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Viridin analogs derived from steroidal building blocks

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ABSTRACT

Naturally occurring furanosteroids such as viridin and wortmannin have long been known as potent inhibitors of the lipid kinase PI-3K. We have been interested in directly accessing analogs of these complex natural products from abundant steroid feedstock materials. In this communication, we describe the synthesis of viridin/wortmannin hybrid molecules from readily available building blocks that function as PI-3K inhibitors and maintain their electrophilic properties. The compounds also show anti-proliferative effects against a breast cancer line.

Over the past decade, there has been an intense interest in the development of potent and specific inhibitors of protein kinases that phosphorylate serine, threonine or tyrosine residues in their target proteins for the treatment of cancer and other diseases. In addition to this large class of protein kinases, there has also been interest in targeting the complementary lipid kinases.[1] Perhaps the most well-studied of these kinases is the family of phosphoinositide kinases, most notably phosphatidyl-inositol-3-kinase (PI-3K), which is responsible for the conversion of phosphatidyl-inositol-4,5-bisphosphate (PIP_2) to the corresponding phosphatidyl-inositol-3,4,5-triphosphate (PIP_3).[2]

Dysregulation of this pathway has been tied to a variety of cancers.[3] There are three PI-3K isoforms; the ability to selectively target specific isozymes remains a high priority.[4] The strong links between over activation of this signaling pathway and cancer has prompted the development of a wide range of small molecule inhibitors with varying degrees of potency and selectivity.[4]

Two of the earliest known PI-3K inhibitors, wortmannin and viridin (Fig. 1), are naturally occurring furanosteroids derived from fungal sources. These unusual steroidal natural products are very potent, albeit non-selective, inhibitors of the enzyme (IC_{50} = 2–10 nM)[3] and function as quasi-irreversible enzyme inhibitors, alkylating a key lysine residue in the enzyme active site through the electrophilic C20-position. In addition to targeting PI-3K, these natural products have also been shown to inhibit structurally related protein kinases such as mTOR, DNA-PK and ATM.[5] Despite the promising anti-proliferative activity of wortmannin, the compound was limited by unfavorable pharmacokinetic properties and hepatotoxicity associated with the protein-reactive inhibitor. Since these initial studies, a variety of reversible, ATP-competitive PI-3K inhibitors have been developed and several are currently in clinical trials against a range of malignancies.[6] There has been renewed interest in the furanosteroid class because of the development of a ring-opened prodrug, PX-866, formed by the simple reaction of wortmannin and diallyl amine.[7] It has been shown that this compound reforms wortmannin in vivo but with an improved pharmacokinetic profile.[8] PX-866 is currently in Phase I clinical trials in patients with solid tumors.

As these compounds are highly complex and synthetically demanding there have been limited attempts to generate analogs in order to improve selectivity and other properties.[9–12] We have been attracted to the use of readily available steroids as starting points for direct and concise syntheses of viridin analogs. Although typical steroids are clearly related to these natural products, there are key differences, specifically in rings A and C. Whereas steroids typically have a carbocyclic A-ring, wortmannin possess a heterocyclic lactone system. Conversely, the viridins maintain the A-ring carbocycle but possess an aromatic C-ring. We envisioned that the use of a readily available steroid such as androstenedione **1** would provide direct access to a class of analogs such as **2** that would represent a hybrid structure between wortmannin and viridin. These analogs would incorporate the saturated (non-aromatic) C-ring found in wortmannin with the carbocyclic A-ring as found in viridin (Scheme 1).

Key to developing these steroid building blocks was the installation of the furan E-ring at the junction of rings A and B, necessitating the introduction of an additional carbon atom on ring A and vicinal oxygenation on the B-ring (Scheme 2).

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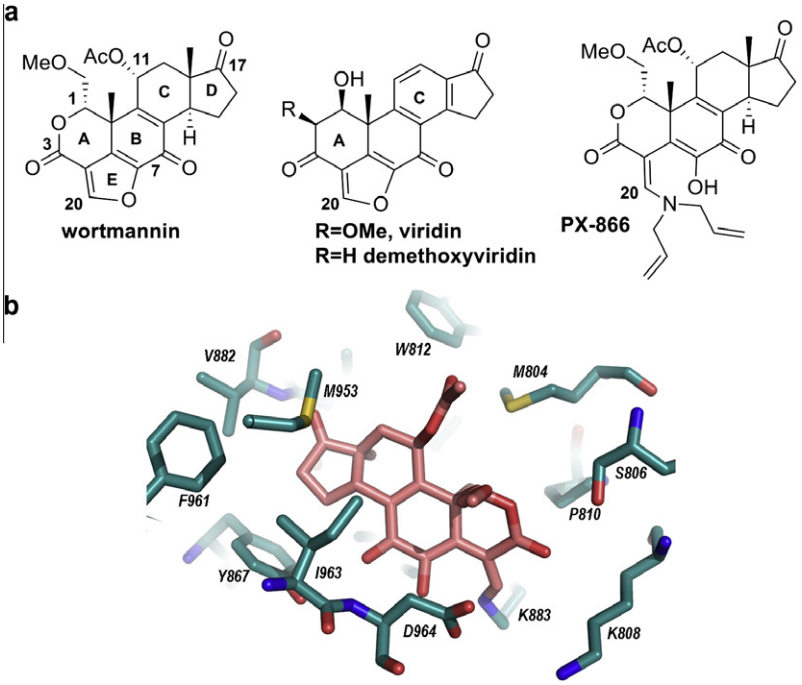
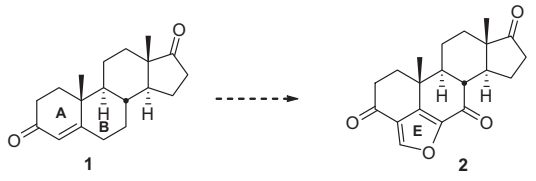
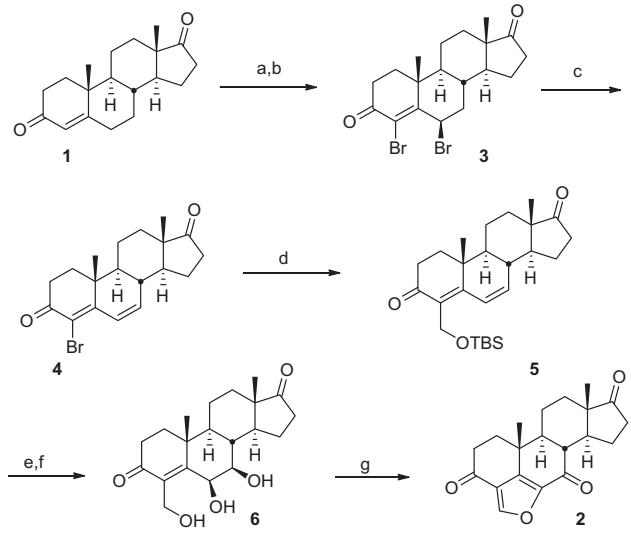


Figure 1. (a) Structure of naturally occurring furanosteroids and a prodrug form; (b) X-ray structure of wortmannin bound to PI-3Kγ, labeled residues are conserved in PI-3Kα.

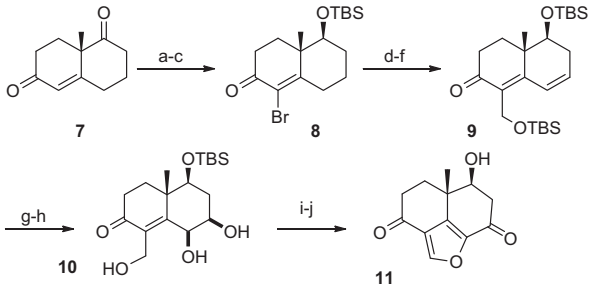


Scheme 1. Design for androgen-derived furanosteroid analogs.



Scheme 2. A-ring functionalization; (a) Br_2/AcOH, Ethylene oxide, −35 °C, 60%; (b) NBS, benzoyl peroxide, CCl_4, reflux, 82%; (c) $LiCO_3$, LiBr, DMF, 90 °C; (d) Bu_3Sn-CH_2-OTBS, $PdCl_2(PPh_3)_2$, dioxane, 90 °C, 68% (e) HF pyr (f) AD-mix β, 5 mol % OsO_4, 5 mol % $(DHAD)_2PHAL$, $MeSO_2NH_2$, $K_2S_2O_8$, *t*-BuOH/ H_2O(1:1), 0 °C to RT; 45% insted of just OsO_4 (g) $COCl_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; 68%.

After considerable experimentation, we were pleased to find that bromination of androstenedione **1** with bromine followed by



Scheme 3. Synthesis of a truncated furanosteroid analog; (a) $NaBH_4$, EtOH; (b)TBSCl, Imidazole, CH_2Cl_2; (c) Br_2/AcOH, Ethylene oxide, −35 °C; (d) NBS, benzoyl peroxide, CCl_4, reflux; (e) $LiCO_3$, LiBr, DMF, 90 °C; (f) Bu_3Sn-CH_2-OTBS, $PdCl_2(PPh_3)_2$, dioxane, 90 °C; (g) AcOH:THF:H_2O(4:1:1); (h) AD-mix β, 5 mol % OsO_4, 5 mol % $(DHAD)_2PHAL$, $MeSO_2NH_2$, $K_2S_2O_8$, *t*-BuOH/ H_2O(1:1), 0 °C to RT; (i) $COCl_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; (j) HF-Pyridine, THF.

a second bromination with N-bromosuccinimide in the presence of a radical initiator led to a smooth conversion to the dibromide **3**.[13] Elimination of the allylic bromide under basic conditions[14] produced bromodienone **4** that contains key ring A/B functionality that allowed direct installation of the diacylfuran moiety. Introduction of the C20-carbon as a formyl equivalent was possible using a palladium catalyzed coupling with an oxygenated stannane to give silylether **5**.[15] Completion of the diacylfuran unit would require selective oxidation of the dienone followed by cyclization to the heterocycle. Deprotection of the silylether was followed by regioselective osmylation of the B-ring olefin to produce the triol **6**. Exposure of the triol to an excess of the Swern reagent[16] effected sequential oxidation and heterocycle formation to produce the key wortmannin analog **2**.

It was also possible to adapt this route to simpler steroid-like building blocks such as the Wieland–Miescher ketone **8** which led to a CD-truncated system with an intact diacylfuran substructure **12** (Scheme 3). This compound was envisioned as a minimal

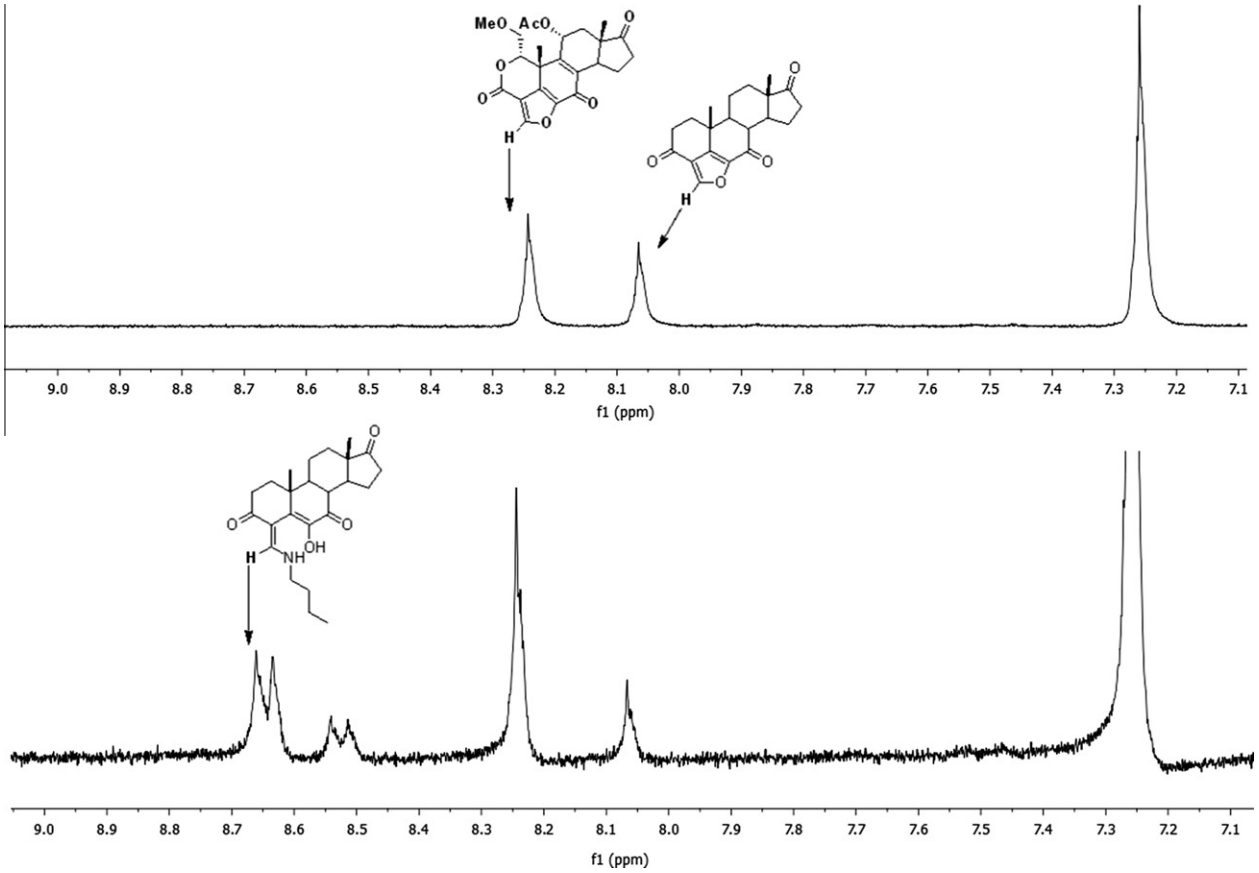


Figure 2. Competitive ring opening study; (a) an equal mixture of **2** and wortmannin and the diagnostic C20 protons (b) addition of *n*-butylamine to the mixture shows preferential attack on **2** relative to wortmannnin, monitored by following the change in diagnostic proton.

pharmacophore model that retains the central furan, activated by the C3 and C7 carbonyl groups but lacks the hydrophobic CD-system of the parent compound.

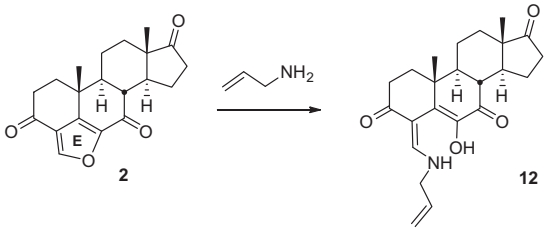
The racemic enone **7** was processed as previously described to produce **9**. Selective deprotection of the primary silylether was followed by regioselective osmylation of the disubstituted olefin to produce the triol **10**. Exposure of the triol to an excess of the Swern reagent effected sequential oxidation and heterocycle formation to produce the truncated wortmannin analog 1**1**.

A central feature of the mechanism of inhibition of PI-3K by wortmannin is the formation of an enzyme adduct through attack of an active site lysine toward the activated, electrophilic furan. Using a simple NMR-based competition experiment, we evaluated the reactivity of **2** towards a limiting amount of a model biological nucleophile (*n*-butylamine) and compared the relative reactivity with that of wortmannin (Fig. 2).

The NMR spectra showed a rapid reaction between the furanosteroid analog and the amine with comparatively less conversion of the natural product. The increase in relative reactivity is likely influenced by the greater withdrawing power of the A-ring ketone relative to the C3-lactone found in wortmannin. Based on the reactivity of **2**, it was straightforward to prepare a ring-opened prodrug analogous to PX-866 by reaction with allyl amine (Scheme 4).[17]

At this point, it was important to conduct a preliminary evaluation of the ability of the three viridin analogs to inhibit the enzyme target and the growth of a cancer cell line. Recombinant human PI-3Kα is a frequently targeted isoform and was chosen for enzyme inhibition assays (Table 1).

All three of the viridin analogs maintained sub-micromolar levels of enzyme inhibition albeit with reduced potency relative to the



Scheme 4. Ring opening of furanosteroid **2**.

natural product. Analysis of the crystal structure[18] of wortmannin bound to PI-3Kγ (PDB ID; 1E7U) provided a rationale for the relative changes in affinity for the synthetic analogs. The key differences between wortmannin and **2** include the absence of the polar functionality at C1 and C11 of wortmannin, the change in hybridization at the BC-ring junction and use of a ketone as a surrogate for the C3-lactone functionality. Major interactions between Tyr867, Ser806, Asp964 and Val882 are predicted to be maintained with compound **2** as well as several hydrophobic interactions with the steroid core. The primary loss of affinity may be ascribed to loss of hydrophobic interactions between the C11 acetate with Trp812/Met804. Interestingly, the minimal, racemic pharmacophore model **11** showed stronger enzyme inhibition than the derivatives with the full steroid core. This compound is still poised to make the strong H-bonding interactions with Tyr867, Ser806, Asp964 but would also lose the hydrophobic interactions with Trp812/Met804 as well as the interaction between Val882 and the D-ring ketonic oxygen. The increased affinity relative to **2**/**12** may result

Table 1
Biological evaluation of viridin analogs

Compound	IC_{50},p110α/p85α (nM)	IC_{50}, MCF-7 (μM)
2	338.9	22.9
11	177.5	>100
12	270.5	37.7
Wortmannin	11.9	>100

from increased flexibility in the binding mode and the placement of an additional polar hydroxyl group. This truncated analog may serve as an excellent starting point for new inhibitor development as the small size would easily allow the introduction of a wide range of substituents to increase potency or selectivity.

The finding that these analogs maintained sub-micromolar levels of enzyme inhibition prompted us to evaluate them for potential antiproliferative effects in a breast cancer cell line (MCF-7). Although there is a strong link between PI-3K dysregulation and breast cancer,[19–21] our results show that wortmannin was not effective against this particular cell line. This observation is in accord with previous reports.[22,23] However, the steroid-derived analog **2** and the ring-opened variant **12** both showed reasonable levels of activity against this line with IC_{50} values of 22.9 and 37.7 μM respectively. The truncated analog **11** proved inactive in the cellular assay which may relate to the poor permeability of this relatively polar compound ($\log P = 0.58$ versus 2.65 for compound **2**). Although the origins of the activity differences between wortmannin and **2** require further investigation, it does illustrate that subtle structural changes can have a significant impact on cellular activity.

The viridins are potent and highly complex inhibitors of PI-3K that act by promoting the addition of a nucleophilic active site lysine to an unusual electrophilic furan. As the potential of PI-3K as a drug target in cancer has received increased attention, there is a need for direct and flexible routes to new inhibitors. We have described the use of abundant steroid and steroid-like building blocks for the preparation of viridin analogs. These first-generation prototype molecules maintain sub-micromolar levels of enzyme inhibition and interestingly show improved anti-proliferative activity against a breast cancer line. The easy availability of the starting materials and relatively short routes should allow for the preparation of several new analogs to improve enzyme potency and anti-proliferative activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.015.

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