, 4 °C, 3 h; d) R_2NCO, CH_2Cl_2, 12 h (R^2 = aryl) or $PhCH_3$, 80 °C, 12 h (R^2 = alkyl); e) DBU, DMA, 12 h; f) TFA/CH_2Cl_2 (1:1), 2 h. DMA* = N,N-*dimethylacetamide; DBU* = *1,8-diazabicyclo[5.4.0]undec-7-ene; NMP* = N-*methylpyrrolidinone.*



Scheme 2. *Intramolecular attack of the amide on the carbodiimide forms the benzoxazine, which can be isomerized to the aminoquinazolinone.*

LC/MS analysis of a portion of the library compounds confirmed that most 2-arylaminoquinazolinones were synthesized in good purity (> 80 %). Minor impurities were mostly due to thiol oxidation. However, the purity of 2-alkylaminoquinazolinones was only modest (40–80 %). The primary impurity result-

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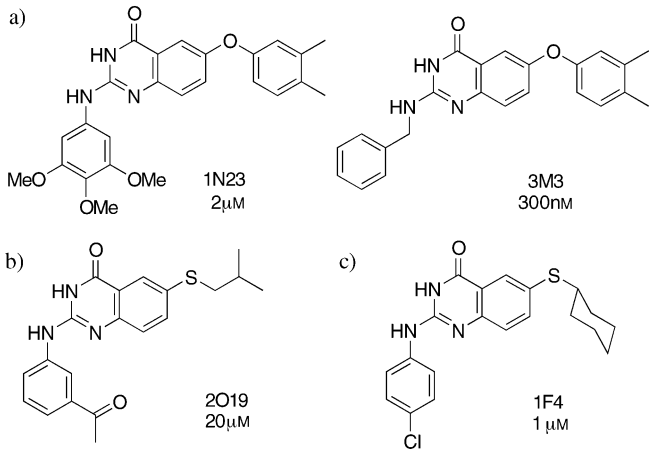
ed from apparent partial hydrolysis of the intermediate benzoxazine.

We screened the library for biological activity on mammalian cells in culture using fluorescence microscopy (estimated concentration ~50 μM). The key to the success of any guanine-mimetic in vivo is solubility, cell-permeability, and selectivity. Guanine itself lacks all three attributes. Happily, since all of our compounds are fluorescent, it was easy to determine that most of our compounds are indeed soluble and cell-permeable.

We chose two broad screens that would reveal disruptions of cell biological processes involving guanine-nucleotide binding proteins. First, to assess the effect of our library compounds on the cytoskeleton and cell cycle progression, we incubated compounds with BS-C-1 (monkey) cells[7] for six hours, followed by fixing and staining for actin, DNA, and microtubules. The most common phenotype was mitotic arrest caused by microtubule depolymerization. Many of the most potent depolymerizers contained a 3,4,5-trimethoxyphenyl group, a common motif among ligands binding in the colchicine-site of tubulin.[8] Curiously, compounds with a benzyl group in lieu of the trimethoxyphenyl ring, for example, **3M3**, were even more potent, and also active on pure tubulin (Scheme 3).



Scheme 3. *Some of the bioactive molecules identified and retested after purification and characterization: a) microtubule depolymerizers; b) disrupter of actin and microtubules; c) compound causing mislocalization of internalized transferrin receptor. Minimum effective concentration shown.*

We also assessed the ability of these compounds to disrupt cellular trafficking. Endocytosis of the transferrin receptor normally proceeds through a perinuclear compartment, the recycling endosome. A number of compounds, exemplified by **1F4**, caused transferrin to accumulate at the tips of cells rather than perinuclearly. Compounds that disrupted exocytosis and Golgi structure were also identified.

Most interesting was the discovery of a small molecule, **2O19**, which disrupted both the actin and microtubule cytoskeleton, but did not arrest cells in mitosis (Figure 1). The disruption of actin and microtubules possibly results from action at a single target, since no structurally similar compounds in this library gave rise to a phenotype that dissociated these two effects.

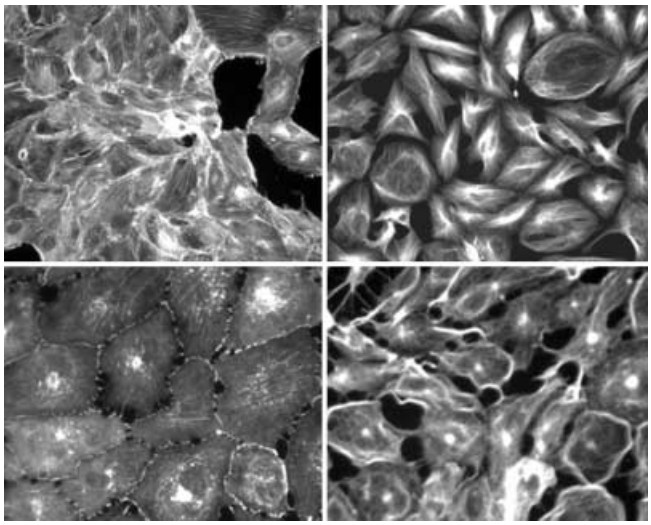


Figure 1. *BS-C-1 cells were treated with TRITC-phalloidin and FITC-DM1α. Top: actin (left) and microtubules (right) in untreated cells. Bottom: actin (left) and microtubules (right) in cells treated with 25 μM 2O19 for 4 h.*

We have identified several putative molecular targets of **2O19** by affinity chromatography from HeLa-cell extract.[9] Notably, no kinases were found to bind. On the other hand, we were happy to find that a prominent bound protein is sepiapterin reductase (SR), the final enzyme in the synthesis of tetrahydrobiopterin. The endogenous substrate for SR is derived from GTP and contains the same hydrogen-bond pattern as guanine.[10] Like most guanine nucleotides, this moiety is recognized by bidentate hydrogen bonds to an aspartate in a hydrophobic pocket. Although we do not believe SR is the phenotypic target of **2O19**, the binding of this protein demonstrates the ability of our library compounds to discriminate between adenine- and guanine-like binding sites. Additionally, the recent report of an aminoquinazolinone-based inhibitor of tRNA-guanine transglycosylase, also recognized by bidentate hydrogen bonds to an aspartate, further demonstrates the suitability of these compounds as guanine mimetics.[11]

Although adenine analogues have been widely used to target kinases and other proteins, guanine-nucleotide binding sites have not been fully exploited by cell-permeable small molecules. The synthetic methodology we describe opens the door to the synthesis of a broad array of different guanine-mimetics. Although modest in size, the unique 270-member guanine-mimetic library reported here nonetheless yielded a surprising number of distinct and interesting bioactive compounds. These compounds should prove to be useful probes of cellular processes. Further work to identify the phenotypic targets of bioactive hits from our library and to synthesize larger and more diverse libraries of guanine-mimetics is currently underway.

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