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Synthesis and biological evaluation of synthetic viridins derived from C(20)-heteroalkylation of the steroidal PI-3-kinase inhibitor wortmannin

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A series of viridin analogs was prepared from wortmannin by nucleophilic ring opening at C(20) and evaluated against the signaling kinases PI-3-kinase and mTOR. Several subnanomolar enzyme inhibitors with orders of magnitude selectivity for PI-3-kinase and strong cytotoxic activity against four cancer cell lines were identified. Among the ten most promising derivatives, six demonstrated lower liver toxicity and greater promise for inhibition of tumor cell growth than the lead structure wortmannin.

Phosphoinositide-3-kinases (PI-3-kinases) represent an ubiquitously expressed enzyme family that catalyzes the addition of a phosphate molecule to the 3-position of the inositol ring of phosphoinositides (Fig. 1).[1]

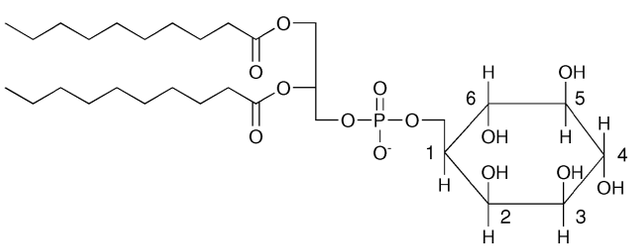

Fig. 1 Structure of phosphatidylinositol (PtdIns).

PI-3-kinase is found in cellular complexes with almost all ligand activated growth factor receptor and oncogene protein tyrosine kinases. PI-3-kinase activity has been found to increase in response to platelet-derived growth factor (PDGF), insulin, insulin-like growth factor 1 (IGF-1), colony stimulating factor 1 (CSF-1), nerve growth factor (NGF), hepatocyte growth factor (HGF), stem cell growth factor (Steel), and epidermal growth factor (EGF).[2] PI-3-kinase activity is found to be elevated in cells transformed by v-src, v-ros, v-yes, and v-abl as well as the polyomavirus middle T antigen/c-src complex.[3,4] PI-3-kinase is also regulated by and associated with members of the non-receptor family of tyrosine-kinases (*i.e. lck, fyn, lyn,* and *c-yes*) in response to cellular activators that stimulate these enzymes.

Approximately 5% of cellular phosphoinositides (PtdIns) are phosphorylated at the 4-position (PtdIns-4-P), and another 5% are phosphorylated at both 4- and 5-positions (PtdIns-4,5-P_2). However, less than 0.25% of the total inositol-containing lipids are phosphorylated at the 3-position, consistent with them having only specific regulatory functions instead of a structural function.[4]

The viridin family of natural products consists of a small group of pentacyclic steroidal antifungal antibiotics isolated as metabolites from a variety of fungi (Fig. 2).[5–7] Unlike other naturally occurring steroids, all members of this family possess a furan ring fused between C(4) and C(6). Both viridin and wortmannin were originally characterized as antifungal agents. Viridin was also found to exhibit antibiotic activity against certain plant pathogens; however, co-metabolites viridiol and demethoxyviridiol exhibited phytotoxic activity, rendering the parent fungal species unsuitable for biocontrol. Wortmannin and its 11-desacetoxy analog were subsequently identified as potent antiinflammatory agents.[8] In the 1970's, researchers at Sandoz AG observed that all of the highly active analogs also exhibited a high degree of acute toxicity rendering them unsuitable for clinical development.[9] Nearly 20 years later, it was discovered that most of the natural viridins are mechanism-based nanomolar inhibitors of PI-3-kinase and since then they have served as important pharmacological probes for the characterization of the cellular functions of these enzymes.[10]

Proteolytic mapping, followed by site-directed mutagenesis of all candidate amino acid residues within the region, revealed Lys-802 to be the target for the covalent binding of wortmannin.[11] This lysine residue resides in the ATP binding site of the p110 catalytic subunit and has a crucial role in the phosphotransfer reaction. The irreversible inhibition of PI-3-kinase occurs by the formation of an enamine following the attack of the lysine on the furan ring at C(20) of wortmannin (Fig. 3). These findings were later supported through structure–activity relationship studies.[12] Wymann *et al.* also proposed a model for the noncovalent interactions of wortmannin with its PI-3-kinase binding site on the basis of the first X-ray crystallographic structures of PI-3-kinase inhibitors bound to the ATP binding pocket.[13]

More recently, wortmannin's selectivity has been called into question as it has been shown to inhibit other serine/threonine kinases of the PI-3-kinase family, such as mTOR and DNA-dependent protein kinase, with IC_{50}'s of 2 and 4 μM respectively, in intact cells (the IC_{50} is considerably lower for the isolated enzymes, 250 nM for mTOR and 16 nM for DNA-PK).[14,15] Functionally, mTOR is involved in the control of protein synthesis through activation of a number of targets including p70 S6 kinase. Recently, a direct link between mTOR and the PI-3-kinase-AKT signalling pathway in transformed cells has been established.[16] Wortmannin is therefore expected to affect other kinases with homologies within the PI-3-kinase Lys-802 region. The natural product has also been reported to be an inhibitor of myosin light chain kinase (MLCK) with an IC_{50} of 0.17 μM and a membrane-bound form of PI-4-kinase at

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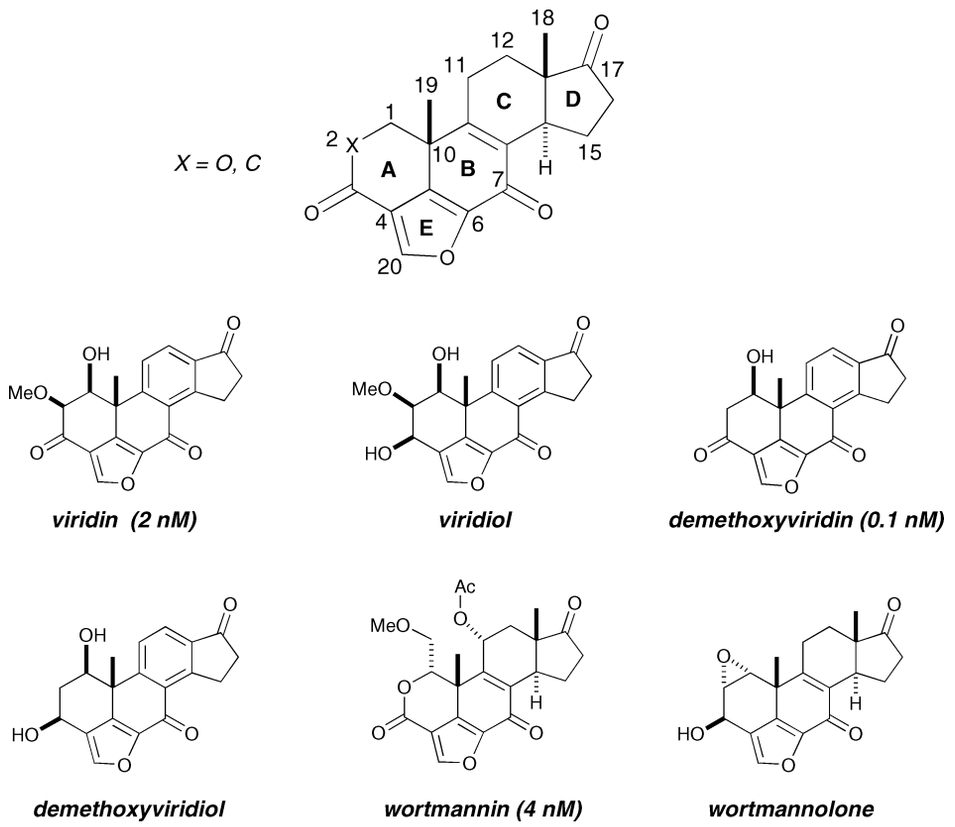


Fig. 2 General molecular scaffold and some specific examples of natural viridin-class steroidal antibiotics. IC_{50} values for PI-3-kinase inhibition are listed in parentheses.

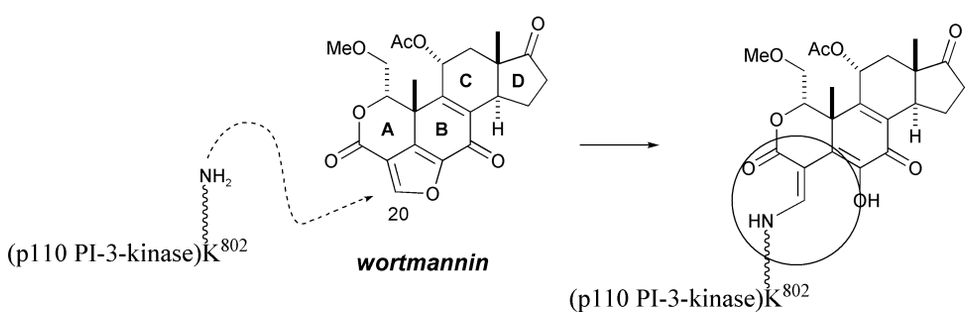


Fig. 3 Proposed mechanism of action of PI-3-kinase inhibition by wortmannin.

high nanomolar concentrations.[17,18] Aside from selectivity and toxicity issues, another obstacle to the use of wortmannin and demethoxyviridin as clinical candidates is their instability. Both compounds, when stored as aqueous solutions at either 37 or 0 °C at neutral pH, are subject to decomposition by hydrolytic opening of the furan ring. This chemical instability is much more pronounced in demethoxyviridin than wortmannin, mirroring their relative potency.

Extensive SAR studies in the mid 1990's by the medicinal chemistry group at Eli Lilly and Company confirmed that the electrophilicity of the furan ring was key to the inhibitory activity of wortmannin.[12,19,20] The reaction of wortmannin with nucleophiles at the C(20)-position followed by furan ring opening provided compounds with a range of biological activities,[21] but its acute toxicity which included hepatotoxicity and biological instability prevented its clinical development as an antitumor agent.

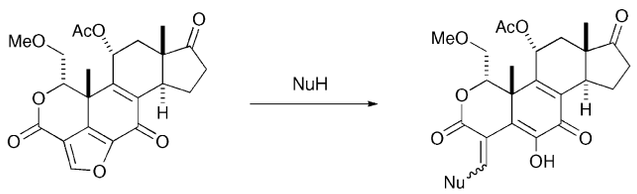
As part of an interdisciplinary program in drug discovery that is based on the use of natural products as lead structures for chemical library synthesis,[22] we selected the viridins as a platform for the development of selective kinase inhibitors. Our specific objectives for this class of compounds were to develop synthetic analogs that possess modified **A**- and **E**-rings with increased chemical stability and structurally simplified **C/D**-ring systems while maintaining biological activity and improving selectivity. We now report a first series of analogs that originate from nucleophilic modification of the furan ring in wortmannin.

A library of 94 C(20)-substituted synthetic viridins was constructed *via* the addition of nucleophiles including primary and secondary amines, anilines, and amino acids to the natural product wortmannin (Table 1). Additionally, a series of five C(20) thioether analogs was prepared by reaction of wortmannin with both alkyl and aryl mercaptans in the presence of triethylamine.

The synthetic viridin library compounds were screened for their ability to inhibit the enzymes PI 3-kinase and mTOR. Samples were also submitted for the National Cancer Institute three-cell prescreen consisting of MCF-7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) cell lines (data reported as percent of growth of the treated cells when compared to the untreated control cells). In addition, growth inhibition of human MCF-7 breast cancer cells was measured over 4 days using the MTT assay. The results of these assays are summarized in Table 2.

A wide range of activities was observed in these assays, with many of the compounds exhibiting potent PI-3-kinase activity at concentrations equal to or lower than wortmannin. Perhaps more important, however, was the increased *in vitro* growth

Table 1 Synthetic viridins obtained by C(20) nucleophilic opening of wortmannin



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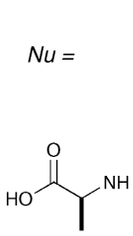

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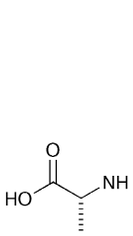

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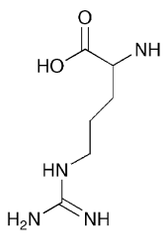

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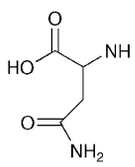

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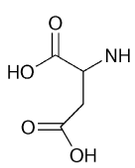

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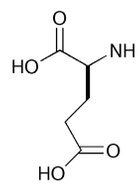

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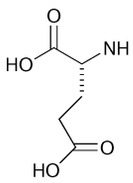

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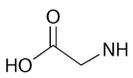

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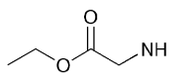

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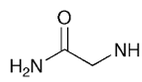

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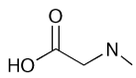

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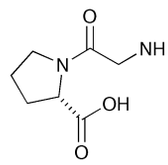

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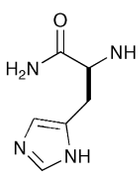

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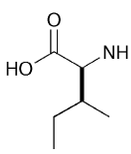

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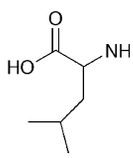

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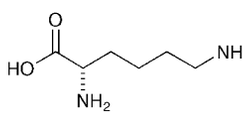

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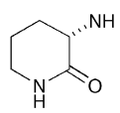

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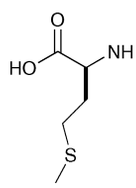

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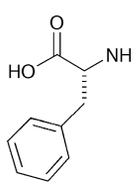

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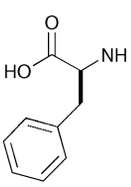

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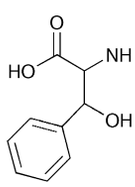

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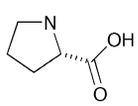

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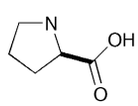

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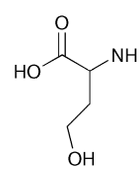

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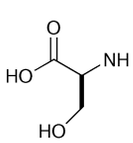

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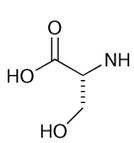

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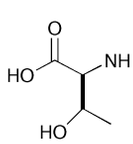

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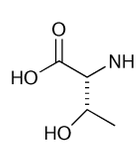

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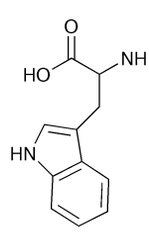

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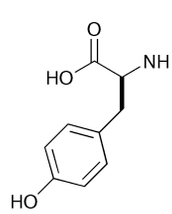

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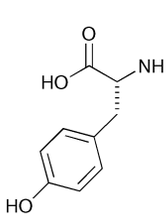

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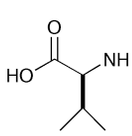

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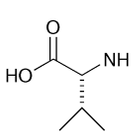

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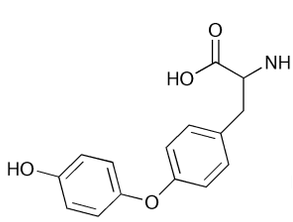

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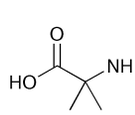

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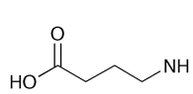

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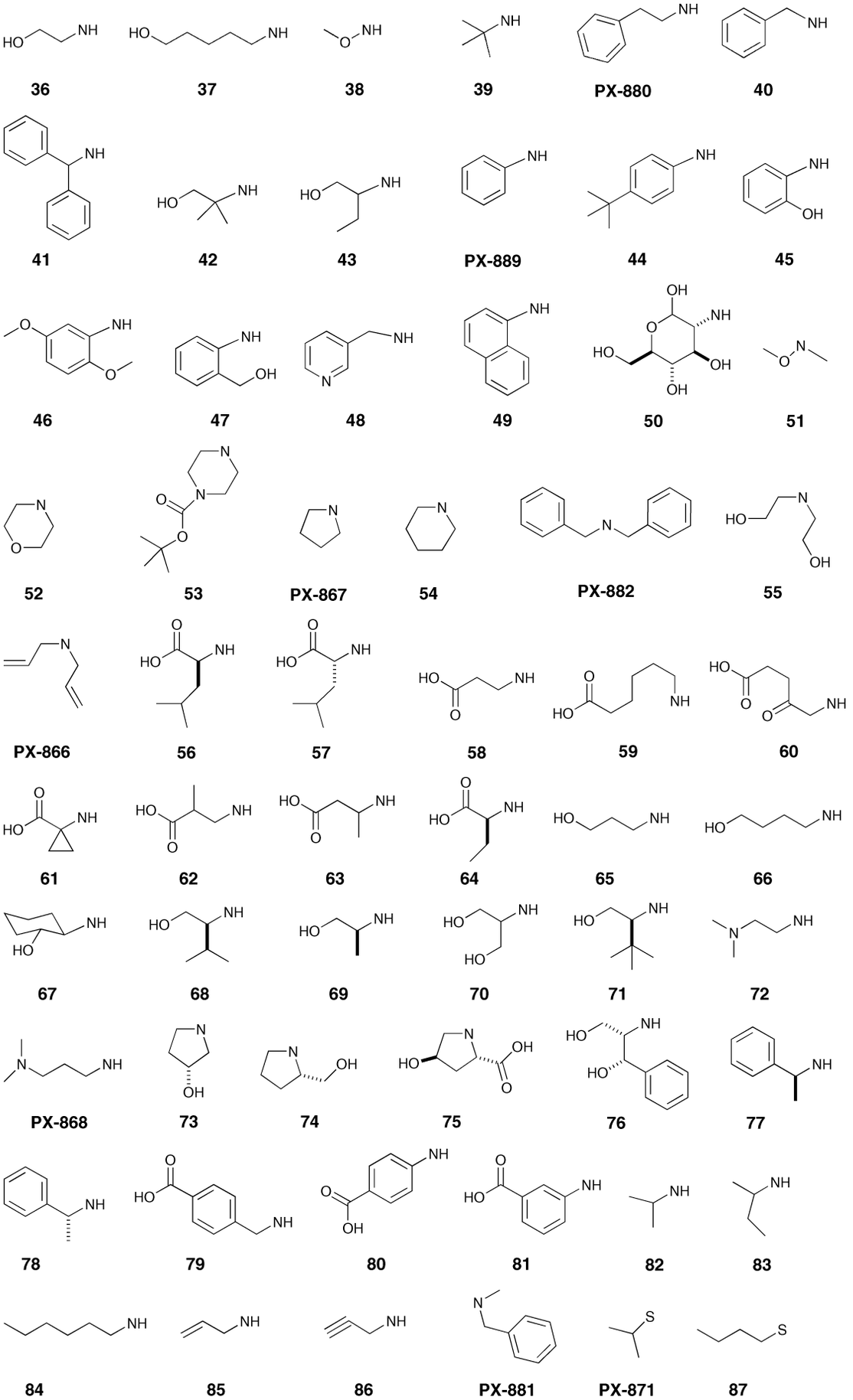

Table 1 (*Contd.*)

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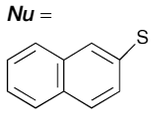		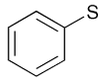
88	**89**	**PX-870**

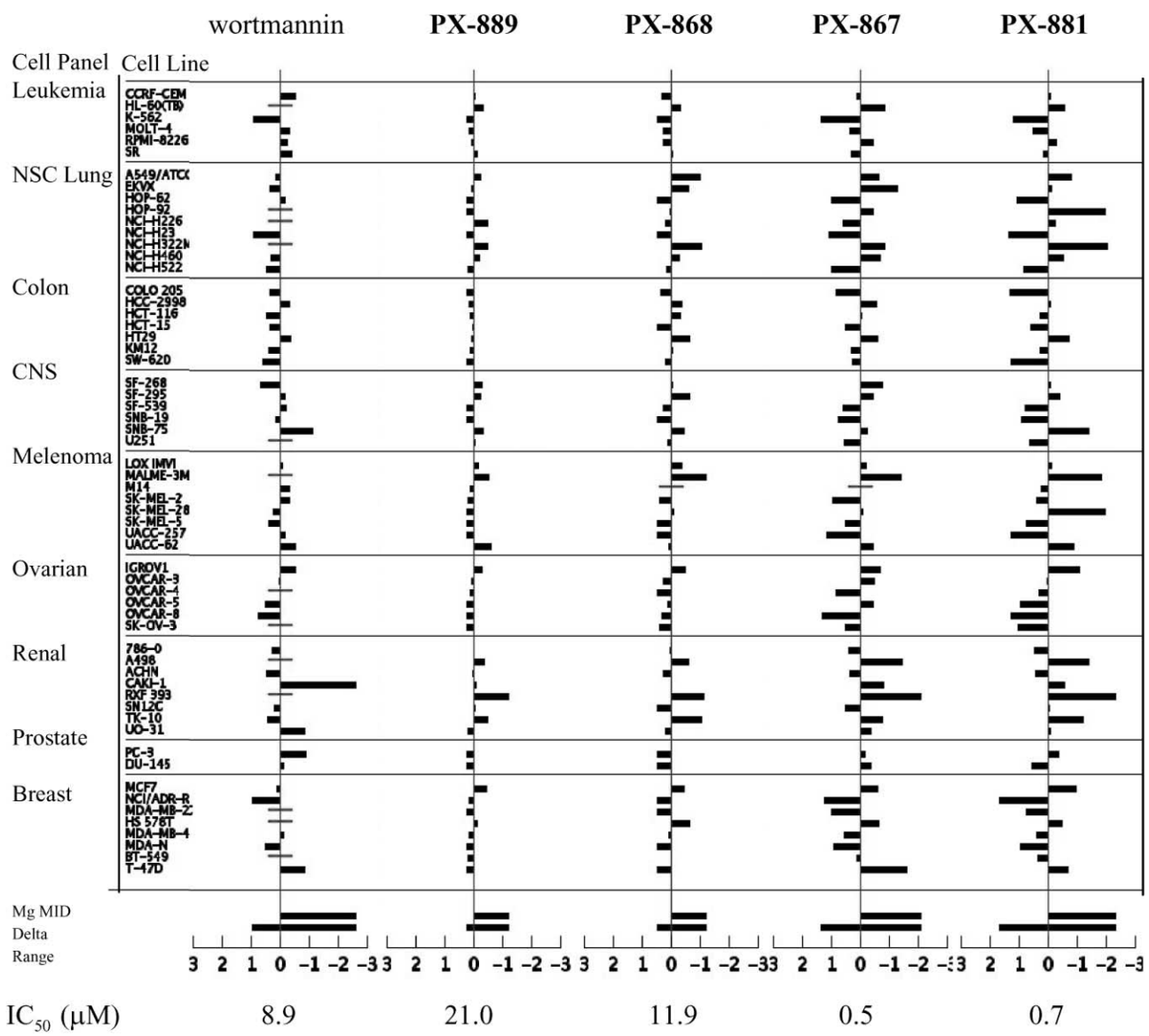

Fig. 4 Cytotoxicity of wortmann and synthetic viridins in the NCI 60 human tumor cell line panel. Cell growth was measured over a 48 h exposure to drug and total protein measured with sulforhodamine D and the response pattern expressed as a mean graph. Growth inhibition was measured as the drug concentration resulting in a 50% reduction in the net protein increase in control cells during the drug incubation (IC_{50}).

inhibition towards MCF-7 cells observed for many analogs *vs.* wortmannin. Numerous derivatives caused 50% inhibition in the nanomolar range, whereas the IC_{50} of wortmannin was found to be around 10 μM.

Data from the NCI human tumor cell line panel shows that as the synthetic viridins increase in cytotoxic potency their selectivity for different cell lines increases (Fig. 4).[23] Wortmannin and **PX-889** which are the least potent compounds show very little selectivity among the different cell lines. **PX-868** which has intermediate potency shows increased selectivity while **PX-867** and **PX-881** which are among the most potent analogs exhibit a high degree of selectivity among different cell lines.

The high level of activity of the synthetic derivatives is probably in part due to their improved chemical stability. In addition, the C(20) substituents can lead to high affinity toward the enzyme by serving as leaving groups upon attack by K^{802} or by providing a better fit in the ATP binding pocket. Three potential interactions may be envisioned to contribute to the latter effect: (1) favorable hydrophobic interactions between the substitutent and the binding site, (2) the introduction of an additional hydrogen bond from the diosphenol hydroxide to the enzyme, and (3) changes in the conformations of the **A**, **B**, **C**, and **D** rings as a consequence of the opening of the furan (**E**) ring that provide a tighter fit to the binding site.

The mean purity of the library compounds was found to be 61% by LC-MS total ion count (TIC) analysis. Ten compounds, **PX-866**, **PX-867**, **PX-868**, **PX-870**, **PX-871**, **PX-880**, **PX-881**,

Table 2 Preliminary biological assays

Compound	PI-3-K: % Enzyme activity remaining at: 0.1 nM	0.5 nM	1.0 nM	10 nM	PI-3-K IC_{50}/nM	mTOR IC_{50}/μM	MCF-7 IC_{50}/μM	NCI 3-cell Mean% *vs.* control
Wortmannin	91.4	49.3	38.4	6.1	**0.80**		>10	
1				77.5			0.1	27
2				77.2			9.0	39
3				80.9			9.0	23
4				100			>10	31
5				42.9		>0.5	>10	34
6				25.8		1.5	>10	23
7	80.4	63.3	40.1	9.0	**0.75**	0.8	>10	31
8				73.5		>0.5	8.0	36
9				37.1		0.9	8.0	28
PX-890				46.6		0.3	0.1	17
10				48.9		0.5	0.1	15
11				100		0.8	8.0	25
12				43.5		2.8	10.0	45
13				100		1	9.0	30
14	48.1		47	35.4	**0.50**	0.1	9.0	
15				81.2		>0.5	10.0	34
16				96.6		>0.5	>10	34
17				100		>0.5	10.0	32
18				65.5		0.8	0.2	30
19				100			10.0	28
20				49.8		>0.5	10.0	22
21				16.9		>0.5	0.4	8
22				59.1		>0.5	0.2	<1
23				91.4		>0.5	>10	39
24				47.8		>0.5	>10	26
26				44.8		>0.5	>10	31
26				39.3		>0.5	6.0	25
27	100	106	95.2	5.4	**4.50**	>0.5	8.0	35
28				89.7		>0.5	>10	28
29				100		0.7	8.0	29
30	49		31	10.0	**0.10**	0.9	0.10	29
31				93.4		>0.5	>10	36
32				85.5			>10	41
33				38.0			>10	25
34				14.9		0.5	4.0	20
35				56.2		4.4	9.0	42
36				40.7		0.9	5.0	30
37				33.2		0.9	5.0	29
38				41.5		0.8	>10	19
39	87.6	94.6	111.5	9.4	**6.0**	0.4	10.0	23
PX-880				48.8		>0.5	0.2	3
40				85.2		>0.5	2.0	14
41				80.8		>0.5	2.0	39
42				84.9		>0.5	0.10	48
43				77.5		>0.5	1.0	53
PX-889				34.7		0.4	8.0	**<1**
44				119.5		>0.5	6.0	14
45				138.5		1.1	0.1	**<1**
46	82.7	69.4	13.2	1.9	**0.5**	0.5	8.0	13
47				16.5		0.4	8.0	18
48				97.9		0.8	8.0	38
49	100	100	100	6.0	**48.0**	0.2	8.0	7
50	100	67.2	43.8	4.9	**0.9**	0.5	1.0	
51	75.5	70.5	58.9	4.1	**2.4**	1.8	1.0	26
52				63.7		0.6	2.0	13
53				80.4		>0.5	8.0	20
PX-867				54.1		1.1	0.2	**<1**
54	82.5	69.8	37.8	3.1	**0.8**	0.5	8.0	19
PX-882	100	44.8	37.4	2.9	**0.4**	0.5	0.4	**<1**
55				48.6		>0.5	1.0	26
PX-866	100	100	100.9	7.0	**0.5**	0.9	0.3	**2**
56	75.4	62.8	4.5	6.3	**0.3**		1.5	34
57	61.7	62.7	51.4	2.7	**1.0**		2.0	36
58				49.4			3.0	74
59				71.4			3.0	83
60	67.8	61.6	37.3	3.3	**0.3**		0.8	
61				45.9			3.0	49
62				61.6			3.0	70
63				76.7			5.0	70
64				42.9			0.80	42
65				100			1.00	60
66				73.9			1.00	45
67				12.4			1.00	31
68				95.2			1.00	79

Table 2 (*Contd.*)

Compound	PI-3-K: % Enzyme activity remaining at:				PI-3-K IC_{50}/nM	mTOR IC_{50}/μM	MCF-7 IC_{50}/μM	NCI 3-cell Mean% *vs.* control
	0.1 nM	0.5 nM	1.0 nM	10 nM				
69				19.6			1.00	69
70				30.5			1.00	75
71				44.8			1.00	88
72	69.7	48.6	40.4	2.3	**0.5**		0.90	27
PX-868	47.8	39.5	31.5	2.0	**0.1**		1.00	30
73				66.8			5.00	13
74				55.0			2.00	17
75	55.8	30.3	27.5	10.5	**0.2**		3.00	20
76				18.6			1.50	71
77	91.7	51.2	39.2	3.3	**0.5**		5.00	17
78	75.1	67.2	60.6	7.2	**5.0**		0.40	20
79	28.1	15.3	9.5	11.0	**0.1**		1.00	29
80				23.1			5.00	33
81				26.4			3.00	28
82				25.0			1.50	
83				58.3			>10	
84				51.6			5.00	
85				53.0			1.50	
86				48.0			2.00	
PX-881					**0.1**		0.7	
PX-871					**0.1**		8.00	37
87					**0.4**		>10	35
88					**0.1**		0.5	<1
89					**1.0**		>10	22
PX-870					**0.1**		0.5	<1

PX-882, **PX-889**, and **PX-890**, were prepared on a 10 mg to 4 g scale and were subjected to advanced screening. The purity of these compounds was consistently >95% (NMR). This re-assay confirmed the promising activities toward PI-3-kinase found in the library screen (Table 3). Since toxicity is a major concern with wortmannin, we also focused on myelosuppression (a specific decrease in lymphocyte count) with this selection of compounds. A high lymphocyte toxicity is often a surrogate for successful clinical inhibition of tumor cell growth. Groups of three C57BL6 mice were administered wortmannin at doses of 1, 2, or 3 mg kg^{-1} or the analogs at 1, 3, 9, or 18 mg kg^{-1} by the intraperitoneal route daily for 4 days. The animals were killed 24 h after the last dose and differential blood counts and serum chemistry were determined.[24] Compounds exhibiting higher cancer cell and lymphocyte toxicity levels relative to wortmannin were selected for further scale up and *in vivo* antitumor testing. The *in vivo* pharmacological profile of **PX-866**, **PX-867**, **PX-868**, **PX-880**, and **PX-881**, and **PX-889** will be discussed elsewhere.[21]

In conclusion, although much information has been gained on the biological function of the viridin class of steroidal antibiotics, there are still many aspects of its cellular action which are not fully understood. In spite of extensive prior medicinal chemistry evaluation of wortmannin and its analogs, clinical efficacy has remained elusive.[25] We have prepared 99 derivatives of wortmannin by nucleophilic opening at C(20) and evaluated their IC_{50}'s against the signaling kinases PI-3 kinase and mTOR. This focused library of synthetic viridins furnished several subnanomolar enzyme inhibitors with orders of magnitude selectivity for PI-3-kinase and high cytotoxic activity against 4 cancer cell lines. Among the ten most promising derivatives, six, namely **PX-866**, **PX-867**, **PX-868**, **PX-880**, **PX-881**, and **PX-889**, demonstrated lower liver toxicity and greater promise for inhibition of tumor cell growth than the lead structure wortmannin. Furthermore, this series of derivatives displayed far higher selectivity for PI-3-kinase relative to mTOR, when compared to the parent compound wortmannin. The synthesis of these derivatives can be scaled up to a multigram level, and *in vivo* testing has been initiated with the goal of identifying a suitable clinical candidate. The results of these advanced pharmacological studies will be reported in due course.

Experimental

General procedure A: construction of a library of C(20) amine adducts of wortmannin

Stock solutions of the amino acids and amines were prepared at 0.06 M in DMSO as well as a stock solution of wortmannin at 0.1 M DMSO. The amino acids that were not soluble at this concentration were diluted to a known concentration in H_2O and warmed at 60 °C until fully dissolved. Amino acids that were not soluble at concentrations of 0.04 M 2 : 1 DMSO–H_2O were sonicated and used as suspensions. One equiv. of NEt_3 from a stock solution was added to solutions of hydrochloride salts or amines or amino acids. The array was assembled in a 1 mL deep-well plate and wortmannin solution was added (42 μL, 4.2 μmol) to each well containing the appropriate amine or amino acid (1.3 eq., 5.4 μmol). The wells were then diluted to 267 μL with DMSO (final concentration 0.0157 M). The plate was covered with foil and heated at 58 °C for 2 h in an oven and then allowed to stand at room temperature overnight. Volatiles were removed *in vacuo* using a SpeedVac© apparatus and daughter plates were prepared consisting of 1.0 μmol (theoretical) of compound in DMSO and were kept frozen until biological testing. Product verification and purity were established through LCMS analysis (APCI, positive mode, 85 : 15 MeOH–H_2O). LCMS samples were prepared by diluting 5 μL of the reaction mixture with MeOH–H_2O (1.0 mL, 95 : 5). Purity was determined through integration of the mass spectral total ion count (TIC) trace. Complete product formation for these reactions was confirmed by thin layer chromatography. Samples were submitted regardless of purity.

General procedure B: synthesis of C(20) thiol adducts of wortmannin

Stock solutions of the thiols were prepared at 1.0 M in CH_2Cl_2. Stock solutions of wortmannin and NEt_3 were prepared at 0.2 M and 0.25 M in CH_2Cl_2, respectively. To a 10 × 75 mm culture tube fitted with a flea bar was added wortmannin solution (100 μL, 20 μmol), the appropriate thiol (40 μL, 40 μmol), and NEt_3 (5 μL, 1.25 μmol) in 200 μL of CH_2Cl_2. The reactions were stirred at room temperature for 3–40 h and monitored by TLC.

Table 3 Activity of scaled and purified synthetic viridins

Compound	PI-3K inhibition IC_{50}/nM	mTOR inhibition IC_{50}/μM	Cytotoxicity MCF-7 cells IC_{50}/μM	Lymphocyte toxicity relative to wortmannin
Wortmannin	0.3	0.3	5.0	1.0
PX-866	0.5	>10	0.5	2.2
PX-867	1.1	>10	0.1	2.4
PX-868	0.1	>3	1.0	0.9
PX-870	1.0	>3	0.4	1.1
PX-871	0.1	>3	8.0	0.7
PX-880	10	2.0	0.2	1.1
PX-881	0.1	>10	ND	1.9
PX-882	0.4	>3	0.5	0.8
PX-889	5	>3	8.1	1.5
PX-890	10	>3	0.2	0.6

The products were purified *via* chromatography on SiO_2 (hexanes–ethyl acetate 1 : 1). Product verification and purity was established through ^{1}H NMR and *via* LCMS (EI+, 65 : 35 MeOH–H_2O). The compounds were recrystallized using hexanes–ethyl acetate to give yellow solids (a modification of this procedure for hindered thiols such as naphthyl, *t*-butyl, or *i*-propyl used $CHCl_3$ as the reaction solvent and the reaction was heated to 55 °C. The reaction was much faster and no bisadduct was observed).

Acetic acid 4-diallylaminomethylene-6-hydroxy-1-α-methoxymethyl-10β,13β-dimethyl-3,7,17-trioxo-1,3,4,7,10,11β,12,13,14α,15,16,17-dodecahydro-2-oxa-cyclopenta[*a*]phenanthren-11-yl ester (PX-866)

To a 0 °C solution of wortmannin (0.89 g, 2.08 mmol) in CH_2Cl_2 (10 mL) was added a freshly prepared 0.2 M stock solution of freshly distilled (from KOH) diallylamine (10.8 mL, 2.16 mmol) in CH_2Cl_2. The reaction mixture was warmed to room temperature and stirred for 1 h. The solvent and excess amine were removed *in vacuo*, providing **PX-866** (1.09 g, 2.08 mmol, quant.) as an orange solid: $[\alpha]_D$ −630 (*c* 0.0015, CH_2Cl_2, 23 °C); IR (KBr) 3391, 1743, 1695, 1685, 1622, 1569, 1222, 1111, 1100 cm^{-1}; ^{1}H NMR δ 8.20 (s, 1 H), 6.81 (s, 1 H), 6.06 (dd, 1 H, *J* = 7.4, 4.8 Hz), 5.85 (br s, 1 H), 5.62 (br, 1 H), 5.44–5.04 (m, 4 H), 4.48 (dd, 1 H, *J* = 7.2, 1.9 Hz), 4.05–3.60 (m, 4 H), 3.26 (s, 3 H), 3.27–3.20 (m, 1 H), 3.16 (dd, 1 H, *J* = 10.9, 7.2 Hz), 3.00–2.90 (m, 2 H), 2.59 (dd, 1 H, *J* = 19.4, 8.6 Hz), 2.40 (dd, 1 H, *J* = 14.4, 7.7 Hz), 2.35–2.07 (m, 2 H), 2.07 (s, 3 H), 1.83 (dd, 1 H, *J* = 14.4, 4.7 Hz), 1.54 (s, 3 H), 0.86 (s, 3 H); ^{13}C NMR δ 217.0, 178.5, 169.6, 164.8, 156.3, 151.5, 139.0, 136.9, 132.2, 131.3, 127.7 (2 C), 119.2, 89.0, 81.9, 73.1, 67.6, 59.1, 50.9 (2 C), 48.9, 42.3, 42.2, 37.5, 36.0, 24.6, 22.2, 20.8, 16.1; MS (EI) *m*/*z* (rel. intensity) 525 (M^+, 11), 466 (17), 391 (15), 350 (14), 323 (13), 266 (17), 239 (17), 60 (100); HRMS (EI) calculated for $C_{29}H_{35}NO_8$ 525.2363, found 525.2386.

Acetic acid 6-hydroxy-1α-methoxymethyl-10β,13β-dimethyl-3,7,17-trioxo-4-pyrrolidin-1-yl-methylene-1,3,4,7,10,11β,12,13,14α,15,16,17-dodecahydro-2-oxa-cyclopenta[*a*]phenanthren-11-yl (PX-867)

To a 0 °C solution of wortmannin (290.4 mg, 0.678 mmol) in CH_2Cl_2 (3.4 mL) was added a freshly prepared 0.2 M stock solution of freshly distilled (from KOH) pyrrolidine (3.75 mL, 0.75 mmol) in CH_2Cl_2. The reaction mixture was warmed to room temperature and stirred for 1 h. The solvent and excess amine were removed *in vacuo*, providing **PX-867** (338.0 mg, 0.677 mmol, quant.) as an orange solid: $[\alpha]_D$ −390 (*c* 0.0073, CH_2Cl_2, 23 °C); IR (KBr) 3337, 1740, 1684, 1617, 1570, 1261, 1221, 1099, 1018 cm^{-1}; ^{1}H NMR δ 8.29 (s, 1 H), 6.72 (s, 1 H), 6.07 (dd, 1 H, *J* = 6.9, 4.8 Hz), 4.47 (dd, 1 H, *J* = 7.0, 1.9 Hz), 3.80–3.70 (m, 2 H), 3.25 (s, 3 H), 3.25–3.14 (m, 2 H), 3.02–2.90 (m, 2 H), 2.69 (br s, 1 H), 2.58 (dd, 1 H, *J* = 19.1, 8.4 Hz), 2.39 (dd, 1 H, *J* = 14.6, 7.8 Hz), 2.32–2.08 (m, 2 H), 2.06 (s, 3 H), 1.99–1.95 (m, 5 H), 1.84 (dd, 1 H, *J* = 14.5, 4.2 Hz), 1.56 (s, 3 H), 0.86 (s, 3 H); ^{13}C NMR δ 217.5, 178.9, 169.9, 164.9, 153.9, 151.3, 137.6, 137.1, 129.2, 89.4, 82.1, 73.3, 67.7, 59.3, 55.2, 49.2 (2 C), 42.6, 42.4, 37.8, 36.3, 25.6 (2 C), 24.5, 22.4, 21.0, 16.3; MS (EI) *m*/*z* (rel. intensity) 499 (M^+, 1), 439 (2), 365 (7), 167 (35), 149 (100); HRMS (EI) calculated for $C_{27}H_{33}NO_8$ 499.2206, found 499.2191.

Acetic acid 4-[(3-dimethylaminopropylamino)methylene]-6-hydroxy-1α-methoxymethyl-10β,13β-dimethyl-3,7,17-trioxo-1,3,4,7,10,11β,12,13,14α,15,16,17-dodecahydro-2-oxa-cyclopenta[*a*]phenanthren-11-yl ester (PX-868)

To a solution of wortmannin (10.7 mg, 25.0 μmol) in CH_2Cl_2 (125 μL) was added a freshly prepared 0.2 M stock solution of 3-dimethylaminopropylamine (138 μL, 27.5 μmol) in CH_2Cl_2. The reaction mixture was stirred at room temperature for 15 min. The solvent and excess amine were removed *in vacuo* to give **PX-868** (11.6 mg, 21.8 μmol, 87%) as an orange oil: $[\alpha]_D$ −500 (*c* 0.008, CH_2Cl_2, 23 °C); IR (KBr) 3306, 3259, 1743, 1669, 1633, 1623, 1573, 1222 cm^{-1}; ^{1}H NMR δ 10.04–10.00 (m, 1 H), 8.54 (d, 1 H, *J* = 14.0 Hz), 5.99 (dd, 1 H, *J* = 7.9, 3.3 Hz), 4.31 (dd, 1 H, *J* = 7.0, 2.1 Hz), 3.76 (q, 2 H, *J* = 6.5 Hz), 3.26 (s, 3 H), 3.26–3.15 (m, 2 H), 2.99–2.84 (m, 2 H), 2.62–2.51 (m, 1 H), 2.40–2.17 (m, 5 H), 2.24 (s, 6 H), 2.04 (s, 3 H), 1.88 (dd, 1 H, *J* = 13.6, 3.4 Hz), 1.82–1.76 (m, 1 H), 1.80 (t, 2 H, *J* = 6.7 Hz), 1.52 (s, 3 H), 0.82 (s, 3 H); ^{13}C NMR δ 218.0, 178.5, 170.2, 165.7, 159.4, 151.0, 137.3, 137.0, 129.3, 88.4, 81.1, 77.8, 73.4, 67.2, 59.3, 56.7, 50.0, 48.6, 45.6, 42.4, 42.3, 38.6, 36.7, 28.4, 26.4, 22.5, 21.1, 16.8; MS (EI) *m*/*z* (rel. intensity) 530 (M^+, 100), 488 (18), 470 (34); HRMS (EI) calculated for $C_{28}H_{38}N_2O_8$ 530.2628, found 530.2609.

Acetic acid 6-hydroxy-1α-methoxymethyl-10β,13β-dimethyl-3,7,17-trioxo-4-phenylsulfanylmethylene-1,3,4,7,10,11β,12,13,14α,15,16,17-dodecahydro-2-oxa-cyclopenta[*a*]phenanthren-11-yl (PX-870)

To a solution of wortmannin (15.0 mg, 35.0 μmol) in CH_2Cl_2 (500 μL) was added thiophenol (10 μL, 118 μmol) and a drop of NEt_3. The reaction mixture was stirred at room temperature for 20 h under N_2. The solvent and excess thiol were removed *in vacuo* and the product was purified by chromatography on SiO_2 (hexanes–ethyl acetate 9 : 1, then 1 : 1) to give **PX-870** (9.7 mg, 18 μmol, 51%) as an orange oil: ^{1}H NMR δ 9.08 (s, 1 H), 7.60–7.57 (m, 2 H), 7.47–7.39 (m, 3 H), 7.34 (s, 1 H), 6.02 (dd, 1 H, *J* = 7.6, 3.5 Hz), 4.50 (dd, 1 H, *J* = 7.4, 1.4 Hz), 3.30 (dd, 1 H, *J* = 11.1, 1.5 Hz), 3.28 (s, 3 H), 3.25–3.16 (m, 1 H), 3.02–2.95 (m, 1 H), 2.89–2.80 (m, 1 H), 2.65–2.52 (m, 1 H), 2.37 (dd, 1 H, *J* = 15.0, 7.9 Hz), 2.31–2.22 (m, 2 H), 2.07 (s, 3 H), 1.91 (dd, 1 H, *J* = 15.1, 3.4 Hz), 1.57 (s, 3 H), 0.82 (s, 3 H); ^{13}C NMR δ 217.4, 179.9, 169.9, 162.6, 160.6, 152.6, 141.3, 137.2, 136.8, 130.4 (2 C), 129.7 (2 C), 128.7, 124.0, 114.4, 82.1, 73.6, 67.2, 59.5, 49.8, 42.9, 42.3, 38.4, 36.5, 25.3, 22.4, 21.1, 16.8; MS (EI) *m*/*z* (rel. intensity) 538 (M^+, 1), 355 (100), 295 (21), 110 (39); HRMS (EI) calculated for $C_{29}H_{30}O_8S$ 538.1661, found 538.1649.

Acetic acid 6-hydroxy-4-isopropylsulfanylmethylene-1α-methoxymethyl-10β,13β-dimethyl-3,7,17-trioxo-1,3,4,7,10,11β,12,-13,14α,15,16,17-dodecahydro-2-oxa-cyclopenta[*a*]phenanthren-11-yl (PX-871)

To a solution of wortmannin (15.0 mg, 35.0 μmol) in $CHCl_3$ (500 μL) was added 2-propanethiol (16.0 μL, 177 μmol) and a drop of NEt_3. The reaction mixture was stirred at room temperature for 20 h under N_2. The solvent and excess thiol were removed *in vacuo* and the product was purified by chromatography on SiO_2 (hexanes–ethyl acetate 9 : 1, then 1 : 1) to give **PX-871** (14.5 mg, 28.7 μmol, 82%) as a yellow oil: ^{1}H NMR δ 9.00 (s, 1 H), 7.27 (s, 1 H), 6.01 (dd, 1 H, J = 7.9, 3.4 Hz), 4.44 (d, 1 H, J = 7.2 Hz), 3.31–3.19 (m, 2 H), 3.26 (s, 3 H), 3.14 (dd, 1 H, J = 11.1, 7.7 Hz), 3.00–2.96 (m, 1 H), 2.89–2.83 (m, 1 H), 2.62–2.54 (m, 1 H), 2.36 (dd, 1 H, J = 15.0, 7.9 Hz), 2.33–2.25 (m, 2 H), 2.05 (s, 3 H), 1.90 (dd, 1 H, J = 15.0, 3.4 Hz), 1.54 (s, 3 H), 1.46 (d, 3 H, J = 6.8 Hz), 1.44 (d, 3 H, J = 6.9 Hz), 0.83 (s, 3 H); ^{13}C NMR δ 217.5, 180.0, 170.0, 162.5, 160.7, 152.6, 141.0, 137.0, 124.7, 113.8, 81.8, 73.6, 67.2, 59.5, 49.8, 42.9, 42.3, 40.7, 38.4, 36.6, 25.3, 23.7, 23.2, 22.4, 21.1, 16.8; MS (EI) *m*/*z* (rel. intensity) 504 (M^+, 18), 370 (100), 355 (82), 313 (65), 323 (31), 295 (32), 266 (33), 239 (43), 165 (29), 152 (35), 129 (35), 115 (40), 91 (53); HRMS (EI) calculated for $C_{26}H_{32}O_8S$ 504.1818, found 504.1824.

Acetic acid 6-hydroxy-1α-methoxymethyl-10β,13β-dimethyl-3,7,17-trioxo-4-(phenethylaminomethylene)-1,3,4,7,10,11β,12,-13,14α,15,16,17-dodecahydro-2-oxa-cyclopenta[*a*]phenanthren-11-yl (PX-880)

To a solution of wortmannin (10.7 mg, 25.0 μmol) in CH_2Cl_2 (125 μL) was added a freshly prepared 0.2 M stock solution of phenethylamine (138 μL, 27.5 μmol) in CH_2Cl_2. The reaction mixture was stirred at room temperature for 1 h. The solvent and excess phenylethylamine were removed *in vacuo* to give **PX-880** (12.9 mg, 23.4 μmol, 94%) as an orange oil: $[\alpha]_D$ −410 (*c* 0.0026, CH_2Cl_2, 23 °C); IR (KBr) 3332, 3266, 1741, 1670, 1644, 1621, 1576, 1223 cm^{-1}; ^{1}H NMR δ 9.95–9.87 (m, 1 H), 8.41 (d, 1 H, J = 13.8 Hz), 7.36–7.20 (m, 5 H), 7.07 (br, 1 H), 5.98 (dd, 1 H, J = 8.1, 3.3 Hz), 4.31 (dd, 1 H, J = 7.5, 1.7 Hz), 3.68–3.60 (m, 2 H), 3.27 (s, 3 H), 3.26–3.21 (m, 1 H), 3.16 (dd, 1 H, J = 11.0, 7.6 Hz), 2.97 (t, 2 H, J = 7.1 Hz), 2.98–2.80 (m, 2 H), 2.63–2.51 (m, 1 H), 2.35 (dd, 1 H, J = 15.1, 7.8 Hz), 2.33–2.24 (m, 2 H), 2.04 (s, 3 H), 1.88 (dd, 1 H, J = 15.1, 3.3 Hz), 1.50 (s, 3 H), 0.82 (s, 3 H); ^{13}C NMR δ 218.0, 178.5, 170.1, 165.8, 159.2, 151.0, 137.5, 137.4, 136.9, 128.9 (5 C), 127.1, 88.6, 81.1, 73.3, 67.2, 59.3, 51.7, 49.9, 42.3 (2 C), 38.5, 37.3, 36.6, 26.3, 22.5, 21.0, 16.8; MS (EI) *m*/*z* (rel. intensity) 549 (M^+, 6), 415 (11), 129 (15), 105 (100); HRMS (EI) calculated for $C_{31}H_{35}NO_8$ 549.2363, found 549.2347.

Acetic acid 4-[(benzylmethylamino)methylene]-6-hydroxy-1α-methoxymethyl-10β,13β-dimethyl-3,7,17-trioxo-1,3,4,7,10,11β,-12,13,14α,15,16,17-dodecahydro-2-oxa-cyclopenta[*a*]phenanthren-11-yl ester (PX-881)

To a 0 °C solution of wortmannin (272.4 mg, 0.635 mmol) in CH_2Cl_2 (3.2 mL) was added a freshly prepared 0.2 M solution of freshly distilled (from KOH) *N*-methylbenzylamine (3.5 mL, 0.70 mmol) in CH_2Cl_2. The reaction mixture was warmed to room temperature and stirred for 1 h. The solvent and excess amine were removed *in vacuo*, providing **PX-881** (348.6 mg, 0.634 mmol, quant.) as an orange solid: $[\alpha]_D$ −835 (*c* 0.0014, CH_2Cl_2, 23 °C); IR (neat) 1742, 1685, 1618, 1589, 1575, 1224 cm^{-1}; ^{1}H NMR δ 8.36 (br s, 1 H), 7.36–7.27 (m, 5 H), 6.60 (br s, 1 H), 6.10–6.00 (m, 1 H), 4.68–4.63 (m, 1 H), 4.53–4.47 (m, 2 H), 3.25 (s, 3 H), 3.25–3.11 (m, 2 H), 2.99–2.84 (m, 2 H), 2.71 (br, 2 H), 2.55 (dd, 1 H, J = 19.5, 8.9 Hz), 2.38 (dd, 1 H, J = 14.4, 7.6 Hz), 2.32–2.05 (m, 2 H), 2.05 (s, 3 H), 1.85 (br s, 1 H), 1.80 (dd, 1 H, J = 14.5, 4.7 Hz), 1.52 (s, 3 H), 0.82 (s, 3 H); ^{13}C NMR δ 217.3, 178.9, 169.9, 164.7, 158.3, 151.7, 138.8, 137.1, 134.9, 129.0 (3 C), 128.6, 128.1 (2 C), 88.7, 82.2, 73.4, 67.9, 64.3, 59.4, 49.1, 42.7, 42.5, 37.8 (2 C), 36.3, 25.2, 22.5, 21.1, 16.3; MS (EI) *m*/*z* (rel. intensity) 549 (M^+, 14), 489 (37), 415 (15), 120 (23), 91 (100); HRMS (EI) calculated for $C_{31}H_{35}NO_8$ 549.2363, found 549.2340.

Acetic acid 4-[(dibenzylamino)methylene]-6-hydroxy-1α-methoxymethyl-10β,13β-dimethyl-3,7,17-trioxo-1,3,4,7,10,11β,12,-13,14α,15,16,17-dodecahydro-2-oxa-cyclopenta[*a*]phenanthren-11-yl ester (PX-882)

To a solution of wortmannin (10.7 mg, 25.0 μmol) in CH_2Cl_2 (125 μL) was added a freshly prepared 0.1 M solution of dibenzylamine (450 μL, 45 μmol) in CH_2Cl_2. The reaction mixture was stirred at room temperature for 6 h. The solvent was removed *in vacuo* and the product was purified *via* chromatography on SiO_2 (hexanes–ethyl acetate, 1 : 9) to give **PX-882** (14.2 mg, 22.6 μmol, 90%) as an orange oil: ^{1}H NMR δ 8.51 (s, 1 H), 7.43–7.31 (m, 8 H), 7.05 (br, 2 H), 6.69 (br, 1 H), 6.00 (dd, 1 H, J = 5.6, 1.4 Hz), 4.58–4.49 (m, 3 H), 4.37–3.34 (m, 1 H), 4.03–4.01 (m, 1 H), 3.27 (s, 3 H), 3.26–3.23 (m, 1 H), 3.19–3.15 (m, 1 H), 2.97–2.90 (m, 2 H), 2.55 (dd, 1 H, J = 19.5, 8.8 Hz), 2.37 (dd, 1 H, J = 14.3, 8.7 Hz), 2.30–2.22 (m, 1 H), 2.06 (s, 3 H), 1.77 (dd, 1 H, J =16.8, 4.9 Hz), 1.34 (s, 3 H), 0.78 (s, 3 H); ^{13}C NMR δ 217.2, 178.9, 169.9, 165.7, 157.1, 152.1, 139.8, 137.1, 135.3, 135.2, 129.2 (2 C), 128.9 (2 C), 128.5 (4 C), 128.0 (3 C), 89.2, 82.6, 73.4, 68.1, 61.3, 59.5, 51.8, 49.1, 42.8, 42.6, 37.7, 36.3, 24.3, 22.5, 21.2, 16.2; MS (EI) *m*/*z* (rel. intensity) 625 (M^+, 1), 371 (11), 106 (19), 91 (100); HRMS (EI) calculated for $C_{37}H_{39}NO_8$ 625.2676, found 625.2657.

Acetic acid 6-hydroxy-1α-methoxymethyl-10β,13β-dimethyl-3,7,17-trioxo-4-(phenylaminomethylene)-1,3,4,7,10,11β,12,13,-14α,15,16,17-dodecahydro-2-oxa-cyclopenta[*a*]phenanthren-11-yl (PX-889)

To a solution of wortmannin (12.7 mg, 30.0 μmol) in CH_2Cl_2 (125 μL) was added a freshly prepared 0.4 M solution of aniline (100 μL, 40 μmol) in CH_2Cl_2. The reaction mixture was stirred at room temperature for 18 h. The solvent was removed *in vacuo*, and the product was purified by chromatography on SiO_2 (hexanes–ethyl acetate 1 : 1, then 1 : 99) to give **PX-889** (11.3 mg, 21.7 μmol, 72%) as a yellow oil: $[\alpha]_D$ −210 (*c* 0.00012, CH_2Cl_2, 23 °C); IR (KBr) 3420, 1743, 1677, 1623, 1574, 1219, 1123 cm^{-1}; ^{1}H NMR δ 11.58 (d, 1 H, J = 13.5 Hz), 9.11 (d, 1 H, J = 13.5 Hz), 7.41 (t, 2 H, J = 7.9 Hz), 7.33 (s, 1 H); 7.20–7.16 (m, 3 H), 6.03 (dd, 1 H, J = 7.9, 3.3 Hz), 4.41 (dd, 1 H, J = 7.6, 1.6 Hz), 3.33–3.28 (m, 1 H), 3.39 (s, 3 H), 3.23 (dd, 1 H, J = 11.1, 7.7 Hz), 3.02–2.85 (m, 2 H), 2.65–2.52 (m, 1 H), 2.38 (dd, 1 H, J = 15.0, 7.9 Hz), 2.39–2.23 (m, 2 H), 2.06 (s, 3 H), 1.90 (dd, 1 H, J = 15.0, 3.3 Hz), 1.58 (s, 3 H), 0.84 (s, 3 H); ^{13}C NMR δ 217.8, 178.9, 170.0, 165.8, 151.5, 150.7, 139.3, 138.3, 137.1, 130.0 (2 C), 127.3, 125.1, 117.2 (2 C), 92.1, 81.6, 73.4, 67.2, 59.4, 49.9, 42.5, 42.3, 38.5, 36.6, 26.2, 22.5, 21.0, 16.7; MS (EI) *m*/*z* (rel. intensity) 521 (M^+, 100), 461 (26), 387 (65), 372 (58), 359 (37), 344 (39), 330 (66); HRMS (EI) calculated for $C_{29}H_{31}NO_8$: 521.2050, found: 521.2043.

Acetic acid 4-[(carbamoylmethylamino)methylene]-6-hydroxy-1α-methoxymethyl-10β,13β-dimethyl-3,7,17-trioxo-1,3,4,7,10,-11β,12,13,14α,15,16,17-dodecahydro-2-oxa-cyclopenta[*a*]-phenanthren-11-yl ester (PX-890)

To a culture tube charged with wortmannin (20.0 mg, 46.7 μmol) was added a homogeneous solution of glycinamide hydrochloride (6.4 mg, 58 μmol) and NEt_3 (8.0 μL, 58 μmol) in dry DMF (1 mL). The reaction mixture was stirred at room temperature for 1 h. The solvent was removed by a stream of N_2 gas and the product was purified by chromatography on SiO_2 (CH_2Cl_2–MeOH 9 : 1) to give **PX-890** (14.6 mg, 29.1 μmol,

62%) as a yellow oil: ^{1}H NMR δ 10.02–9.94 (m, 1 H), 8.50 (d, 1 H, J = 13.7 Hz), 7.26 (s, 1 H), 6.51 (br s, 1 H), 6.22 (br s, 1 H), 5.99 (dd, 1 H, J = 7.8, 3.1 Hz), 4.32 (dd, 1 H, J = 6.5, 2.7 Hz), 4.12 (d, 2 H, J = 6.2 Hz), 3.25 (s, 3 H), 3.25–3.12 (m, 2 H), 2.98–2.81 (m, 2 H), 2.61–2.51 (m, 1 H), 2.38–2.20 (m, 3 H), 2.04 (s, 3 H), 1.87 (dd, 1 H, J = 15.0, 3.1 Hz), 1.52 (s, 3 H), 0.81 (s, 3 H); ^{13}C NMR δ 217.9, 178.8, 170.5, 170.1, 166.0, 159.6, 151.1, 138.0, 137.1, 127.6, 90.3, 81.2, 73.3, 67.2, 59.3, 52.0, 49.9, 42.2 (2 C), 38.5, 36.6, 26.1, 22.5, 21.1, 16.8; MS (EI) *m/z* (rel. intensity) 502 (M^+, 9), 442 (15), 368 (18), 167 (20), 146 (69), 60 (100); HRMS (EI) calculated for $C_{25}H_{30}N_2O_9$ 502.1951, found 502.1940.

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