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Synthetic and computational studies on liphagal: a natural product inhibitor of PI-3K

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ABSTRACT

The natural product liphagal has been shown to function as a reasonably potent and selective inhibitor of the key signaling enzyme PI-3Kα. We have been interested in developing an analog class of PI-3K inhibitors based upon this unusual terpenoid natural product. Toward that end, we have evaluated the binding of the natural product to its target protein computationally and formulated a class of simplified analogs based on the structural analysis. Utilizing the cycloadduct derived from tetrabromocyclopropene and furan, we were able to generate a key, versatile scaffold upon which to pursue this analog design.

The enzyme phosphatidylinositol-3-kinase (PI-3K) is an important mediator of key cell signaling cascades and functions by transferring a phosphate from ATP to a hydroxyl function on the cyclitol head group of phosphoinositides.[1] Dysregulation of PI-3K and other regulators of this pathway have been linked to a wide variety of malignancies and as such, there has been considerable interest in the development of effective inhibitors of the enzyme.[2] PI-3K is found in several different isoforms and the development of isozyme-selective inhibitors is considered to be an important step in the development of highly effective therapeutic agents.[3]

Recently, a new natural product inhibitor of PI-3K was isolated from marine sources and named liphagal.[4] This interesting terpenoid is structurally related to the frondosins[5] which have been identified as inhibitors of the serine/threonine kinase protein kinase C (PKC) (Fig. 1).

Liphagal was shown to be a relatively potent inhibitor of PI-3Kα (IC_{50} = 100 nM) and, interestingly, showed a 10-fold selectivity over the inhibition of PI-3Kγ as well as antiproliferative effects against several cancer cell lines. We have been interested in the development of isozyme-selective inhibitors of PI-3K and feel that this natural product may serve as an excellent template for new discovery efforts. In this Letter, we wish to report on both computational and synthetic studies[6] of this natural product that will serve as the basis for the development of new PI-3K inhibitors.

As our ultimate interest is in the development of inhibitors with greater levels of potency and selectivity, we wanted to utilize the rich structural information available concerning PI-3K in complex with various inhibitors to help guide the design of new analogs

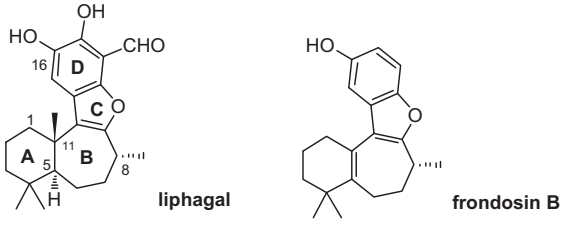

Figure 1. Structures of terpenoid kinase inhibitors.

based on this natural product. In order to assess the interactions of liphagal with PI-3K, we first generated an accurate docking model of PI-3K bound to compounds with measured inhibition values.[7] Nine inhibitors of PI-3K: liphagal, wortmannin, LY294002, quercetin, staurosporine, myricetin, 039, 090, 093, and QYT were flexibly docked using Surflex-Dock as implemented by Sybyl 7.2[8] to crystal structures of PI-3K[9] with and without ordered water molecules in the active site. For wortmannin, a covalent inhibitor, three forms with different representations of the electrophilic furan (intact, ring-opened, and a surrogate form replacing C21 with a hydrogen-bond accepting keto-oxygen to simulate the covalent bond with Lys 833) were docked. Scoring was based on hydrophobic, polar, electrostatic repulsive, entropic, and solvation terms.[10] The docking results were assessed for correct orientation of the ligands in the active site and relative docking scores as compared to experimentally measured inhibition values.

The orientation of the ligand in the active site, which determined the number of possible satisfied hydrogen bonding interactions, is an important contributor to the docking score. The docking model based on the structure of PI-3K bound to wortmannin

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(1E7U[9]) yielded the most accurate results. This model shows the correct co-crystallized ligand placement, the highest number of total ligands oriented correctly in the active site, and proper ranking of wortmannin, with the highest docking score, relative to the other and less potent ligands. The inclusion of the ordered water molecules did not affect the results. Figure 2 shows the docked structure of liphagal to the kinase.

Using this model, it is possible to interpret the many interactions of liphagal with PI-3K. Liphagal forms lipophilic interactions with Ile963, Ile879, the aromatic region of Tyr867, Ile831, Ile881, Val882, and Trp812. The cyclohexyl A-ring projects up from the aromatic plane and into the upper pocket of the lipophilic region. The polar region consists of several hydrogen bonds made between the heteroatom rich aromatic D-ring and Lys833, Asp964, Lys807, and Ser806 (Fig. 3).

Based on our computational studies, we envisioned a generalized tricyclic scaffold that would give access to both the polar and hydrophobic regions of the enzyme while maintaining a relatively rigid core structure. This would require a modular synthesis whereby differentially substituted benzofuran units could be easily fused to a suitably functionalized seven-membered ring (Scheme 1).

We hoped to access this type of intermediate from the oxa-bridged enone **1**, a readily available and highly flexible compound with which we have worked for several years[11] and one that can be prepared in enantiomerically pure form.[12] Key to utilizing this intermediate would be the development of a direct method for annulations of the fused benzofuran unit, stereoselective installation of the secondary methyl group on the cycloheptyl ring, and facile opening of the bridging ether atom.

The initial studies began with developing a straightforward method to annulate a benzofuran heterocycle on to the bridged synthon **1** by taking advantage of the differential reactivity of the two halogen atoms in the dibromoenone function.[13] It was anticipated that the increased reactivity of the β-bromide would allow an effective sequential metal-mediated cross-coupling reaction with a suitable aromatic partner. As a model unit, we studied the Suzuki coupling of 2-hydroxyphenyl boronic acid with the dibromoenone. The coupling proceeded exclusively to deliver the phenol **2** in good yield under the optimized conditions (Scheme 2).

The ability to directly cross-couple the free phenol to the dibromoenone set the stage for a second metal-mediated coupling to close the benzofuran ring. Exposure of the phenol to stoichiometric copper(I) iodide effected a high yielding Ullman-type coupling to give **3**. The regiochemical outcome of the annulation was confirmed by crystallographic analysis of ketone **3** (Fig. 4).

This direct, two-step method for the annulation of a benzofuran onto the dibromoenone should allow access to a wide variety of

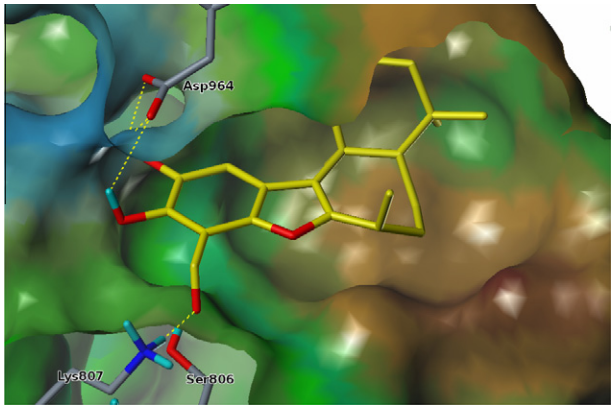


Figure 2. Docked complex of liphagal and PI-3K.

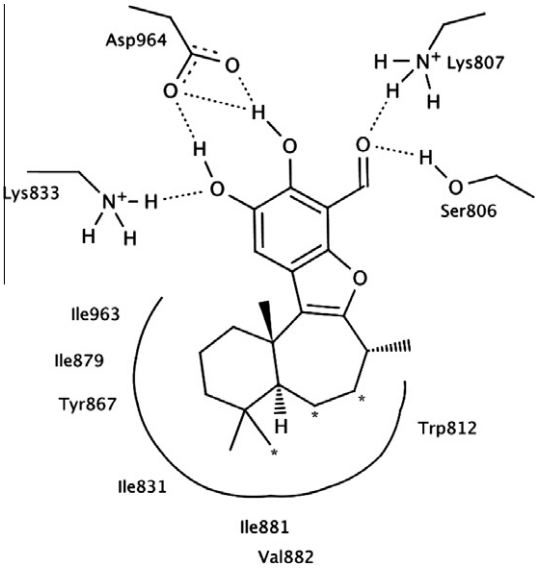
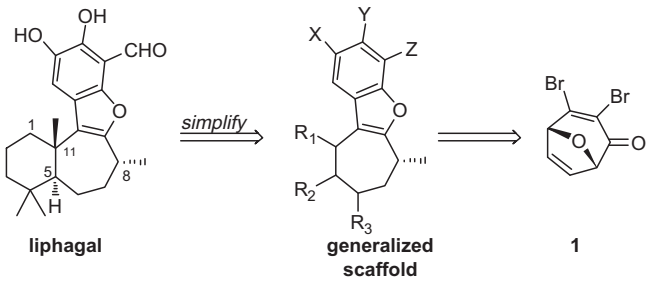
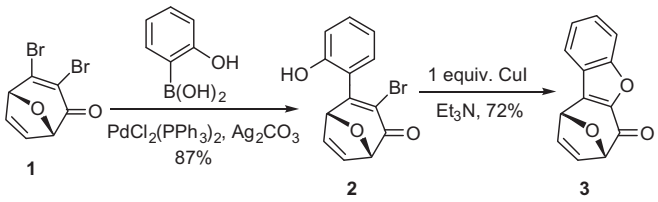


Figure 3. Interaction map of liphagal with PI-3K.



Scheme 1. Synthetic strategy for analog generation.



Scheme 2. Two-step benzofuran annulation.

analogs in the aromatic region. We were also pleased to see that a small modification to the protocol allowed the annulation to be carried out with a more oxidized aromatic precursor to install a key phenolic oxygen at C16 (Scheme 3).

It was found that incorporation of the additional methoxy group rendered the standard boronic acid coupling partner prone to proto-deboronation. Coupling of **1** to an excess of 2-hydroxy, 4-methoxyphenyl boronic acid produced low yields (<50%) of the expected Suzuki product. Moreover, the coupled product and the deboronated phenol were very tedious to separate. Fortunately, switching to the trifluroboronate derivative developed by Molander and co-workers[14] allowed for a productive coupling. Using only a slight excess, the previously unreported phenol **4**[15] was effectively cross-coupled in good yield and in sufficient purity to be taken on without the benefit of further purification to give **5** in 79% overall yield for the two-steps. With a successful route to the benzofuran in place, our attention turned to installing key functionality for liphagal analogs on to the central seven-membered ring.

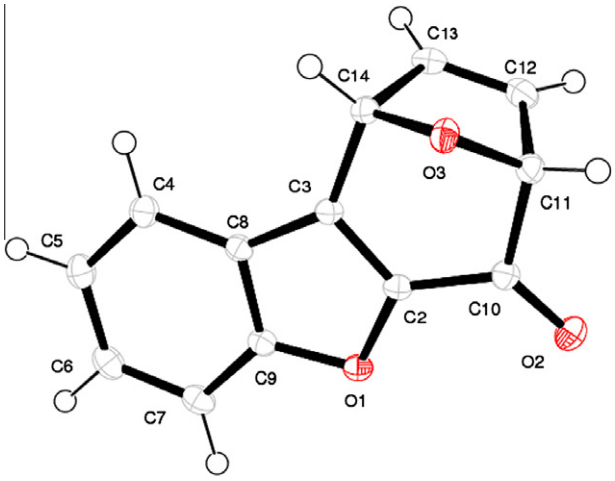
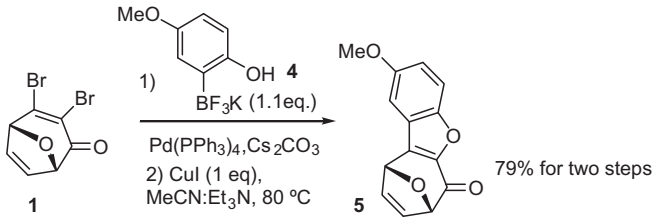
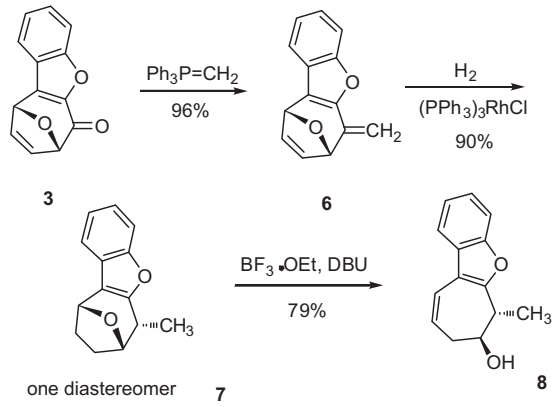


Figure 4. X-ray structure of intermediate **3**.



Scheme 3.



Scheme 4.

The B-ring ketone provides convenient functionality for the installation of the C8-secondary methyl group common to both liphagal and the frondosins (Scheme 4).

We anticipated that the facial bias introduced through the bridging ether could be exploited to control the stereoselectivity during reduction of an exocyclic olefin. Treatment of ketone **3** with a Wittig reagent gave rise to the *exo*-methylene derivative **6**. Pleasingly, catalytic hydrogenation of **6** in the presence of Wilkinson's catalyst led to saturation of both olefins to give **7** as the exclusive diastereomer where reduction of the exocyclic methylene group occurred selectively from the *exo*-face of the bicyclic framework. This suggests that simply using a single isomer of the starting dibromoenone will allow us to transfer the stereochemistry from the initial cycloaddition to the secondary methyl group on the B-ring, a key stereogenic center in liphagal and the sole stereogenic center in frondosin B. To further elaborate ring-B toward the desired intermediates, the ability to effectively open the ether bridge was evaluated. Treatment of **7** with boron triflouride etherate led to a very facile opening of the bridged ether to give **8**. The regiochemistry of the opening is controlled by the stabilization of an intermediate carbonium ion by the annulated benzofuran. The olefinic and hydroxyl functionality proved versatile for the introduction of various appendages needed for the generation of analogs.

In conclusion, we have made efforts toward using the structure of the natural product liphagal to develop isozyme-selective inhibitors of PI-3K. Utilizing high-resolution structures of the kinase, a docked complex of liphagal and the protein was created. Analysis of this complex led to the design of a basic simplified tricyclic scaffold for development of new inhibitors. A route to these types of scaffolds was developed using a key bridged bicyclic intermediate. Current efforts are directed toward synthesis and evaluation of simplified liphagal analogs.

Acknowledgment

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15. Prepared from 4-methoxyphenol by bromination and protection of the phenol as a THP ether (80% overall) followed by lithiation (*t*-BuLi) and reaction with $B(OiPr)_3$ and treatment with 2 N HCl then KHF_2 (85% overall).