Synthesis and Biological Evaluation of Purealin and Analogues as Cytoplasmic Dynein Heavy Chain Inhibitors

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Received October 13, 2005

Cytoplasmic dynein plays important roles in membrane transport, mitosis, and other cellular processes. A few small-molecule inhibitors of cytoplasmic dynein have been identified. We report here the first synthesis of purealin, a natural product isolated from the sea sponge *Psammaplysilla purea*, which is known to inhibit axonemal dynein. Also described are the first syntheses, by modular amide coupling reactions, of the natural product purealidin A (a component of purealin) and a small library of analogues. The library was examined for inhibition of cytoplasmic dynein heavy chain and cell growth. The compounds showed effective antiproliferative activity against a mouse leukemia cell line but selective activities against human carcinoma cell lines. Purealin and some of the analogues inhibited the microtubule-stimulated ATPase activity of recombinant cytoplasmic dynein heavy chain motor domain. The inhibitory effect of purealin was concentration dependent and uncompetitive, supporting the hypothesis that it does not compete with the binding of ATP.

Introduction

Dyneins are protein motor complexes that generate forces toward the minus ends of microtubules (MTs).[a] Among the 15 forms of dynein found in vertebrates, only two are cytoplasmic. These are cytoplasmic dynein-1, initially described as microtubule-associated protein 1C (MAP1C),[2] and cytoplasmic dynein-2.[3−5] Cytoplasmic dynein-1 (hereafter, cytoplasmic dynein) is a multisubunit protein complex with a heavy chain (HC) polypeptide ≥500 kDa that is responsible for ATPase and motor activities. There are also several intermediate chains (ICs), light intermediate chains (LICs), and light chains (LCs) that are responsible for subcellular localization and recognition and binding of the various forms of cargo carried by dynein.[2]

Cytoplasmic dynein is involved in fundamental cellular processes such as the return of vesicles to the MT organizing center (MTOC) and the retention of the vesicles at this subcellular locale. It is intricately involved in the maintenance of the Golgi apparatus and in the trafficking of membrane-encapsulated vesicles and proteins such as membrane-bound receptors. Cytoplasmic dynein has roles in various stages of mitosis, including nuclear envelope breakdown at late prometaphase, chromosome segregation, and mitotic spindle formation.[6] Cytoplasmic dynein has also been implicated in retinitis pigmentosa,[7] lissencephaly,[8−10] virus transport,[11,12] and neurodegenerative diseases.[13,14]

Purealin (**1**) is a bromotyrosine-derived natural product isolated from the sea sponge *Psammaplysilla purea*.[15] It inhibits the ATPase activity of isolated axonemal dynein and skeletal muscle myosin without competing for the ATP sites on these motor proteins.[16,17] Other small molecules that can inhibit dynein include the vanadate anion and several ATP analogues. Some redox-active and thiol-perturbing agents are also inhibitors because dynein has a thioredoxin-like domain including vicinal thiols. The structures of purealin and other related natural products are shown in Figure 1. Relatives of purealin **1** include lipopurealins A (**2**), B (**3**), and C (**4**), and purealidin A (**5**).[18−20] Common substructures within these compounds include the dibromotyrosine oxime (in an open form or the oxidatively closed spiroisoxazoline form) and the aminohistamine side chain. In purealin, a spiroisoxazoline side chain is coupled to the bromotyrosine amine, whereas in lipopurealins A, B, and C, fatty acid side chains are coupled to the bromotyrosine amine.

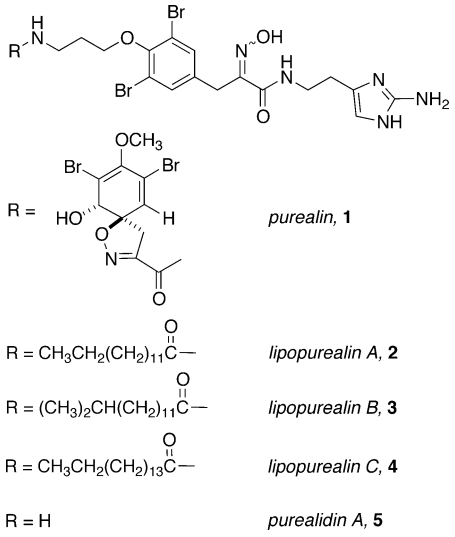

Figure 1. Bromotyrosine-derived alkaloids.

The effects of the lipopurealins and purealidins on dynein are unknown. What is known about these natural products is that purealidin A (**5**) has weak inhibitory activity (22% inhibition

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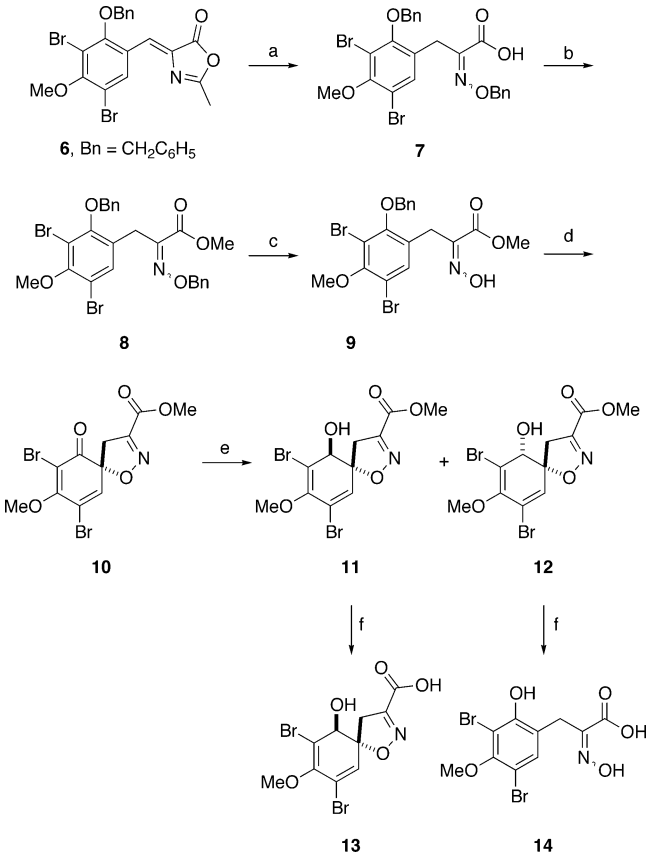
[a] Abbreviations: MTs, microtubules; MAP1C, microtubule-associated protein 1C; HC, heavy chain; ICs, intermediate chains; LICs, light intermediate chains; MTOC, MT organizing center; HPLC, high performance liquid chromatography.

10.1021/jm051030l CCC: $33.50
Published on Web 02/17/2006

Scheme 1. Synthesis of Spiroisoxazoline Acid **13**



Reagents and conditions: (a) $Ba(OH)_2$, $BnONH_2 \cdot HCl$, dioxane/H_2O, 60 °C, 64%. (b) $TMSCH_2N_2$, benzene/MeOH, rt, 92%. (c) H_2, Pd-black, AcOH/dioxane, rt, 81%. (d) NBS, DMF, rt. (e) $Zn(BH_4)_2$, CH_2Cl_2/Et_2O, rt, **11**: 24% in two steps; **12**: 14% in two steps. (f) LiOH, MeOH/H_2O, 94%.

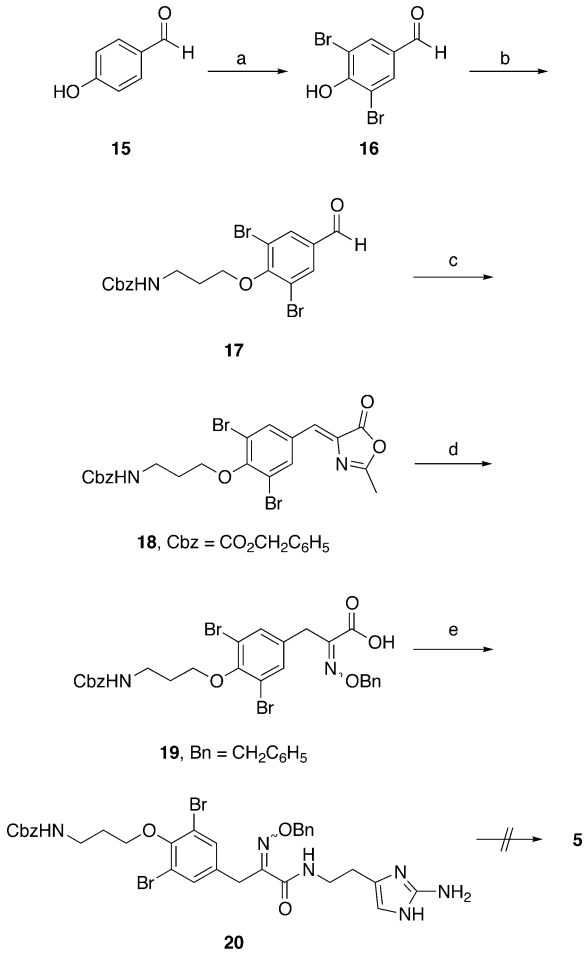
at 100 μM) against Na,K-ATPase and exhibits cytotoxicity against L1210 murine leukemia cells with an IC_{50} value of 1.9 μM.[19] Lipopurealins A, B, and C exhibit inhibitory activities against Na,K-ATPase purified from porcine brains and dog kidneys, with lipopurealin B being the most potent inhibitor.[20]

We report herein the synthesis of a small bromotyrosine library designed around the natural product purealin. The first syntheses of purealin and purealidin A are described. Several chemical groups were modified to examine the effects of relative hydrophilicity in the compound library, and some alterations in regioconnections were examined. The biological activity of the resulting library members were examined and the results are begining to point the way to the design of more potent dynein inhibitors.

Results and Discussion

From a retrosynthetic perspective, purealin comprises the spiroisoxazoline acid[21] subunit and purealidin A (**5**). In turn, purealidin A comprises the dibromotyrosine and the aminohistamine subunits. The starting point for the synthesis of key spiroisoxazoline acid **13** was azlactone **6** (Scheme 1), which was prepared from 2-hydroxy-4-methoxybenzaldehyde in three steps.[22] Azlactone **6** was hydrolyzed with $Ba(OH)_2$ in the presence of *O*-benzyl hydroxylamine to provide dibenzyl oxime-acid **7** in 64% yield. Acid **7** was treated with $TMSCHN_2$ to provide methyl ester **8** in 92% yield. Hydrogenolysis of **8** with palladium-black in acetic acid and dioxane afforded *O*-phenolic oxime-methyl ester (**9**) in 81% yield.[23] Oxime-methyl ester **9** was cyclized with NBS in DMF to afford spirosioxazoline **10** in 92% crude yield. Because **10** degraded on silica gel, it was

Scheme 2. Synthesis of Protected Purealidin **20** and Attempted Deprotection

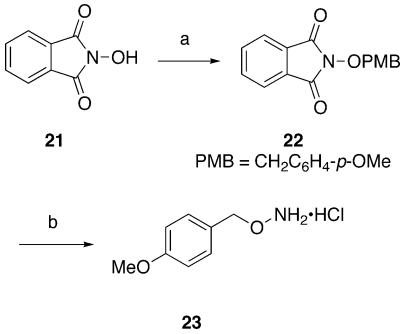


Reagents and conditions: (a) Br_2, AcOH, NaOAc, rt, 96%. (b) $CbzNHCH_2CH_2CH_2Br$, K_2CO_3, DMF, 90 °C, 71%. (c) acetylglycine, Ac_2O, NaOAc, 120 °C, 96%. (d) $Ba(OH)_2$, $BnONH_2 \cdot HCl$, dioxane/H_2O, 60 °C, 34%. (e) aminohistamine, DCC, HOBt, Et_3N, $CHCl_3$/DMF, rt, 66%.

directly reduced with excess $Zn(BH_4)_2$ (prepared with $ZnCl_2$ and $NaBH_4$ as a 0.1 N solution in diethyl ether)[24] to give corresponding stereoisomers **11** and **12**. These were separated by preparative TLC or careful column chromatography. Trans isomer **11** was obtained in 24% yield over two steps, and cis isomer **12** was obtained in 14% yield. The modest overall yield (38%) is probably due to the instability of **10**. Characterization data for **11** and **12** were consistent with literature.[21a] Ester **11** was easily saponified to requisite acid **13** in 94% yield with LiOH,[25] but hydrolysis of ester **12** under the same conditions led to decomposition.

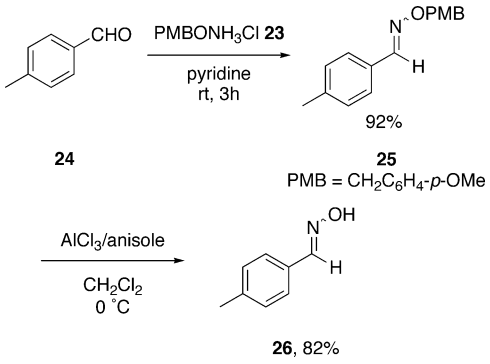
The synthesis of purealidin A (**5**) is shown in Scheme 2. The bromination of 4-hydroxybenzaldehyde (**15**) afforded dibromoaldehyde **16** in 96% yield.[26] The treatment of **16** with 3-(*N*-benzyloxycarbonylamino)propyl bromide afforded **17** in 71% yield.[27] The heating of a mixture of aldehyde **17** and *N*-acetylglycine to 120 °C in acetic anhydride for 4 h gave azlactone **18** as a yellow solid in 96% yield after isolation by filtration. Azlactone **18** was saponified with $Ba(OH)_2$ in the presence of *O*-benzylhydroxylamine in aqueous dioxane to afford benzyloxime acid **19** in 34% yield. Acid **19** was converted to amide **20** by coupling with aminohistamine, which was made according to the known procedure.[28] The extractive workup of the reaction mixture containing **20** was problematic, so the reaction solvent was simply removed by lyophilization. Amide **20** was obtained in 66% isolated yield after flash chromatog-

Scheme 3. Synthesis of *p*-Methoxybenzylhydroxylamine **23**



Reagents and conditions: (a) PMBCl, Et_3N, DMF, 90 °C, 68%. (b) hydrazine, 60 °C, then HCl, 50%.

Scheme 4. Deprotection of the PMB Group in Model System **25**



raphy by using 15:9:1:1 EtOAc/acetone/formic acid/H_2O as the eluent.

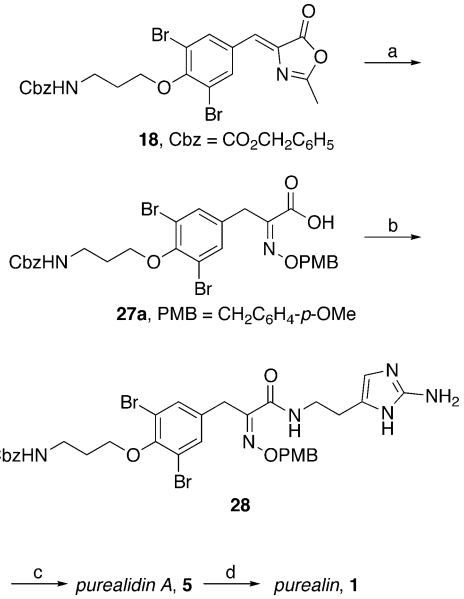
At this stage, it proved impossible to remove the protecting groups from **20** to give **5**. Attempts at hydrogenolysis of the benzyl and Cbz protecting groups on amide **20** with palladium-black in acetic acid and dioxane[23] only returned the starting material. Hydrogenation with Pd/C in methanol or ethanol led to decomposition, whereas the use of acetic acid and dioxane yielded no conversion. Treatment of amide **20** with TMSCl/NaI[29] only removed the Cbz group.

The benzyl oxime-protecting group was therefore replaced with the more easily removable *p*-methoxybenzyl (PMB) group. *O*-PMB hydroxyamine (**23**) was synthesized as shown in Scheme 3.[30] *N*-Hydroxyphthalimide (**21**) was treated with PMBCl in DMF at 90 °C to afford PMB hydroxyphthalimide (**22**) in 68% yield. This was hydrolyzed in the presence of hydrazine to give *O*-PMB-hydroxyamine (**23**) in 50% yield.

Before the coupling reaction between acid **27** and amino-histamine was performed, model reactions for the removal of the PMB group were conducted (Scheme 4). Model oxime **25** was readily made from *p*-tolualdehyde (**24**) and PMB-hydroxyl-amine (**23**). Attempted deprotections of **25** with TMSCl/NaI, DDQ,[31] or $AlCl_3$ in acetonitrile were not successful. However, treatment of **25** with $AlCl_3$ and anisole in CH_2Cl_2 removed the PMB group to give **26** in 82% yield.[32] A similar reaction in 1:1 CH_2Cl_2/CH_3NO_2 gave **26** in 79% yield.

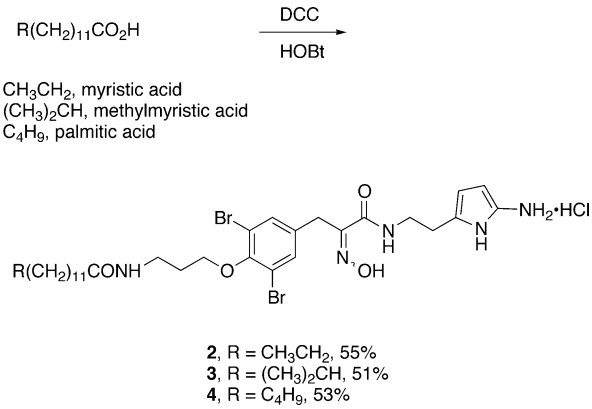
O-PMB-protected oxime acid **27a** was then prepared in 35% yield by treatment of azlactone **18** with *O*-PMB-protected hydroxyamine under basic conditions (Scheme 5). Acid **27a** was treated with aminohistamine in the presence of DCC and HOBt to afford amide **28** in 70% yield. The PMB and Cbz groups of **28** were removed by exposure to $AlCl_3$/anisole to afford purealidin A (**5**) in 94% yield. ^1H and ^{13}C NMR and mass spectra of synthetic **5** were identical to those reported for the

Scheme 5. Synthesis of Purealidin A (**5**) and Purealin (**1**)



Reagents and conditions: (a) $Ba(OH)_2$, dioxane/H_2O, $PMBONH_2$•HCl, 60 °C, 35%. (b) aminohistamine, DCC, HOBt, Et_3N, DMF/$CHCl_3$, rt, 68−72%. (c) $AlCl_3$, anisole, CH_2Cl_2/CH_3NO_2, rt, 94%. (d) **13**, DCC, HOBt, DMF/CH_2Cl_2, rt, 52%.

Scheme 6. Synthesis of Lipopurealins A (**2**), B (**3**), and C (**4**)



Reagents and conditions: (a) **5**, DCC, HOBt, DMF/CH_2Cl_2, rt.

natural product.[33] Racemic purealin **1** was synthesized subsequently in 52% yield by coupling amine **5** with spiroisoxazoline acid **13**. Again, the spectroscopic data for synthetic **1** was identical to the literature values for the natural product.

With purealidin A (**5**) in hand, lipopurealins A, B, and C **2**−**4** were synthesized by DCC/HOBt-mediated coupling to myristic, methylmyristic, and palmitic acids, respectively (Scheme 6). The ^1H NMR spectra of these three compounds were identical to those in the literature.[34]

A 16 member purealidin A library was next prepared by coupling the 4 bromophenyl oxime acids and the 4 amines shown in Figure 2. The oxime acids were 3,5-dibromo-4-butoxyphenyl oxime acid (**27a**), 3-bromo-4-butoxyphenyl oxime acid (**27b**), 3,5-dibromo-2-butoxyphenyl oxime acid (**27c**), and 3,5-dichloro-2-butoxyphenyl oxime acid (**27d**), and the amines were phenethylamine (**29a**), 2-(4-methoxyphenyl) amine (**29b**), 2-(4-chlorophenyl) amine (**29c**), and tyrosine (**29d**).

The synthesis of acid **27b** (Scheme 7) was analogous to that of acid **27a**. 3-Bromo-4-hydroxybenzaldehyde (**30**) was protected by 3-(*N*-benzyloxycarbonylamino)propyl bromide to afford phenyl ether **31** in 79% yield. This was treated with acetylglycine in acetic anhydride at 120 °C for 4 h to afford

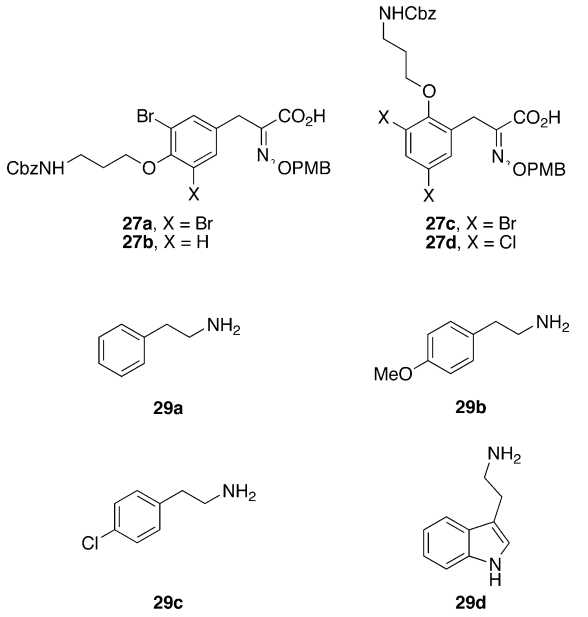
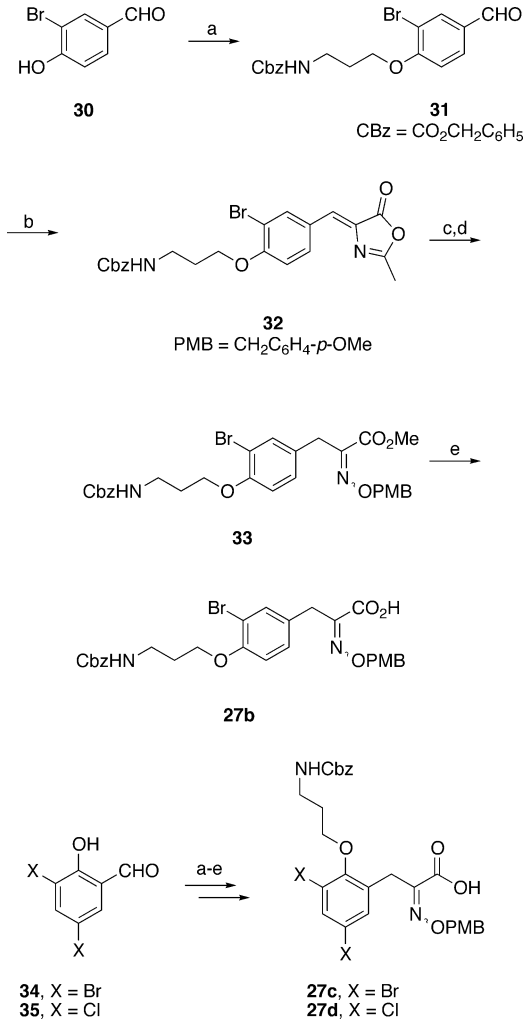


Figure 2. Four acids and four amines used in the library synthesis.

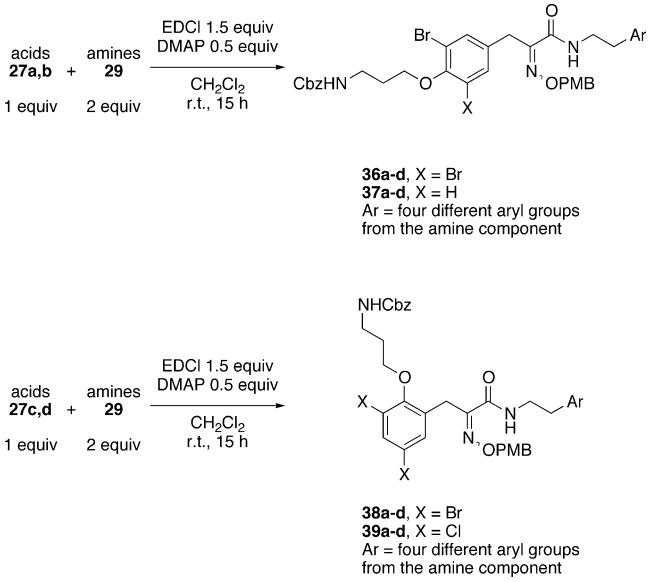
Scheme 7. Synthesis of Bromotyrosine Acids **27b**−**d**



Reagents and conditions: (a) $CbzNHCH_2CH_2CH_2Br$, K_2CO_3, DMF, 100 °C, 79%. (b) acetylglycine, Ac_2O, NaOAc, 125 °C, 85%. (c) $Ba(OH)_2$, dioxane/H_2O, $PMBONH_2$·HCl, 60 °C. (d) $TMSCHN_2$, benzene/MeOH. (e) LiOH, H_2O/MeOH, rt, three steps, 32%.

azlactone **32** in 85% yield. The treatment of **33** with $Ba(OH)_2$ in the presence of $PMBONH_2$·HCl provided oxime acid **27b**, which was an oil that was difficult to purify. The treatment of acid **27b** with $TMSCHN_2$ followed by hydrolysis with LiOH afforded acid **27b** as a white solid in 32% yield over three steps. Acids **27c** and **d** were synthesized by similar procedures.

Scheme 8. Sixteen Coupling Reactions of Amines with Oxime Acids



All of the 16 coupling reactions were achieved by the same protocol (Scheme 8).[35] A mixture of 1 equiv of the acid, 2 equiv of the amine, 1.5 equiv of EDCI, and 0.5 equiv of DMAP in CH_2Cl_2 was stirred at room temperature for 15 h. Diethyl ether was added, and the mixture was washed with 5% HCl to remove EDCI and DMAP. HPLC analyses showed a single peak for each of the 16 desired amides. Table 1 shows the reaction yields and the HPLC retention times of the 16 products. LC−MS analyses further verified the identities and purities of the amides. The removal of the PMB and Cbz groups from the 16 amides provided the 16 purealidin A analogues **40a**−**d**, **41a**−**d**, **42a**−**d**, and **43a**−**d** shown in Figure 3. All 16 library components were purified by flash chromatography followed by preparative HPLC.

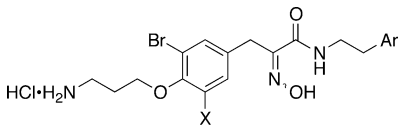
The 21 purealidins and purealin were assessed for antiproliferative activity against a small panel of human carcinoma cells (MDA-MB231 (breast), PC-3 (prostate), 2008 (ovarian), and HT-29 (colon)) as well as against the mouse L1210 leukemia cell line. The L1210 cells were used to compare data obtained here against that reported in the literature for purealidin A.[19] The 50% growth inhibitory (GI_{50}) values obtained (Table 2) showed purealin **1** and purealidin A (**5**) to be inactive against the human cell lines. However, some of the purealidin A analogues, for example, **43c** and **d**, showed low micromolar antiproliferative activity. The mouse leukemia cells, however, were uniformly sensitive to the individual library components.

The abilities of the library components to inhibit the MT-stimulated ATPase activity of a recombinant form of the full motor domain fragment of the rat cytoplasmic dynein heavy chain[36] were examined. First, the library components were screened against 50 μg/mL of rat cytoplasmic dynein motor domain (hereafter dynein motor domain) in the presence of 5 mg/mL of paclitaxel-induced tubulin polymer. Purealin **1** and purealidin A (**5**) gave IC_{50} values of 35 and 42 μM, respectively. The following compounds showed some inhibitory actions, but their IC_{50} values were greater than 50 μM for **2**, **41c** and **d**, **42c** and **d**, and **43d**. Then, inhibitor/protein ratios for the best ATPase inhibitory ranges were examined. Three compounds

Table 1. Yields and HPLC Retention Times of Sixteen Synthetic Amides Prepared as Precursors to the Test Agent Library

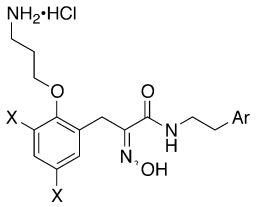
compd	yield	retention time (min)[a]	final product	compd	yield	retention time (min)[a]	final product
36a	93%	9.8	**40a**	**38a**	82%	12.8	**42a**
36b	93%	9.0	**40b**	**38b**	90%	11.5	**42b**
36c	96%	12.6	**40c**	**38c**	79%	16.8	**42c**
36d	91%	8.2	**40d**	**38d**	81%	9.9	**42d**
37a	89%	7.0	**41a**	**39a**	91%	11.2	**43a**
37b	95%	6.4	**41b**	**39b**	88%	10.0	**43b**
37c	91%	8.7	**41c**	**39c**	87%	14.5	**43c**
37d	88%	5.7	**41d**	**39d**	86%	8.6	**43d**

[a] 7:13 CH_3CN/H_2O (0.1% CF_3CO_2H), Symmetry C18 column, l mL/min.



Ar, X

40a	phenyl, Br
40b	4-methoxyphenyl, Br
40c	4-chlorophenyl, Br
40d	3-indolyl, Br
41a	phenyl, H
41b	4-methoxyphenyl, H
41c	4-chlorophenyl, H
41d	3-indolyl, H



Ar, X

42a	phenyl, Br
42b	4-methoxyphenyl, Br
42c	4-chlorophenyl, Br
42d	3-indolyl, Br
43a	phenyl, Cl
43b	4-methoxyphenyl, Cl
43c	4-chlorophenyl, Cl
43d	3-indolyl, Cl

Figure 3. Structures of 16 purealidin A analogues.

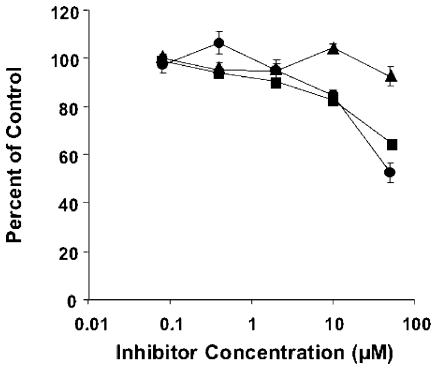


Figure 4. Dynein motor domain ATPase inhibitory activities of purealin **1**, purealidin A (**5**), and compound **41c**. Dynein motor domain (5 μg/mL) and 1 mg/mL of paclitaxel-induced tubulin polymer were used in this assay. The reaction was initiated by the addition of 2 mM ATP: **1** (●); **5** (■); and **41c** (▲). Each point is the mean of four determinations ± SD.

within the library, purealin **1**, purealidin A (**5**), and compound **41c** (the latter chosen because of its antiproliferative effects) showed ATPase inhibitory properties (Figure 4).

The concentration-dependent inhibition of MT-stimulated ATPase by purealin **1** against the dynein motor domain is shown

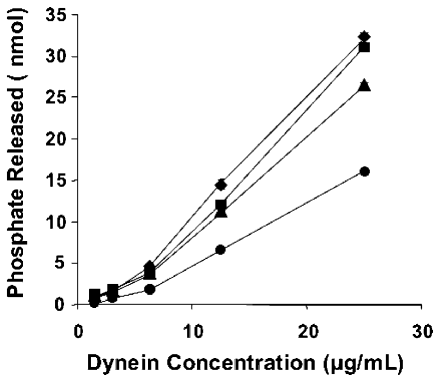


Figure 5. Inhibition by purealin **1** of dynein motor domain catalyzed release of free phosphate from ATP. The dynein motor domain was used in the presence of the paclitaxel-induced tubulin polymer (1 mg/mL) and a test agent. ATP (2 mM) was added to initiate the reaction, and the concentration of free phosphate was determined. No inhibitor (♦); 2 μM **1** (■); 10 μM **1** (▲); and 50 μM **1** (●). Each point is the mean of four determinations ± SD (some of the error bars are smaller than the symbols used).

in Figure 5. To investigate the kinetics in the presence of inhibitors, purealin **1**, purealidin A (**5**), and **41c** were each incubated at 50 μM with different concentrations of the substrate, ATP, to determine the Michaelis−Menten parameters of the dynein motor domain (Figure 6a). The calculated V^{app}_{max}, K^{app}_{M} and K_i values are given in Table 3. In a typical dynein motor domain ATPase kinetic assay, the basal ATPase activity was 0.41s^{-1} (V_{max}), whereas the MT-stimulated ATPase activity was 2.89 s^{-1} (V_{max}), 7-fold of the basal activity. These results further show the inhibitory effects of purealin and analogues on the MT-stimulated dynein motor domain ATPase activity. The rank order of inhibition was purealin **1** > purealidin A (**5**) ≥ **41c**. An examination of the kinetics in the Hanes−Woolf format (Figure 6b), wherein no significant change in the *y* intercept was noted, indicated that the compounds displayed uncompetitive inhibition, supporting the hypothesis that purealins do not bind to the ATP site.

Conclusions

Dynein plays an important role in variety of cellular processes. Recent work is beginning to uncover the mechanism of dynein movement and its power stroke.[36−38] A variety of molecules involved in cell growth control, including p53, Bim, cdc2 kinase, HDAC6, HSP70, and HSP90 have also been reported to interact with or be under the control of cytoplasmic dynein.[39−44] Motor activity and motor-cargo interactions in dynein and other motor proteins, such as kinesin and myosin, might represent attractive drug targets and are certainly of interest in the development of chemical biology tools. Recently, RNA interference technology has been applied against dynein as well as its regulatory polypeptides,[45−49] showing that lowering the level of the cytoplasmic dynein heavy chain (and therefore its activity in

Table 2. Antiproliferative Activities of the Purealin/Purealidin Library

compd	GI_{50} (μM)				
	2008	MDA-MB231	HT29	PC3	L1210
40a	29 ± 2	27 ± 0	31 ± 3	31 ± 3	6.4 ± 2.2
40b	24 ± 3	17 ± 2	20 ± 5	21 ± 5	2.8 ± 2.8
40c	29 ± 1	27 ± 2	31 ± 1	30 ± 4	7.8 ± 2.5
40d	27 ± 1	24 ± 2	26 ± 4	26 ± 9	5.8 ± 1.2
41a	30 ± 5	>50	27 ± 4	31 ± 6	6.2 ± 0.9
41b	31 ± 12	48 ± 14	30 ± 9	39 ± 4	9.9 ± 6.1
41c	27 ± 3	26 ± 1	27 ± 1	33 ± 4	6.5 ± 2.0
41d	26 ± 2	25 ± 1	30 ± 1	32 ± 6	7.6 ± 1.9
42a	19 ± 5	5.7 ± 0.8	5.3 ± 0.2	19 ± 6	6.3 ± 0.4
42b	25 ± 2	8.8 ± 0.6	4.9 ± 0.6	35 ± 15	6.2 ± 0.9
42c	5.6 ± 0.9	5.6 ± 0.7	5.3 ± 0.2	15 ± 7	6.3 ± 0.8
42d	7.5 ± 0.4	6.7 ± 1.0	6.0 ± 0.1	31 ± 6	5.4 ± 0.9
43a	8.8 ± 2.7	7.6 ± 3.2	5.6 ± 0.2	31 ± 7	7.5 ± 0.9
43b	29 ± 1	24 ± 2	6.2 ± 1.7	32 ± 11	6.9 ± 0.5
43c	5.4 ± 0.6	5.2 ± 0.9	5.5 ± 1.8	7.8 ± 0.9	5.9 ± 0.5
43d	5.7 ± 0.1	5.3 ± 0.0	6.0 ± 0.6	5.0 ± 1.1	5.8 ± 1.1
2	28 ± 3	28 ± 1	29 ± 2	30 ± 4	9.0 ± 1.9
3	29 ± 1	28 ± 3	30 ± 0	25 ± 9	12 ± 4
4	5.7 ± 1.2	7.0 ± 0.4	20 ± 3	32 ± 3	9.7 ± 1.6
5	>50	>50	>50	>50	4.7 ± 1.6
1	>50	>50	>50	>50	3.2 ± 1.2
discodermolide (nM)	2.7 ± 1.7	40 ± 7	28 ± 9	52 ± 8	9.2 ± 4.3

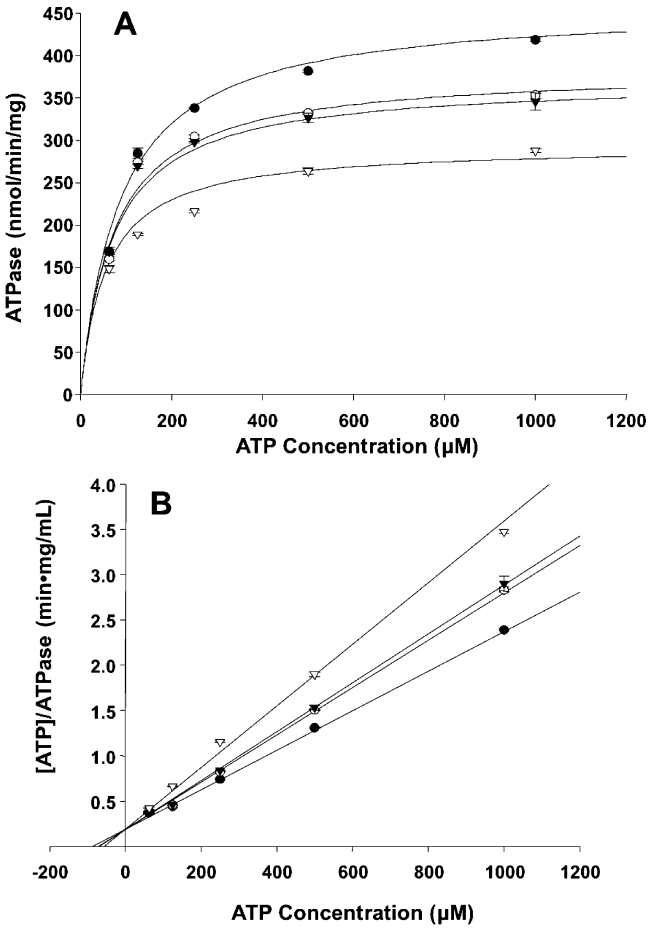


Figure 6. Michaelis−Menten (A) and Hanes−Woolf (B) plots of dynein motor domain ATPase activity in the presence of purealin **1**, purealidin A (**5**), and compound **41c**. The reaction was initiated with different concentrations of ATP in the presence of 12.5 μg/mL of recombinant dynein heavy chain and 1 mg/mL of paclitaxel-induced tubulin polymer. No inhibitor (●); 50 μM **41c** (○); 50 μM **5** (▼); 50 μM **1** (▽).

bulk) leads to a block in mitosis.[45] Effective small-molecule dynein inhibitors could therefore be useful for further investigation of the cellular function of dynein in vivo.

Table 3. V^{app}_{max}, K^{app}_{M}, and K_i Values of the Microtubule-Stimulated ATPase Activity of Recombinant Dynein Heavy Chain Motor Domain in the Presence of 50 μM **1**, **5**, or **41c**

	V^{app}_{max} (s^{-1})	K^{app}_{M} (mM)	K_i (mM)
no inhibitor	2.89	0.091	N/A
41c	2.45	0.073	0.30
5	2.39	0.070	0.26
1	1.84	0.061	0.85

Very little is known, however, about the structural requirements of small molecules to inhibit dynein. Agents that competitively inhibit the binding of ATP to the protein are the most studied, and redox active agents will also inhibit the protein. The only non-ATP, noninorganic and nonredox active agent known to inhibit the axonemal isoform of cytoplasmic dynein is the natural product purealin **1**,[16] which acted as our seminal lead in these studies. Our results showed that synthetic purealin **1** and two of its analogues inhibit MT-stimulated cytosolic dynein heavy chain's ability to hydrolyze ATP. Thus, purealins might be useful for further physiological investigation of dynein function.

Kon and co-workers[50] showed that ATP binding and its hydrolysis only at the P1 site are essential for the motor activities of cytoplasmic dynein and suggested that the motor activities are also regulated by the other nucleotide-binding/hydrolysis sites. For example, nucleotide binding at the P3 site is also critical for MT-activated ATPase and the motile activities of cytoplasmic dynein.[50] It is known that purealin **1** inhibits the ATPase activity of axonemal dynein but does not compete for the ATP binding site.[16] Here, we found it to inhibit cytoplasmic-dynein motor domain ATPase activity and examined the kinetics of inhibition by **1** and by some of its analogues (Figure 6). Plotting the data in the Hanes−Woolf format showed no significant change in the *y* intercept, which represents the value of K_m/V_{max}, in the presence of purealin **1**, purealidin A (**5**), and **41c.** This indicates that the binding pattern of the purealins to the cytoplasmic dynein motor domain is uncompetitive, meaning the inhibitors bind to a site that becomes available only after ATP has bound to the P1 site, giving rise to the conclusion that purealins do not compete for the binding of ATP or its hydrolysis at the P1 site. The binding site of purealins is still unknown.

Although purealin **1** and purealidin A (**5**) were inactive as human cancer cell antiproliferative agents, their inhibition of cytoplasmic dynein ATPase activity supports the hypothesis that purealin/purealidin and analogues are good leads for finding small molecules to inhibit this target. Compound **41c** showed reasonable antiproliferative activity against human carcinoma cell lines as well as the mouse leukemia cell line and inhibited dynein motor domain ATPase activity, providing correlative support of the hypothesis that small molecule inhibitors of the molecular motor can have antiproliferative effects. Compared to the effects on human cancer cell lines, the growth of the mouse leukemia L1210 cell line was uniformly sensitive to low micromolar concentrations of the purealin/purealidin library components. Future work will include more detailed cell-based experiments to begin to determine the mechanisms by which these bromotyrosine-containing agents exert their antiproliferative effects and whether the mechanisms include the inhibition of cellular cytoplasmic dynein.

Experimental Details

Chemistry. General Procedures. All reactions were performed under an atmosphere of argon, unless the reaction solvent contained H_2O. Benzene was distilled from Na/benzophenone. CH_2Cl_2, THF, Et_2O, and toluene were dried by passing through activated alumina.[51] High-resolution mass spectra were obtained on a V/G 70/70 double focusing instrument. High-performance liquid chromatography (HPLC) was performed using the indicated ratios of CH_3CN to H_2O, both containing 0.1% CF_3CO_2H (TFA), flowing at a rate of 1 mL/min through a Symmetry C_{18} column, and analytes were detected by UV absorption. Percent purities given represent the relative purity from the reactions (the purities of compounds used for biological evaluations was >99%).

3-(2-Benzyloxy-3,5-dibromo-4-methoxyphenyl)-2-benzyloxyiminopropanoic Acid (7). A mixture of azlactone **6** (12 g, 25 mmol) and $Ba(OH)_2$ (30 g, 175 mmol) in 1:1 dioxane/H_2O (360 mL) was stirred for 1 h at 60 °C. *O*-Benzyl hydroxylamine hydrochloride (12 g, 75 mmol) was added at 60 °C, and the mixture was stirred vigorously at the same temperature for 14 h. The reaction mixture was cooled to 0 °C, the solution was acidified with 15% HCl, and a yellow solid precipitated. After filtration, the solid was washed with cold 1:1 pentane/Et_2O to yield **7** as a pale-yellow solid (10.5 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62−7.59 (m, 2H), 7.52−7.45 (m, 6H), 7.34−7.30 (m, 2H), 7.37 (s, 1H), 5.33 (s, 2H), 5.12 (s, 2H), 4.02 (s, 2H), 4.00 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 162.3, 154.5, 154.3, 149.3, 136.5, 135.6, 132.0, 128.8, 128.75, 128.5, 128.3, 128.2, 127.9, 114.7, 112.9, 78.6, 74.9, 60.7, 25.2.

Methyl 3-(2-benzyloxy-3,5-dibromo-4-methoxyphenyl)-2-benzyloxyiminopropanoate (8). $TMSCHN_2$ (12 mL, 2.0 N in hexane) was added to a solution of acid **7** (9.0 g, 16 mmol) in 3:1 benzene/MeOH (160 mL) at 0 °C. The reaction mixture was stirred for 45 min at room temperature. 5% HCl (10 mL) was added at 0 °C. A yellow solid precipitated after most of the organic solvents were removed. After filtration, the solid was washed with cold 1:1 pentane/Et_2O to yield **8** (8.03 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49−7.46 (m, 2H), 7.39−7.29 (m, 6H), 7.21−7.17 (m, 2H), 7.19 (s, 1H), 5.23 (s, 2H), 4.93 (s, 2H), 3.89 (s, 2H), 3.86 (s, 3H), 3.69 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 163.6, 154.2, 153.8, 149.9, 136.5, 136.0, 132.0, 128.6, 128.5, 128.4, 128.35, 128.2, 128.1, 127.9, 114.5, 112.7, 78.0, 74.4, 60.6, 52.8, 26.4.

Methyl 3-(2-hydroxy-3,5-dibromo-4-methoxyphenyl)-2-hydroxyiminopropanoate (9). A solution of benzyl ester **8** (7.4 g, 12.8 mmol) in 1:1 AcOH/dioxane (180 mL) was hydrogenated over Pd-black (1.21 g) under H_2 (1 atm) at room temperature for 24 h. After filtration, the solvent was removed under reduced pressure. EtOAc (200 mL) was added, and the solution was washed with H_2O, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. The crude product was purified by column chromatography (2:1 hexane/EtOAc) to afford **9** (4.19 g, 82%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42 (s, 1H), 3.94 (s, 2H), 3.91 (s, 3H), 3.86 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 164.5, 153.7, 151.5, 149.9, 133.4, 119.4, 107.6, 60.6, 53.5, 25.5.

Methyl 7,9-dibromo-8-methoxy-10-oxo-1-oxo-2-azospirol[4.5]-deca-2,6,8-triene-3-carboxylate (10). A mixture of NBS (940 mg, 5.29 mmol) and *O*-phenolic oxime acid **9** (1.40 g, 3.53 mmol) in DMF (15 mL) was stirred at room temperature for 3 h. After the addition of Et_2O (200 mL), the solution was washed successively with H_2O, 5% aqueous NaS_2O_3, and H_2O, then dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure to give spiroisoxazoline **10** (1.29 g, 92%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.78 (s, 1H), 4.18 (s, 3H), 3.91 (s, 3H), 3.61 (d, J = 18.0 Hz, 1H), 3.30 (d, J = 18.0 Hz, 1H).

Methyl 7,9-dibromo-8-methoxy-10-hydroxy-1-oxo-2-azospirol-[4.5]deca-2,6,8-triene-3-carboxylate (*trans*-11 and *cis*-12). Zn-$(BH_4)_2$ (0.1 N solution in Et_2O, 35 mL) was added to a solution of crude spiroisoxazoline **10** (1.1 g, 2.78 mmol) in CH_2Cl_2 (35 mL) at 0 °C over a 10 min period. The reaction mixture was stirred for another 5 min at room temperature. After the addition of saturated aqueous NH_4Cl, the mixture was extracted with Et_2O (3 × 100 mL). The extracts were washed with H_2O, dried over $MgSO_4$, and filtered. The crude product was purified by column chromatography (2:1 hexane/EtOAc) to afford **11** (290 mg, 26.3%) and **12** as pale-yellow solids (170 mg, 15.5%). ***trans*-11:** ^{1}H NMR (300 MHz, acetone-d_6) δ 6.53 (s, 1H), 5.42 (d, J = 8.1 Hz, 1H), 4.21 (d, J = 8.1 Hz, 1H), 3.84 (d, J = 18.0 Hz, 1H), 3.83 (s, 3H), 3.72 (s, 3H), 3.20 (d, J = 18.0 Hz, 1H), 2.83 (s, 1H); ^{13}C NMR (75 MHz, acetone-d_6) 161.2, 152.5, 148.8, 132.2, 122.2, 113.8, 92.5, 75.2, 60.2, 52.8, 40.0. ***cis*-12:** ^{1}H NMR (300 MHz, acetone-d_6) δ 6.31 (s, 1H), 5.07 (brs, 1H), 4.55 (brs, 1H), 3.82 (s, 3H), 3.73 (s, 3H), 3.46 (d, J = 18.0 Hz, 1H), 3.39 (d, J = 18.0 Hz, 1H); ^{13}C NMR (75 MHz, acetone-d_6) 161.3, 152.3, 146.2, 131.7, 127.6, 124.0, 90.5, 74.47, 60.5, 52.9, 43.4.

7,9-Dibromo-8-methoxy-10-hydroxy-1-oxo-2-azospirol[4.5]-deca-2,6,8-triene-3-carboxylic Acid (13). A mixture of spiroisoxazoline ester **11** (198 mg, 0.5 mmol) and $LiOH\cdot H_2O$ (63 mg, 1.5 mmol) in 1:1 MeOH/H_2O (30 mL) was stirred for 30 min at room temperature. After the addition of 5% HCl (3 mL), the solution was concentrated under reduced pressure. EtOAc (50 mL) was added. The mixture was washed with H_2O, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure to afford **13** (189 mg, 98%) as a pale-yellow solid, mp 110−112 °C. IR (KBr, cm^{-1}): 3500 (broad), 1723, 1589; ^{1}H NMR (300 MHz, CD_3OD): δ 6.44 (s, 1H), 4.91 (s, 3H), 4.11 (s, 1H), 3.75 (d, J = 18.0 Hz, 1H), 3.73 (s, 3H), 3.10 (d, J = 18.0 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) 162.7, 153.9, 149.3, 132.2, 122.8, 114.2, 93.1, 75.5, 60.4, 40.2; MS (EI) *m*/*z*: ($M^{+\bullet}$) 381 ($^{79}Br_2$, 12), 365 (34), 351 (57), 69-(100); HRMS (EI) *m*/*z*: ($M^{+\bullet}$) calcd for $C_{10}H_9{}^{79}Br_2NO_5$, 380.8847; found, 380.8865.

Purealin (1). A mixture of acid **13** (11.5 mg, 0.03 mmol), purealidin A **5** (22 mg, 0.04 mmol, free amine), DCC (9.3 mg, 0.045 mmol), and HOBt (6.1 mg, 0.045 mmol) in CH_2Cl_2 (3 mL) and DMF (3 mL) was stirred at room temperature for 20 h. After the solvent was removed under reduced pressure, the crude product was purified by flash chromatography (15:9:1:1 EtOAc/acetone/HCO_2H/H_2O). The product was dissolved in MeOH and acidified with 0.5 N HCl. Solvents were removed to afford **1** as a pale-yellow solid (14 mg, 54%), mp 139−141 °C. IR (KBr, cm^{-1}): 3450 (broad), 1655, 1541; ^{1}H NMR (300 MHz, CD_3OD): δ 7.47 (s, 2H), 6.51 (s,1H), 6.42 (d, J = 0.6 Hz, 1H), 4.08 (d, J = 0.6 Hz, 1H), 4.04 (t, J = 6.0 Hz, 2H), 3.83 (s, 2H), 3.78 (d, J = 18.3 Hz, 1H), 3.72 (s, 3H), 3.58 (t, J = 6.9 Hz, 2H), 3.46 (t, J = 6.9 Hz, 2H), 3.09 (t, J = 18.3 Hz, 1H), 2.70 (t, J = 6.9 Hz, 2H), 2.10 (quin, J = 6.6 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) 163.1, 158.9, 154.5, 150.8, 150.75, 147.1, 146.8, 136.2, 132.8, 131.2, 124.1, 120.7, 117.2, 113.1, 109.0, 90.2, 73.5, 71.2, 59.6, 37.3, 36.2, 29.3, 27.8, 24.3; MS (ESI) $[M + H]^+$ *m*/*z*: 880 ($^{79}Br_4$, 6), 882 ($^{79}Br_3{}^{81}Br$, 16), 884 ($^{79}Br_2{}^{81}Br_2$, 17), 886 ($^{79}Br^{81}Br_3$, 13), 888 ($^{81}Br_4$, 5); HRMS (ESI) $[M + H]^+$ calcd for $C_{27}H_{29}{}^{79}Br_4N_7O_7$ *m*/*z*: 879.8884; found, 879.8935. HPLC analysis: 8:17 CH_3CN (0.1% TFA)/H_2O (0.1% TFA), t_R = 19.9 min, 98% purity.

3,5-Dibromo-4-hydroxybenzaldehyde (16). Bromine (30.2 g, 190 mmol) in AcOH (50 mL) was added to a mixture of 4-hydroxybenzaldehyde (11 g, 90 mmol) and sodium acetate (22.9 g, 279 mmol) in AcOH (200 mL) at room temperature over 20 min. The reaction mixture was stirred for 1 h at room temperature. A solid precipitated after H_2O (200 mL) was added. After filtration, the solid was washed with H_2O and dried in a vacuum desiccator with P_2O_5 overnight to afford **16** as a pale-yellow solid (24.1 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.80 (s, 1H), 8.00 (s, 2H), 6.43 (s, 1H); ^{13}C NMR (75 MHz, acetone-d_6) 189.8, 157.0, 135.0, 132.7, 112.4.

General Procedure B: Synthesis of Phenol Ethers. Benzyl [3-(2,6-dibromo-4-formylphenoxy)propyl]carbamate (17). A mixture of phenol **16** (2.8 g, 10 mmol), K_2CO_3 (3.45 g, 25 mmol), and **16** (3.0 g, 11 mmol) in DMF was stirred at 100 °C for 4 h. The reaction mixture was extracted with Et_2O (5 × 40 mL). The extracts were washed with H_2O, dried over $MgSO_4$, filtered, and the solvent removed under vacuum. The crude product was purified by column chromatography (2:1 hexane/EtOAc) to afford **17** as a white solid (3.32 g, 70.5%). IR (KBr, cm^{-1}): 3311, 3063, 1693, 1544; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.86 (s, 1H), 8.03 (s, 2H), 7.38−7.35 (m, 5H), 5.16 (brs, 1H), 5.13 (s, 2H), 4.15 (t, J = 5.8 Hz, 2H), 3.56 (q, J = 6.3 Hz, 2H), 2.12 (quin, J = 6.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 188.3, 157.8, 156.4, 136.5, 134.2, 133.8, 128.4, 128.0, 119.3, 71.6, 66.6, 38.4, 29.9; MS (EI) m/z: ($M^{+\bullet}$) 468 ($^{79}Br_2$, 30), 277 (65), 192 (40), 91 (100); HRMS (EI) m/z: ($M^{+\bullet}$) calcd for $C_{18}H_{16}{}^{79}Br_2NO_4$, 467.9446; found, 467.9464.

General Procedure C: Synthesis of Azlactones. Benzyl {3-[2,6-dibromo-4-(5-oxo-2-methyloxazol-4-ylidenemethyl)phenoxy]-propyl}carbamate (18). A mixture of aldehyde **17** (3.02 g, 6.28 mmol), sodium acetate (515 mg, 6.28 mmol), and *N*-acetylglycine (735 mg, 6.28 mmol) in Ac_2O (12.5 mL) was stirred at 120 °C for 4 h. A yellow solid precipitated after the reaction mixture was cooled to room temperature. After filtration, the solid was washed with cold 1:1 pentane/Et_2O to yield **18** as a yellow solid (3.35 g, 97%). IR (KBr, cm^{-1}): 3316, 3066, 1807, 1772, 1682; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (s, 2H), 7.38−7.33 (m, 5H), 6.92 (s, 1H), 5.21 (brs, 1H), 5.13 (s, 2H), 4.12 (t, J = 5.7 Hz, 2H), 3.55 (q, J = 6.3 Hz, 2H), 2.44 (s, 3H), 2.11 (quin, J = 6.0 Hz, 2H);^{13}C NMR (75 MHz, $CDCl_3$): 167.3, 167.0, 156.4, 154.7, 136.6, 135.8, 133.7, 131.8, 128.5, 128.1, 127.0, 118.6, 71.5, 66.6, 38.5, 29.9, 15.7; MS (ESI) m/z: [M + Na]$^+$ 573 ($^{79}Br_2$, 50), 575 ($^{79}Br^{81}Br$, 100), 553 (35), 551 (15); HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{22}H_{20}{}^{79}Br_2N_2O_5Na$, 572.9614; found, 572.9565.

3-[4-(3-Benzyloxycarbonylaminopropoxy)-3,5-dibromophenyl]-2-benzyloxyiminopropanoic Acid (19). A mixture of azlactone **18** (2.2 g, 4 mmol) and $Ba(OH)_2$ (4.8 g, 28 mmol) in 1:1 dioxane/H_2O (56 mL) was stirred at 60 °C for 1 h. *O*-Benzylhydroxylamine hydrochloride (2.05 g, 12.8 mmol) was added at 60 °C, and the mixture was stirred vigorously at the same temperature for 6 h. The reaction mixture was cooled to 0 °C and acidified with 10% HCl. The mixture was extracted with EtOAc. The extracts were washed with H_2O, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. The crude product was purified by column chromatography (10:1 EtOAc/MeOH) to afford **19** (850 mg, 34%) as a pale-yellow solid, mp 118−120 °C. IR (KBr, cm^{-1}): 3437, 2933, 1763, 1721, 1513, 1455, 1369, 1231; ^{1}H NMR (300 MHz, 1:2 $CDCl_3$/CD_3OD) δ 7.47 (s, 2H), 7.35 (brs, 10H), 5.31 (s, 2H), 5.10 (s, 2H), 4.02 (t, J = 5.7 Hz, 2H), 3.82 (s, 2H), 3.27 (t, J = 6.6 Hz, 2H), 2.07 (quin, J = 6.0, 2H); ^{13}H NMR (75 MHz, 1:2 $CDCl_3$/CD_3OD) 164.2, 156.8, 151.2, 149.7, 136.3, 135.7, 134.5, 133.0, 128.3, 128.0, 127.6, 127.4, 117.4, 77.7, 70.8, 66.1, 38.0, 29.4; MS (ESI) m/z: [M + Na]$^+$ 655($^{79}Br_2$, 15), [M + H]$^+$ 635-($^{79}Br_2$, 10), 591(100), 547(20), 439(15); HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{27}H_{26}{}^{79}Br_2N_2O_6Na$, 655.0021; found, 655.0055.

Benzyl [3-(4-{2-[(2-amino-3*H*-imidazol-4-ylmethyl)carbamoyl]-2-benzyloxyiminoethyl}-2,6-dibromophenoxy)propyl]carbamate (20). A mixture of the acid **19** (320 mg, 0.5 mmol), aminohistamine (200 mg, 1 mmol), HOBt (135 mg, 1 mmol), DCC (192 mg, 1 mmol), and Et_3N (2.8 mL) in 1:1 DMF/$CHCl_3$ (30 mL) was stirred for 48 h at room temperature. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography (15:9:1:1 EtOAc/acetone/HCO_2H/H_2O) to afford amide **20** (245 mg, 66%) as a pale-yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ 7.39 (s, 2H), 7.37−7.29 (m, 10H), 6.48 (s, 1H), 5.25 (s, 2H), 5.07 (s, 2H), 3.98 (t, J = 6.0 Hz, 2H), 3.79 (s, 3H), 3.48 (t, J = 6.1 Hz, 2H), 3.40 (t, J = 6.1 Hz, 2H), 2.70 (t, J = 6.1 Hz, 2H), 2.00 (quin, J = 6.1 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) 164.7, 158.9, 153.0, 152.7, 148.7, 138.4, 138.0, 136.7, 134.5, 129.6, 129.5, 129.4, 128.9, 128.8, 126.2, 78.7, 72.1, 67.4, 39.0, 31.3, 29.7, 25.8; MS (ESI) m/z: [M + H]$^+$ 741 ($^{79}Br_2$, 40), 743 ($^{79}Br^{81}Br$, 100); HRMS (ESI) m/z: [M + H]$^+$ calcd for $C_{32}H_{35}{}^{79}Br_2N_6O_5$, 741.1018; found, 741.1030.

2-(4-Methoxybenzyloxy)isoindole-1,3-dione (22). A mixture of *N*-hydroxyphthalimide (26.1 g, 160 mmol), PMBCl (25 g, 160 mmol), and Et_3N (53 mL, 384 mmol) in DMF (400 mL) was stirred for 40 min at 90 °C. A solid precipitated after the mixture was poured into ice water (500 mL). The solid was collected by filtration. The solid was washed with H_2O and dried under vacuum to yield **22** as a pale-yellow solid (30.8 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73−7.70 (m, 2H), 7.44 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.14 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 163.4, 160.3, 134.3, 131.5, 128.7, 125.7, 123.3, 113.8, 79.4, 55.1.

***O*-(4-Methyoxybenzyl)hydroxylamine Hydrochloride (23).** Compound **22** (18.7 g, 66 mmol) in 12:25 DMF/MeOH (270 mL) was heated to 60 °C and treated with hydrazine hydrate (10 mL). After 5 min, the mixture was cooled to room temperature. H_2O (100 mL) was added, and most of the MeOH was removed under reduced pressure. The solution was extracted with EtOAc (4 × 100 mL), washed with H_2O, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. The crude product was purified by column chromatography (2:1 hexane/EtOAc) to provide an oil. MeOH (30 mL) and concentrated HCl (6 mL) were added to the oil at 0 °C, and the solvent was removed under reduced pressure to afford **23** as a white solid (6.2 g, 50%). ^{1}H NMR (300 MHz, CD_3OD): δ 7.37 (d, J = 6.9 Hz, 2H), 6.98 (d, J = 6.9 Hz, 2H), 4.95 (s, 2H), 3.82 (s, 3H).

4-Methylbenzaldehyde *O*-(4-methoxybenzyl)oxime (25). A mixture of amine **23** (379 mg, 2 mmol) and 4-methylbenzaldehyde **24** (180 mg, 1.5 mmol) in pyridine (20 mL) was stirred for 3 h at room temperature. EtOAc (50 mL) was added after the solvent was removed under reduced pressure. The solution was washed successively with H_2O, saturated aqueous $CuSO_4$ and H_2O, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. The crude product was purified by column chromatography (20:1 hexane/EtOAc) to afford **25** as a white solid (350 mg, 92%). IR (KBr, cm^{-1}): 2931, 1612, 1584, 1511, 1441; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07 (s, 1H), 7.45 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.0 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 5.12 (s, 2H), 3.78 (s, 3H), 2.34 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 159.4, 148.9, 139.9, 130.1, 129.5, 129.4, 129.3, 127.0, 113.8, 76.0, 55.2, 21.4; MS (EI) m/z: 255 ($M^{+\bullet}$, 30), 122 (100), 91 (46), 77 (65); HRMS (EI) m/z: ($M^{+\bullet}$) calcd for $C_{16}H_{17}NO_2$, 255.1257; found, 255.1259.

4-Methylbenzaldehyde Oxime (26). Anhydrous $AlCl_3$ was added to a solution of PMB ether **25** (25.5 mg, 0.1 mmol) in anisole (43 mg, 0.4 mmol) and dry CH_2Cl_2 (4 mL) at 0 °C. The reaction was quenched by the addition of H_2O after stirring for 40 min at room temperature. The resulting mixture was extracted with CH_2Cl_2 (10 mL), and the organic phase was washed with H_2O, dried with $MgSO_4$, filtered, and the solvent removed. The crude product was purified by flash chromatography (5:1 hexane/EtOAc) to afford **26** as a colorless oil (11 mg, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12 (s, 1H), 8.02 (s, 1H), 7.47 (d, J = 8.1 Hz, 2H), 7.19 (d, J = 8.1 Hz, 2H), 2.37 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 150.4, 140.2, 129.5, 129.1, 127.0, 21.3.

4-[4-(3-Benzyloxycarbonylaminopropoxy)-3,5-dibromophenyl]-2-(4-methoxybenzyloxyimino)propanoic Acid (27a). A mixture of azlactone **18** (2.2 g, 4 mmol) and $Ba(OH)_2$ (4.8 g, 28 mmol) in 1:1 dioxane/H_2O (56 mL) was stirred at 60 °C for 1 h. *O*-PMB-hydroxylamine hydrochloride (1.51 g, 8 mmol) was added at 60

°C, and the mixture was stirred vigorously at the same temperature for 13 h. The reaction mixture was cooled to 0 °C and acidified with 10% HCl. The reaction mixture was extracted with EtOAc. The extracts were washed with H_2O, dried over $MgSO_4$, and filtered, and the solvent was evaporated. The crude product was purified by column chromatography (6:1 CH_2Cl_2/MeOH) to afford **27a** (934 mg, 33.5%) as an oil. IR (KBr, cm^{-1}): 3320 (broad), 2946, 1698, 1688, 1612, 1543, 1514, 1456, 1257; ^1H NMR (300 MHz, 3:1 $CD_3OD/CDCl_3$) δ 7.39 (s, 2H), 7.38−7.35 (m, 5H), 7.24 (d, J = 7.5 Hz, 2H), 6.86 (d, J = 7.5 Hz, 2H), 5.11 (brs, 1H), 5.06 (s, 2H), 4.86 (s, 2H), 3.96 (t, J = 5.9 Hz, 2H), 3.77 (s, 3H), 3.40 (t, J = 6.7 Hz, 2H), 2.02 (quin, J = 6.3 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD:$CDCl_3$ = 3:1) 160.7, 158.5, 152.3, 138.0, 137.2, 134.3, 130.9, 130.0, 129.2, 128.7, 128.5, 118.4, 114.8, 77.7, 71.9, 67.2, 55.7, 39.0, 31.3, 30.9: MS (ESI) *m/z*: $[M + Na]^+$ 685 ($^{79}Br_2$, 43), 687 ($^{79}Br^{81}Br$, 100), 621 (35), 211 (46); HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{28}H_{28}{}^{79}Br_2N_2O_7Na$, 685.0175; found, 685.0161.

Benzyl (3-{4-[2-[2-(2-amino-3*H*-imidazol-4-yl)ethylcarbamoyl]-2-(4-methoxybenzyloxyimino)ethyl]-2,6-dibromophenoxyl}-propyl)carbamate (28). A mixture of acid **27a** (220 mg, 0.33 mmol), aminohistamine (135 mg, 0.66 mmol), HOBt (89 mg, 0.66 mmol), DCC (192 mg, 1 mmol), and Et_3N (2.8 mL) in 1:1 DMF/$CHCl_3$ (30 mL) was stirred for 26 h at room temperature. The solvent was removed under reduced pressure, and the crude product was subjected to column chromatography (15:9:1:1 EtOAc/acetone/HCO_2H/H_2O) to provide **28** as a pale-yellow solid (152 mg, 68%). IR (KBr, cm^{-1}): 3387 (broad), 1676, 1527, 1455, 1256; ^1H NMR (300 MHz, CD_3OD): δ 7.34 (s, 2H), 7.32−7.23 (m, 5H), 7.24 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 6.51 (s, 1H), 3.96 (t, J = 5.8 Hz, 2H), 3.77 (s, 3H), 3.75 (s, 2H), 3.49 (t, J = 6.6 Hz, 2H), 3.39 (t, J = 6.9 Hz, 2H), 2.72 (t, J = 6.6 Hz, 2H), 2.01 (quin, J = 6.5 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) 164.8, 161.3, 158.8, 152.9, 152.5, 149.0, 138.4, 136.7, 134.5, 131.2, 130.0, 129.4, 128.9, 128.7, 126.1, 118.8, 115.1, 110.7, 39.2, 39.0, 31.3, 29.7, 25.8; MS (ESI) *m/z*: $[M + H]^+$ 771 ($^{79}Br_2$, 20), 773 ($^{79}Br^{81}Br$, 100), 239 (80); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{33}H_{37}{}^{79}Br_2N_6O_6$, 771.1110; found, 771.1141.

Purealidin A (5). A mixture of amide **28** (130 mg, 0.168 mmol), $AlCl_3$ (335 mg, 2.52 mmol) and anisole (272 mg, 2.52 mmol) in 1:1 CH_3NO_2/CH_2Cl_2 (12 mL) was stirred for 4 h at room temperature, then H_2O was added. The solvents were removed under reduced pressure and the crude product was subjected to column chromatography (2:1 CH_2Cl_2/CH_3OH) to provide **5** as a pale yellow solid (84 mg, 94%): IR (KBr, cm^{-1}): 3387 (broad), 1679, 1661, 1529, 1458; ^1H NMR (300 MHz, DMSO-d_6) δ 12.11 (s, 1H), 12.09 (s, 1H), 8.18 (brs, 1H), 7.45 (s, 2H), 7.36 (s, 2H), 6.56 (s, 1H), 3.99 (t, J = 6.6 Hz, 2H), 3.75 (s, 2H), 3.04−3.01 (m, 2H), 2.60 (t, J = 6.6 Hz, 2H), 2.09 (quin, J = 6.6 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) 163.1, 150.8, 150.5, 146.8, 136.4, 132.9, 124.2, 117.2, 109.1, 70.5, 37.3, 36.3, 27.9, 27.7, 24.3; MS (ESI) *m/z*: $[M + H]^+$ 517 ($^{79}Br_2$, 30), 519($^{79}Br^{81}Br$, 80), 521($^{81}Br_2$, 64); HRMS (ESI) $[M + H]^+$ calcd for $C_{17}H_{23}{}^{79}Br_2N_6O_3$ *m/z*: 517.0174; found, 517.0198. HPLC: C_{18}, 91%, 3:17 CH_3CN (0.1% TFA)/H_2O (0.1% TFA), t_R = 11.5 min.

Lipopurealin A (2). A mixture of methyl myristic acid (9.7 mg, 0.04 mmol), purealidin A **5** (21 mg, 0.05 mmol), DCC (12.5 mg, 0.08 mmol), HOBt (8.1 mg, 0.08 mmol), and Et_3N (0.1 mL) in 1:1 CH_2Cl_2/DMF (6 mL) was stirred at room temperature for 20 h. After the solvent was removed under reduced pressure, the crude product was purified by flash chromatography (15:9:1:1 EtOAc/acetone/HCO_2H/H_2O). The product was dissolved in MeOH and acidified with 0.5 N HCl. The solvents were removed to afford **2** as a pale-yellow solid (15 mg, 52%), mp 93−95 °C. IR (KBr, cm^{-1}): 3310 (broad), 2922, 2851, 1677, 1541, 1456; ^1H NMR (300 MHz, CD_3OD): δ 7.47 (s, 2H), 6.51 (s, 1H),4.01 (t, J = 6.2 Hz, 2H), 3.83 (s, 2H), 3.47 (t, J = 7.0 Hz, 2H), 3.45 (t, J = 7.1 Hz, 2H), 2.70 (t, J = 6.9 Hz, 2H), 2.20 (t, J = 7.4 Hz, 2H), 2.04 (quin, J = 6.6 Hz, 2H), 1.61 (quin, J = 6.9 Hz, 2H), 1.28 (brs, 20H), 0.89 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) 176.5, 165.6, 152.9, 152.1, 148.7, 137.4, 134.5, 126.2, 118.8, 110.8, 72.4, 39.0, 37.8, 37.2, 33.1, 30.9, 30.8, 30.7, 30.7, 30.6, 30.5, 30.4, 30.4, 30.3, 28.8, 27.1, 25.9, 23.7, 14.4; MS (ESI) *m/z*: $[M + H]^+$ 727 ($^{79}Br_2$, 50), 729 ($^{79}Br^{81}Br$, 100), 731 ($^{81}Br_2$, 5); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{31}H_{49}{}^{79}Br_2N_6O_4$, 727.2151; found, 727.2182. HPLC: 93%, C_{18}, 3:2 CH_3CN (0.1% TFA)/H_2O (0.1% TFA), t_R = 31.1 min.

Lipopurealin B (3). Using a procedure similar to that for the preparation of compound **2**, compound **3** was obtained as a pale-yellow solid (12 mg, 53%), mp 91−93 °C. IR (KBr, cm^{-1}): 3416 (broad), 2924, 1674, 1642, 1543, 1457; ^1H NMR (300 MHz, CD_3OD): δ 7.47 (s, 2H), 6.51 (s, 1H),4.01 (t, J = 6.2 Hz, 2H), 3.83 (s, 2H), 3.47 (t, J = 6.9 Hz, 2H), 3.44 (t, J = 6.9 Hz, 2H), 2.70 (t, J = 6.8 Hz, 2H), 2.20 (t, J = 7.2 Hz, 2H), 2.04 (quin, J = 6.6 Hz, 2H), 1.61 (quin, J = 6.9 Hz, 2H), 1.54−1.49 (m, 1H), 1.28 (brs, 16H), 1.19−1.15 (m, 2H), 0.87 (d, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, CD_3OD) 176.4, 165.6, 152.9, 152.1, 148.6, 137.3, 134.5, 126.1, 118.8, 110.8, 72.4, 40.2, 39.0, 37.9, 37.7, 37.2, 31.0, 30.9, 30.8, 30.7, 30.7, 30.6, 30.4, 30.3, 29.2, 29.1, 28.8, 28.5, 27.1, 25.8, 23.0; MS (ESI) *m/z*: 741($^{79}Br_2$, 46), 743($^{79}Br^{81}Br$, 100), 745($^{81}Br_2$, 55). HPLC: 92%, C_{18}, 3:2 CH_3CN (0.1% TFA)/H_2O (0.1% TFA), t_R = 17.9 min.

Lipopurealin C (4). Using a procedure similar to that for the preparation of compound **2**, compound **4** was obtained as a pale-yellow solid (16 mg, 55%), mp 104−105 °C; IR (KBr, cm^{-1}): 3310 (broad), 2916, 2849, 1679, 1642, 1543, 1457; ^1H NMR (300 MHz, CD_3OD): δ 7.47 (s, 2H), 6.51 (s, 1H), 4.01 (t, J = 6.2 Hz, 2H), 3.83 (s, 2H), 3.47 (t, J = 7.0 Hz, 2H), 3.44 (t, J = 7.0 Hz, 2H), 2.70 (t, J = 6.9 Hz, 2H), 2.19 (t, J = 7.4 Hz, 2H), 2.04 (quin, J = 6.6 Hz, 2H), 1.61 (quin, J = 6.7 Hz, 2H), 1.28 (brs, 24H), 0.90 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) 176.5, 165.6, 152.9, 152.1, 148.7, 137.4, 134.5, 126.2, 118.8, 110.8, 72.4, 39.0, 37.8, 37.2, 33.1, 30.9, 30.8, 30.7, 30.7, 30.6, 30.5, 30.4, 30.4, 30.3, 28.8, 27.1, 25.9, 23.7, 14.4; MS (ESI) *m/z*: $[M + H]^+$ 755 ($^{79}Br_2$, 16), 757 ($^{79}Br^{81}Br$, 32), 759 ($^{81}Br_2$, 16); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{33}H_{53}{}^{79}Br_2N_6O_4$, 755.2525; found, 755.2495. HPLC: 95%, C_{18}, 3:2 CH_3CN (0.1% TFA)/H_2O (0.1% TFA), t_R = 12.2 min.

General Procedure A: Synthesis of *O*-PMB Oxime Acids. 3-[-4-(3-Benzyloxycarbonylaminopropoxy)-3-bromophenyl]-2-(4-methoxybenzyloxyimino)propanoic Acid (27b). A mixture of ester **33** (599 mg, 1 mmol) (see below) and $LiOH\cdot H_2O$ (126 mg, 3 mmol) in 3:1 MeOH/H_2O (60 mL) was sonicated for 3 h at room temperature. The reaction mixture was acidified with 5% HCl (2 mL). A white solid precipitated after concentrating the mixture under reduced pressure. Filtration afforded acid **27b** (567 mg, 98%) as a white solid, mp 80−82 °C. IR (KBr, cm^{-1}): 3321, 2940, 1701, 1681, 1533, 1514, 1456, 1254; ^1H NMR (300 MHz, $CDCl_3$): δ 7.42 (d, J = 2.0 Hz, 1H), 7.36−7.30 (m, 5H), 7.29 (d, J = 8.6 Hz, 2H), 7.13 (d, J = 8.3 Hz, 1H), 6.92 (d, J = 8.6 Hz, 2H), 6.73 (d, J = 8.3 Hz, 1H), 5.56 (brs, 1H), 5.24 (s, 2H), 5.12 (s, 2H), 4.03 (t, J = 5.7, 2H), 3.82 (s, 2H), 3.80 (s, 2H), 3.47 (q, J = 5.7, 2H), 2.06−2.02 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 163.2, 160.0, 156.6, 153.9, 136.7, 133.9, 130.2, 129.3, 129.2, 128.4, 128.0, 127.9, 127.8, 78.2, 67.8, 66.6, 55.3, 39.1, 29.25, 29.2.

3-[2-(3-Benzyloxycarbonylaminopropoxy)-3,5-dibromophenyl]-2-(4-methoxybenzyloxyimino)propanoic Acid (27c). Using general procedure A, 538 mg of the title compound was prepared in 98% yield as a white solid. IR (KBr, cm^{-1}): 3422, 3312, 1706, 1684, 1611, 1546, 1514, 1450; ^1H NMR (300 MHz, $CDCl_3$): δ 7.52 (d, J = 1.9 Hz, 1H), 7.36−7.30 (m, 5H), 7.13 (d, J = 8.7 Hz, 2H), 7.11 (d, J = 1.9 Hz, 1H), 6.86 (d, J = 8.7 Hz, 2H), 5.26 (brs, 1H), 5.15 (s, 2H), 5.10 (s, 2H), 3.94 (t, J = 5.7 Hz, 2H), 3.86 (s, 2H), 3.80 (s, 3H), 3.42 (q, J = 6.0 Hz, 2H), 1.99 (quin, J = 6.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 163.1, 160.0, 156.7, 153.3, 149.1, 136.6, 134.5, 132.9, 132.6, 130.2, 128.5, 128.1, 127.6, 118.1, 117.1, 114.1, 78.3, 71.2, 66.8, 55.3, 38.4, 30.1, 26.1.

3-[2-(3-Benzyloxycarbonylaminopropoxy)-3,5-dichlorophenyl]-2-(4-methoxybenzyloxyimino)propanoic Acid (27d). Using general procedure A, 433 mg of the title compound was prepared in 98% yield as a white solid. IR (KBr, cm^{-1}): 3312, 2949, 1705, 1684, 1611, 1543, 1514, 1455, 1257; ^1H NMR (300 MHz, $CDCl_3$): δ 7.35−7.30 (m, 5H), 7.22 (d, J = 2.0, 1H), 8.3 (d, J =

8.3 Hz, 2H), 6.93 (brs, 1H), 8.3 (d, J = 8.3 Hz, 2H), 5.23 (brs, 1H), 5.14 (s, 2H), 5.11 (s, 2H), 3.96 (t, J = 5.6 Hz, 2H), 3.85 (s, 2H), 3.81 (s, 3H), 3.42 (quin, J = 6.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 162.8, 160.0, 156.7, 151.8, 149.1, 136.6, 132.4, 130.2, 129.3, 129.1, 128.9, 128.6, 128.5, 128.1, 127.6, 114.1, 78.3, 71.3, 66.8, 55.3, 38.4, 30.1, 26.0.

Benzyl [3-(2-bromo-4-formylphenoxy)propyl]carbamate (31). Using general procedure B, 7.8 g of the title compound was prepared in 79% yield as a white solid, mp 70 °C. IR (KBr, cm^{-1}): 3423, 3310, 1684, 1595, 1542; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.81 (s, 1H), 8.04 (d, J = 1.9 Hz, 1H), 7.77 (dd, J = 8.4, 1.7 Hz, 1H), 7.34 (brs, 5H), 6.97 (d, J = 8.4 Hz, 1H), 5.49 (brs, 1H), 5.10 (s, 2H), 4.18 (t, J = 5.7 Hz, 2H), 3.48 (q, J = 6.0 Hz, 2H), 2.10 (quin, J = 6.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 189.5, 156.0, 156.4, 136.5, 134.3, 131.1, 130.6, 128.4, 127.9, 127.8, 112.7, 112.1, 67.7, 66.4, 38.5, 28.9; MS (EI) m/z: 391 ($M^{+\bullet}$, ^{79}Br, 0.2), 300 (0.3), 192 (30), 91 (100).

Benzyl {3-[2-bromo-4-(2-methyl-5-oxo-oxazol-4-ylidenemethyl)-phenoxy]propyl}carbamate (32). Using general procedure C, 6.4 g of the title compound was prepared in 85% yield as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 8.41 (d, J = 1.8 Hz, 1H), 7.94 (dd, J = 8.6, 1.8 Hz, 1H), 7.37−7.32 (m, 5H), 7.00 (s, 1H), 6.91 (d, J = 8.6 Hz, 1H)H, 5.44 (brs, 1H), 5.12 (s, 2H), 4.17 (t, J = 5.7 Hz, 2H), 3.49 (q, J = 6.0 Hz, 2H), 2.41 (s, 3H), 2.13−2.08 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 167.6, 165.8, 157.1, 156.6, 136.8, 133.2, 131.7, 129.4, 128.5, 128.0, 128.0, 127.7, 67.8, 66.7, 38.9, 29.2, 15.6.

General Procedure D: Synthesis of *O*-PMB Oxime Methyl Esters. Methyl 3-[2-(3-benzyloxycarbonylaminopropoxy)-3,5-dibromophenyl]-2-(4-methoxybenzyloxyimino)propanoate. A mixture of benzyl {3-[2,4-dibromo-6-(2-methyl-5-oxooxazol-4-ylidenemethyl)phenoxy]propyl}carbamate (1.89 g, 4 mmol) (see below) and $Ba(OH)_2$ (4.8 g, 28 mmol) in 1:1 dioxane/H_2O (56 mL) was stirred at 60 °C for 1 h. *O*-PMB-Hydroxylamine hydrochloride (1.52 g, 8 mmol) was added at 60 °C, and the mixture was stirred vigorously at the same temperature for 13 h. The reaction mixture was cooled to 0 °C and acidified with 10% HCl. The reaction mixture was extracted with EtOAc. The extracts were washed with H_2O, dried over $MgSO_4$, filtered, and the solvent evaporated. The crude product in dry benzene (40 mL) and MeOH (15 mL) was treated with $TMSCHN_2$ (3 mL, 2.0 N in hexane) at 0 °C. The mixture was stirred for 40 min at room temperature. The organic solvents were removed after the addition of saturated aqueous NH_4-Cl. The residue was extracted with Et_2O, and the extracts were dried over $MgSO_4$, filtered, and the solvent evaporated. The crude product was purified by column chromatography (2:1 hexane/EtOAc) to afford the title compound (752 mg, 32%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.52 (d, J = 2.4 Hz, 1H), 7.36−7.32 (m, 5H), 7.18 (d, J = 8.7 Hz, 2H), 7.07 (d, J = 2.4 Hz, 1H), 6.87 (d, J = 8.7 Hz, 2H), 5.26 (brs, 1H), 5.20 (s, 2H), 5.10 (s, 2H), 3.93 (t, J = 6.0 Hz, 2H), 3.89 (s, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 3.43 (q, J = 6.0 Hz, 2H), 1.99−1.95 (m, 2H).

Methyl 3-[4-(3-benzyloxycarbonylaminopropoxy)-3-bromophenyl]-2-(4-methoxybenzyloxyimino)propanoate. Using general procedure D, 752 mg of the title compound was prepared in 32% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42 (d, J = 1.8 Hz, 1H), 7.36−7.28 (m, 5H), 7.29 (d, J = 8.6 Hz, 2H), 7.11 (dd, J = 8.4, 1.8 Hz, 1H), 6.91 (d, J = 8.6 Hz, 2H), 6.71 (d, J = 8.4 Hz, 1H), 5.50 (brs, 1H), 5.25 (s, 2H), 5.10 (s, 2H), 4.05 (t, J = 5.7 Hz, 2H), 3.84 (s, 2H), 3.81(s, 5H), 3.47 (q, J = 5.7 Hz, 2H), 2.07−2.03 (m, 2H).

Benzyl [3-(2,4-dibromo-6-formylphenoxy)propyl]carbamate. Using general procedure B, 9.5 g of the title compound was prepared in 80% yield as a white solid, mp 60 °C. IR (KBr, cm^{-1}): 3297, 3059, 1691, 1574, 1551; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.18 (s, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.87 (d, J = 2.4 Hz, 1H), 7.35−7.29 (m, 5H), 5.26 (brs, 1H), 5.10 (s, 2H), 4.07 (t, J = 6.0 Hz, 2H), 3.51(q, J = 6.0 Hz, 2H), 2.08 (quin, J = 6.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 187.6, 157.6, 156.4, 141.2, 136.4, 131.6, 131.2, 128.5, 128.1, 126.9, 119.5, 118.2, 74.4, 66.7, 38.1, 30.1.

Benzyl [3-(2,4-dichloro-6-formylphenoxy)propyl]carbamate. Using general procedure B, 4.03 g of the title compound was prepared in 78% yield as a white solid, mp 56 °C. IR (KBr, cm^{-1}): 3319, 3066, 1687, 1678, 1534; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.22 (s, 1H), 7.69 (d, J = 2.7 Hz, 1H), 7.61 (d, J = 2.7 Hz, 1H), 7.35−7.32 (m, 5H), 5.27 (brs, 1H), 5.10 (s, 2H), 4.09 (t, J = 6.0 Hz, 2H), 3.49 (q, J = 6.0 Hz, 2H), 2.07 (quin, J = 6.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 187.6, 156.4, 156.2, 136.4, 135.6, 131.2, 130.5, 129.9, 128.4, 128.0, 127.2, 126.8, 74.1, 66.2, 38.1, 30.1; MS (ESI) m/z: $[M + H]^+$ 382 ($^{35}Cl_2$, 20), 338 (14), 192 (28), 91 (100); HRMS (EI) m/z: $[M + H]^+$ calcd for $C_{18}H_{18}{}^{35}Cl_2NO_4$, 382.0613; found, 382.0619.

Benzyl {3-[2,4-dibromo-6-(2-methyl-5-oxooxazol-4-ylidenemethyl)phenoxy]propyl}carbamate. Using general procedure C, 6.98 g of the title compound was prepared in 85% yield as a yellow solid, mp 167 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.74 (d, J = 2.4 Hz, 1H), 7.74 (d, J = 2.4 Hz, 1H), 7.36−7.26 (m, 6H), 5.27 (brs, 1H), 5.11 (s, 2H), 3.97 (t, J = 5.8 Hz, 2H), 3.53 (q, J = 6.0 Hz, 2H), 2.43 (s, 3H), 2.10 (quin, J = 6.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 167.9, 167.1, 156.5, 155.2, 137.8, 136.5, 134.7, 134.1, 129.9, 128.5, 128.0, 122.2, 118.5, 117.9, 73.3, 66.6, 38.2, 29.9, 15.7.

Benzyl {3-[2,4-dichloro-6-(2-methyl-5-oxooxazol-4-ylidenemethyl)phenoxy]propyl}carbamate. Using general procedure C, 4.23 g of the title compound was prepared in 92% yield as a yellow solid, mp 159 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (d, J = 2.5 Hz, 1H), 7.44 (d, J = 2.5 Hz, 1H), 7.36−7.32 (m, 6H), 5.24 (brs, 1H), 5.11 (s, 2H), 4.0 (t, J = 6.0 Hz, 2H), 3.53 (q, J = 6.3 Hz, 2H), 2.45 (s, 3H), 2.10 (quin, J = 6.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 167.8, 167.1, 156.5, 153.7, 136.5, 134.6, 132.3, 130.4, 130.1, 129.5, 129.0, 128.5, 128.0, 122.2, 73.2, 66.6, 38.2, 30.0, 15.7.

Methyl 3-[2-(3-benzyloxycarbonylaminopropoxy)-3,5-dichlorophenyl]-2-(4-methoxybenzyloxyimino)propanoate. Using general procedure D, 770 mg of the title compound was prepared in 33% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37−7.32 (m, 5H), 7.22 (d, J = 2.0 Hz, 1H), 7.17 (d, J = 8.3 Hz, 2H), 6.90 (d, J = 2.0 Hz, 1H), 6.86 (d, J = 8.3 Hz, 2H), 5.26 (brs, 1H), 5.21 (s, 2H), 5.11 (s, 2H), 3.95 (t, J = 5.9 Hz, 2H), 3.90 (s, 2H), 3.84 (s, 2H), 3.81 (s, 3H), 3.43 (q, J = 6.0 Hz, 2H) 1.97 (quin, J = 6.0 Hz, 2H); MS (ESI) m/z: 611 $[M + Na]^+$ ($^{35}Cl_2$, 100), 589 $[M + H]^+$ ($^{35}Cl_2$, 6).

General Procedure E. 3-[4-(3-Aminopropoxy)-3,5-dibromophenyl]-2-hydroxyimino-*N*-phenethylpropanamide (40a). A mixture of amide **36a** (71 mg, 0.095 mmol), $AlCl_3$ (134 mg, 1 mmol), and anisole (108 mg, 1 mmol) in 1:1 CH_3NO_2−CH_2Cl_2 (20 mL) was stirred for 4 h at room temperature. The mixture was stirred after H_2O was added. The solvents were removed under reduced pressure, and the crude product was subjected to flash chromatography (10:6:1:1 EtOAC/acetone/H_2CO_2H/H_2O) to afford an oil, which was acidified with 5% HCl to give the hydrochloride salt of **40a** (50 mg, 95%) as a pale-yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ 7.50 (s, 2H), 7.27−7.16 (m, 5H), 4.11 (t, J = 5.7 Hz, 2H), 3.84 (s, 2H), 3.46 (t, J = 7.2 Hz, 2H), 3.31 (t, J = 7.2 Hz, 2H), 2.80 (t, J = 7.2 Hz, 2H), 2.21 (quin, J = 6.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.2, 152.2, 152.0, 140.3, 137.9, 134.6, 129.8, 129.5, 127.4, 118.6, 71.6, 42.0, 38.9, 36.5, 29.0, 28.8; MS (ESI) m/z: $[M + H]^+$ 512 ($^{79}Br_2$, 53), 514 ($^{79}Br^{81}Br$, 100), 516 ($^{81}Br_2$, 50); HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{20}H_{24}{}^{79}Br_2N_3O_3$, 512.0814; found, 512.0815. HPLC: C_{18}, 99%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), t_R = 4.0 min.

3-[4-(3-Aminopropoxy)-3,5-dibromophenyl]-2-hydroxyimino-*N*-[2-(4-methoxyphenyl)-ethyl]propanamide (40b). Using general procedure E, 39 mg of the title compound was obtained in 85% yield as a pale-yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ 7.51 (s, 2H), 7.08 (d, J = 5.0 Hz, 2H), 6.80 (d, J = 5.0 Hz, 2H), 4.12 (t, J = 5.7 Hz, 2H), 3.84 (s, 2H), 3.75 (s, 3H), 3.43 (t, J = 7.5 Hz, 2H), 3.31 (t, J = 7.5 Hz, 2H), 2.73 (t, J = 7.5 Hz, 2H), 2.13 (quin, J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.3, 159.8, 152.3, 152.2, 137.9, 134.6, 132.4, 130.7, 118.6, 115.0, 71.7, 55.7, 42.1, 39.0, 35.6, 29.0, 28.8; MS (ESI) m/z: $[M + H]^+$ 542 ($^{79}Br_2$, 60),

544 ($^{79}Br^{81}Br$, 100), 546 ($^{81}Br_2$, 50); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{21}H_{26}{}^{79}Br_2N_3O_4$, 542.0290; found, 542.0290. HPLC: C_{18}, 98%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 3.5$ min.

3-[4-(3-Aminopropoxy)-3,5-dibromophenyl]-*N*-[2-(4-chlorophenyl)ethyl]-2-hydroxyiminopropanamide (40c). Using general procedure E, 44 mg of the title product was obtained in 89% yield as a pale-yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.48 (s, 2H), 7.23 (d, $J = 8.4$ Hz, 2H), 7.15 (d, $J = 8.4$ Hz, 2H), 4.08 (t, $J = 5.7$ Hz, 2H), 3.82 (s, 2H), 3.46 (t, $J = 6.9$ Hz, 2H), 3.17 (t, $J = 4.8$ Hz, 2H), 2.79 (t, $J = 7.0$ Hz, 2H), 2.13 (quin, $J = 6.9$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.3, 152.3, 152.1, 139.2, 137.9, 134.6, 133.2, 133.4, 129.5, 118.6, 71.7, 41.6, 39.0, 35.7, 29.0, 28.8; MS (ESI) *m/z*: $[M + H]^+$ 546 ($^{79}Br_2$, 43), 548 ($^{79}Br^{81}Br$, 100), 550 ($^{81}Br_2$, 70); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{20}H_{23}Br_2N_3O_3Cl$, 545.9795; found, 545.9802. HPLC: C_{18}, 100%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 3.6$ min.

3-[4-(3-Aminopropoxy)-3,5-dibromophenyl]-2-hydroxyimino-*N*-[2-(1*H*-indol-3-yl)ethyl]propanamide (40d). Using general procedure E, 26 mg of the title compound was obtained in 50% yield as a pale-yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.54 (d, $J = 8.1$ Hz, 1H), 7.53 (s, 2H), 7.32 (d, $J = 8.1$ Hz, 1H), 7.07 (t, $J = 8.1$ Hz, 1H), 7.06 (s, 1H), 6.98 (t, $J = 8.1$ Hz, 1H), 4.10 (t, $J = 5.7$ Hz, 2H), 3.85 (s, 2H), 3.29 (t, $J = 7.4$ Hz, 2H), 2.96 (t, $J = 7.5$ Hz, 2H), 2.10 (quin, $J = 6.6$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.3, 152.3, 152.1, 138.2, 138.0, 134.6, 128.8, 123.5, 122.6, 119.7, 119.3, 118.6, 117.3, 113.2, 112.2, 71.7, 41.4, 39.0, 29.0, 28.9, 16.3; MS (ESI) *m/z*: $[M + H]^+$ 551 ($^{79}Br_2$, 68), 553($^{79}Br^{81}Br$, 100), 555 ($^{81}Br_2$, 80); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{22}H_{25}{}^{79}Br_2N_4O_3$, 551.0293; found, 551.0289. HPLC: C_{18}, 92%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 3.6$ min.

3-[4-(3-Aminopropoxy)-3-bromophenyl]-2-hydroxyimino-*N*-phenethylpropanamide (41a). Using general procedure E, 45 mg of the title compound was obtained in 89% yield as a pale-yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.46 (d, $J = 1.8$ Hz, 1H), 7.46−7.15 (m, 6H), 6.94 (d, $J = 8.4$ Hz, 2H), 4.15 (t, $J = 5.5$ Hz, 2H), 3.82 (s, 2H), 3.44 (t, $J = 7.0$ Hz, 2H), 3.21 (t, $J = 7.0$ Hz, 2H), 2.78 (t, $J = 7.2$ Hz, 2H), 2.18 (quin, $J = 6.0$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.6, 154.7, 153.0, 140.4, 134.7, 132.8, 130.6, 129.8, 127.3, 114.7, 112.7, 67.9, 41.9, 39.0, 36.5, 28.8, 28.3; MS (ESI) *m/z*: $[M + H]^+$ 434 (^{79}Br, 90), 436(^{81}Br, 100); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{20}H_{25}{}^{79}BrN_3O_3$, 434.1079; found, 434.1079. HPLC: C_{18}, 99%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 2.6$ min.

3-[4-(3-Aminopropoxy)-3-bromophenyl]-2-hydroxyimino-*N*-[2-(4-methoxyphenyl)ethyl]propanamide (41b). Using general procedure E, 49 mg of the title compound was obtained in 95% yield as a pale-yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.47 (d, $J = 2.0$ Hz, 1H), 7.20 (dd, $J = 8.4$ Hz, 2.0 Hz, 1H), 7.07 (d, $J = 8.5$ Hz, 2H), 6.94 (d, $J = 8.5$ Hz, 1H), 6.80 (d, $J = 8.6$ Hz, 2H), 4.16 (t, $J = 6.6$ Hz, 2H), 3.82 (s, 2H), 3.75 (s, 3H), 3.41 (t, $J = 6.9$ Hz, 2H), 3.22 (t, $J = 7.2$ Hz, 2H), 2.72 (t, $J = 7.2$ Hz, 2H), 2.18 (quin, $J = 6.0$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.5, 159.7, 154.6, 152.9, 134.7, 132.6, 132.3, 130.8, 130.6, 114.9, 114.4, 112.5, 67.6, 55.6, 42.1, 38.9, 35.6, 28.7, 28.2; MS (ESI) *m/z*: $[M + H]^+$ 464 (^{79}Br, 55), 466 (^{81}Br, 50); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{21}H_{27}{}^{79}BrN_3O_4$, 464.1185; found, 464.1187. HPLC: C_{18}, 99%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 2.6$ min.

3-[4-(3-Aminopropoxy)-3-bromophenyl]-*N*-[2-(4-chlorophenyl)ethyl]-2- hydroxyiminopropanamide (41c). Using general procedure E, 45 mg of the title product was obtained in 96% yield a pale-yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.43 (s, 1H), 7.23−7.12 (m, 6H), 6.95 (d, $J = 8.2$ Hz, 1H), 4.16 (t, $J = 6.9$ Hz, 2H), 3.81 (s, 2H), 3.44 (t, $J = 6.9$ Hz, 2H), 3.22 (t, $J = 6.9$ Hz, 2H), 2.77 (t, $J = 6.9$ Hz, 2H), 2.18 (brs, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.5, 154.6, 152.8, 139.2, 134.7, 133.0, 132.4, 131.5, 130.5, 129.4, 114.4, 112.5, 106.5, 67.6, 41.6, 38.8, 35.7, 28.7, 28.2; MS (ESI) *m/z*: $[M + H]^+$ 468 (^{79}Br, 70), 470 (^{81}Br, 100); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{20}H_{24}{}^{79}BrN_3O_3Cl$, 468.0690; found, 468.0692. HPLC: C_{18}, 97%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 4.30$ min.

3-[4-(3-Aminopropoxy)-3-bromophenyl]-2-hydroxyimino-*N*-[2-(1H-indol-3-yl)ethyl]propanamide (41d). Using general procedure E, 40 mg of the title compound was obtained in 87% yield as a pale-yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.54 (d, $J = 7.9$ Hz, 1H), 7.48 (d, $J = 1.9$ Hz, 1H), 7.32 (d, $J = 8.1$ Hz, 1H), 7.21 (dd, $J = 8.1$ Hz, 1.8 Hz, 1H), 7.07 (t, $J = 7.2$ Hz, 1H), 7.04 (s, 1H), 6.97 (t, $J = 7.2$ Hz, 1H), 6.92 (d, $J = 8.4$ Hz, 1H), 4.14 (t, $J = 5.6$ Hz, 2H), 3.83 (s, 2H), 3.53 (t, $J = 7.3$ Hz, 2H), 3.20 (t, $J = 7.1$ Hz, 2H), 2.94 (t, $J = 7.2$ Hz, 2H), 2.16 (quin, $J = 6.0$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.5, 154.6, 152.9, 138.1, 134.7, 132.6, 130.6, 128.7, 123.4, 122.3, 119.6, 119.3, 114.4, 113.1, 112.5, 112.2, 67.6, 41.4, 38.9, 28.2, 26.3; MS (ESI) *m/z*: $[M + H]^+$ 473 (^{79}Br, 92), 475 (^{81}Br, 100); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{22}H_{26}{}^{79}BrN_3O_3$, 473.1188; found, 473.1194. HPLC: C_{18}, 98%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 2.4$ min.

3-[2-(3-Aminopropoxy)-3,5-dibromophenyl]-2-hydroxyimino-*N*-phenethylpropanamide (42a). Using general procedure E, 44 mg of the title compound was obtained in 89% yield as a pale-yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.62 (d, $J = 2.3$ Hz, 1H), 7.24−7.14 (m, 6H), 4.09 (t, $J = 6.0$ Hz, 2H), 3.95 (s, 2H), 3.47 (t, $J = 6.9$ Hz, 2H), 3.10 (t, $J = 7.0$ Hz, 2H), 2.80 (t, $J = 7.2$ Hz, 2H), 2.10 (quin, $J = 6.6$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.4, 154.3, 152.3, 140.4, 135.6, 135.1, 133.5, 129.8, 129.5, 127.4, 119.2, 118.4, 72.1, 42.0, 39.1, 36.5, 29.0, 24.7; MS (ESI) *m/z*: $[M + H]^+$ 512 ($^{79}Br_2$, 50), 514($^{79}Br^{81}Br$, 100), 516($^{81}Br_2$, 50); HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{20}H_{23}{}^{79}Br_2N_3O_3Na$, 534.0004; found, 534.0012. HPLC: C_{18}, 91%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 9.0$ min.

3-[2-(3-Aminopropoxy)-3,5-dibromophenyl]-2-hydroxyimino-*N*-[2-(4-methoxyphenyl)-ethyl]propanamide (42b). Using general procedure E, 45 mg of the title compound was obtained in 90% yield as a pale-yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.62 (d, $J = 2.3$ Hz, 1H), 7.25 (d, $J = 2.3$ Hz, 1H), 7.07 (d, $J = 8.6$ Hz, 2H), 6.78 (d, $J = 8.6$ Hz, 2H), 4.11 (t, $J = 5.9$ Hz, 2H), 3.96 (s, 2H), 3.74 (s, 3H), 3.44 (t, $J = 7.0$ Hz, 2H), 3.28 (t, $J = 7.1$ Hz, 2H), 2.73 (t, $J = 7.2$ Hz, 2H), 2.21 (quin, $J = 6.3$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.3, 159.7, 154.3, 152.3, 135.6, 135.0, 133.4, 132.3, 130.8, 119.2, 118.4, 114.9, 71.9, 55.7, 42.2, 38.9, 35.6, 29.0, 24.6; MS (ESI) *m/z*: $[M + H]^+$ 542 ($^{79}Br_2$, 50), 544($^{79}Br^{81}Br$, 100), 546($^{81}Br_2$, 50); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{21}H_{26}{}^{79}Br_2N_3O_4$, 542.0290; found, 542.0291. HPLC: C_{18}, 100%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 7.7$ min.

3-[2-(3-Aminopropoxy)-3,5-dibromophenyl]-*N*-[2-(4-chlorophenyl)ethyl]-2-hydroxyiminopropanamide (42c). Using general procedure E, 46 mg of the title compound was obtained in 90% yield as a pale-yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.63 (d, $J = 2.2$ Hz, 1H), 7.22 (d, $J = 2.2$ Hz, 1H), 7.20 (d, $J = 8.7$ Hz, 2H), 7.13 (d, $J = 8.6$ Hz, 2H), 4.11 (t, $J = 5.7$ Hz, 2H), 3.95 (s, 2H), 3.47 (t, $J = 7.1$ Hz, 2H), 3.28 (t, $J = 7.2$ Hz, 2H), 2.78 (t, $J = 7.0$ Hz, 2H), 2.21 (quin, $J = 6.3$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.4, 154.3, 152.3, 139.2, 135.5, 135.1, 133.4, 131.4, 129.5, 129.4, 119.2, 118.4, 72.0, 41.6, 39.0, 35.8, 29.0, 24.7; MS (ESI) *m/z*: $[M + H]^+$ 546 ($^{79}Br_2$, 50), 548 ($^{79}Br^{81}Br$, 100), 550 ($^{81}Br_2$, 70); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{20}H_{23}{}^{79}Br_2N_3O_3Cl$, 545.9795; found, 545.9774. HPLC: C_{18}, 98%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 15.7$ min.

3-[2-(3-Aminopropoxy)-3,5-dibromophenyl]-2-hydroxyimino-*N*-[2-(1H-indol-3-yl)ethyl]propanamide (42d). Using general procedure E, 26 mg of the title compound was obtained in 87% yield as a pale-yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 7.62 (d, $J = 2.3$ Hz, 1H), 7.54 (d, $J = 7.8$ Hz, 1H), 7.32 (d, $J = 7.8$ Hz, 1H), 7.29 (d, $J = 2.3$ Hz, 1H), 7.09−7.07 (m, 1H), 7.04 (s, 1H), 7.00−6.95 (m, 1H), 4.08 (t, $J = 5.7$ Hz, 2H), 3.96 (s, 2H), 3.56 (t, $J = 7.2$ Hz, 2H), 3.25 (t, $J = 7.1$ Hz, 2H), 2.96 (t, $J = 7.2$ Hz, 2H), 2.19 (quin, $J = 6.3$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.3, 154.2, 152.1, 138.0, 135.6, 134.9, 133.5, 128.6, 123.5, 122.3, 119.5, 119.3, 119.2, 118.3, 113.0, 112.2, 71.9, 46.0, 38.9, 29.0, 26.2, 24.8; MS (ESI) *m/z*: $[M + H]^+$ 551 ($^{79}Br_2$, 54), 553 ($^{79}Br^{81}Br$, 100), 555 ($^{81}Br_2$, 42); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for

$C_{22}H_{25}{}^{79}Br_2N_4O_3$, 551.0293; found, 551.0283. HPLC: C_{18}, 98%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 7.6$ min.

3-[2-(3-Aminopropoxy)-3,5-dichlorophenyl]-2-hydroxyimino-*N*-phenethylpropanamide (43a). Using general procedure E, 38 mg of the title compound was obtained in 91% yield as a pale-yellow solid: ^{1}H NMR (300 MHz, CD_3OD): δ 7.34 (d, $J = 2.5$ Hz, 1H), 7.26−7.15 (m, 5H), 7.07 (d, $J = 2.5$ Hz, 1H), 4.12 (t, $J = 5.7$ Hz, 2H), 3.96 (s, 2H), 3.48 (t, $J = 6.9$ Hz, 2H), 3.27 (t, $J = 7.1$ Hz, 2H), 2.80 (t, $J = 7.2$ Hz, 2H), 2.04 (quin, $J = 6.0$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.4, 152.8, 152.3, 140.4, 135.2, 130.8, 129.8, 129.7, 129.5, 129.3, 127.4, 72.0, 41.9, 39.0, 36.5, 29.1, 24.6; MS (ESI) *m*/*z*: $[M + H]^+$ 424 ($^{35}Cl_2$, 100), 426 ($^{35}Cl^{37}Cl$, 65); HRMS (ESI) *m*/*z*: $[M+Na]^+$ calcd for $C_{20}H_{24}{}^{35}Cl_2N_3O_3$, 424.1195; found, 424.1198. HPLC: C_{18}, 99%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 7.0$ min.

3-[2-(3-Aminopropoxy)-3,5-dichlorophenyl]-2-hydroxyimino-*N*-[2-(4-methoxyphenyl)ethyl]propanamide (43b). Using general procedure E, 37 mg of the title compound was obtained in 88% yield as a pale-yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ 7.34 (d, $J = 2.3$ Hz, 1H), 7.09 (d, $J = 2.3$ Hz, 1H), 7.07 (d, $J = 8.6$ Hz, 2H), 6.78 (d, $J = 8.5$ Hz, 2H), 4.12 (t, $J = 5.7$ Hz, 2H), 3.95 (s, 2H), 3.74 (s, 3H), 3.44 (t, $J = 7.0$ Hz, 2H), 3.27 (t, $J = 7.1$ Hz, 2H), 2.73 (t, $J = 7.2$ Hz, 2H), 2.20 (quin, $J = 6.3$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.4, 159.8, 152.8, 152.3, 135.2, 132.3, 130.7, 129.8, 129.7, 129.3, 115.0, 72.0, 55.7, 42.1, 39.0, 35.6, 29.0, 24.6; MS (ESI) *m*/*z*: $[M + H]^+$ 454 ($^{35}Cl_2$, 100), 456 ($^{35}Cl^{37}Cl$, 70); HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{21}H_{26}{}^{35}Cl_2N_3O_4$, 454.1300; found, 454.1313. HPLC: C_{18}, 99%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 6.1$ min.

3-[2-(3-Aminopropoxy)-3,5-dichloro-phenyl]-*N*-[2-(4-chlorophenyl)ethyl]-2-hydroxyiminopropanamide (43c). Using general procedure E, 38 mg of the title compound was obtained in 87% yield as a pale-yellow solid: ^{1}H NMR (300 MHz, CD_3OD): δ 7.34 (d, $J = 2.4$ Hz, 1H), 7.20 (d, $J = 8.7$ Hz, 2H), 7.14 (d, $J = 8.7$ Hz, 2H), 7.05 (d, $J = 2.4$ Hz, 1H), 4.11 (t, $J = 5.7$ Hz, 2H), 3.94 (s, 2H), 3.47 (t, $J = 7.2$ Hz, 2H), 3.27 (t, $J = 7.1$ Hz, 2H), 2.79 (t, $J = 7.0$ Hz, 2H), 2.20 (quin, $J = 6.3$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.4, 152.8, 152.4, 139.2, 135.1, 133.1, 131.5, 130.7, 129.8, 129.7, 129.4, 71.9, 41.6, 38.9, 35.8, 29.0, 24.5; MS (ESI) *m*/*z*: $[M + H]^+$ 458 ($^{35}Cl_3$, 100), 460 ($^{35}Cl_2{}^{37}Cl$, 100); HRMS (ESI) *m*/*z*: $[M+Na]^+$ calcd for $C_{20}H_{22}{}^{35}Cl_3N_3O_3Na$, 480.0624; found, 480.0627. HPLC: C_{18}, 99%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 13.0$ min.

3-[2-(3-Aminopropoxy)-3,5-dichlorophenyl]-2-hydroxyimino-*N*-[2-(1H-indol-3-yl)ethyl]propanamide (43d). Using general procedure E, 32 mg of the title compound was obtained in 64% yield as a pale-yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ 7.54 (d, $J = 7.8$ Hz, 1H), 7.34 (d, $J = 2.5$ Hz, 1H), 7.31 (d, $J = 7.8$ Hz, 1H), 7.11 (d, $J = 2.5$ Hz, 1H), 7.07 (t, $J = 7.8$ Hz, 1H), 7.04 (s, 1H), 6.97 (t, $J = 7.8$ Hz, 1H), 4.08 (t, $J = 5.7$ Hz, 2H), 3.96 (s, 2H), 3.56 (t, $J = 7.1$ Hz, 2H), 3.23 (t, $J = 7.1$ Hz, 2H), 2.96 (t, $J = 7.2$ Hz, 2H), 2.17 (quin, $J = 6.3$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 165.6, 153.0, 152.5, 138.4, 135.4, 130.9, 130.1, 129.5, 123.7, 122.5, 119.8, 119.5, 113.3, 112.4, 72.2, 41.5, 39.1, 29.2, 26.4, 24.8; MS (ESI) *m*/*z*: $[M + H]^+$ 463 ($^{35}Cl_2$, 100), 465 ($^{35}Cl^{37}Cl$, 75); HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{22}H_{25}{}^{35}Cl_2N_4O_3$, 463.1304; found, 463.1310. HPLC: C_{18}, 99%, 7:13 CH_3CN (0.1% TFA)/H_2O (0.1%TFA), $t_R = 6.1$ min.

Biology. Antiproliferative Assay. The cells were maintained as exponentially growing cultures in an RPMI 1640 medium with 10% FBS, 1% penicillin, and 1% glutamine. For the human cancer cell lines, the cells were trypsinized and washed, and a cell suspension in RPMI-1640 plus additives was plated into 96-well microtiter plates at 500−2000 cells/well (depending on the cell line). The cells were allowed to attach and grow for 72 h before treatment with vehicle (DMSO) or test agent for an additional 72 h. For the L1210 line, the cells were plated into 96-well plates at 7500 cells/well and incubated with vehicle or test agents for 48 h. In each case, one plate consisted entirely of cells in medium and medium alone to determine time zero cell numbers. The other plates in a given determination contained eight wells of control cells and eight wells of medium, and each agent concentration was tested in quadruplicate. Cell viability was determined with the MTS assay, and 50% growth-inhibitory concentrations (GI_{50}) were calculated.[52] Discodermolide was used as a positive control in these assays.

Recombinant Cytoplasmic Dynein Motor Domain Expression and Purification. The gene encoding the 380 kDa motor domain fragment [Gly(1286)-Glu(4644)] of rat cytoplasmic dynein with a *C*-terminal in-frame hexahistidine tag was inserted into the baculovirus expression vector pVL1393 (BD biosciences) as described.[36] The Hi5 cells were infected with the virus for 40 h. The cells were washed and resuspended in PBS, and the recombinant motor domain fragment was extracted from the cells by homogenization in the extraction buffer described above supplemented with 1 mM DTT and a protease inhibitor cocktail (Sigma-Aldrich). The cytosolic extract was spun at 5000*g* for 10 min and 100 000*g* for 30 min. The supernatant was applied on a Ni^{2+}-affinity column (Ni-NTA superflow, Qiagen) equilibrated in an extraction buffer supplemented with 20 mM imidazole. Unbound material was washed out with 16 volumes of the extraction buffer supplemented with 10 mM imidazole, and the protein was eluted in 5 volumes of the elution buffer (50 mM Pipes + 50 mM Hepes, pH 7.2, containing 2 mM $MgSO_4$, 2 mM EGTA, 125 mM imidazole, and 1 mM DTT). The eluted protein was dialyzed extensively against the extraction buffer to remove the imidazole. Protein concentration was determined by the Bradford method using albumin as a standard.[53] A typical batch of six dishes of confluent Hi5 cells produced 2−4 mg of recombinant protein that had no visible sign of contaminants or degradation. Peak fractions were pooled, flash-frozen in liquid nitrogen and subsequently stored at −80 °C until use.

Dynein Motor Domain ATPase Inhibition Assay. The ability of the purealin analogues to inhibit the MT-stimulated ATPase activity of the recombinant motor domain was determined colorimetrically with the malachite green assay.[54] Paclitaxel-induced tubulin polymer (5 mg/mL final concentration based on soluble tubulin incubated with 1 mM paclitaxel), dynein motor domain (50 μg/mL final concentration), and the test agents (50, 10, 2, 0.4, and 0.08 μM) as well as the positive control (EHNA, an adenosine analogue known to inhibit dynein ATPase activity, 5 mM final concentration) and the negative control (DMSO only), were premixed in a reaction buffer (10 mM PIPES and 10 mM HEPES, pH 7.0, containing 0.4 mM $MgCl_2$ and 0.2 mM EDTA). ATP (2 mM) was added to initiate the reaction. The other controls, including a no enzyme control (all but dynein motor domain), no substrate control (all but ATP), and no enzyme/substrate control (all but ATP and dynein motor domain), were also prepared. The reaction mixture was incubated at 37 °C for 30 min. The malachite green solution (1 mM malachite green, 8.5 mM ammonium molybdate ,and 0.8 M HCl) and 34% aqueous sodium citrate were added. The absorbance at 655 nm was determined in a 96-well microtiter plate reader. Absorbance values from the control wells were subtracted from those from wells containing the replete set of reaction components. The amount of free phosphate released by the dynein heavy chain was calculated from a sodium phosphate standard curve. The percentage of control was calculated from the ratio of the ATPase activity of cytoplasmic dynein motor domain with a specific concentration of an inhibitor and the ATPase activity of cytoplasmic dynein motor domain only.

Kinetics of Dynein Inhibition. Dynein motor domain (12.5 μg/mL final concentration) and paclitaxel-induced tubulin polymer (1 mg/mL final concentration with 10 μg/mL paclitaxel) were premixed with the test agents in the extraction buffer as described. Different concentrations of ATP (1000, 500, 250, 125, and 62.5 μM) were added to the system to initiate the reaction. The mixtures were incubated at 37 °C for 30 min. The reaction was stopped by the addition of the malachite-green solution, and the absorbance at 655 nm was determined in a 96-well microtiter plate reader.

Acknowledgment. This work was supported in part by NIH grants CA078039 and GM047434.

Supporting Information Available: Representative HPLC chromatograms for key compounds subjected to biological testing.

This material is available free of charge via the Internet at http://pubs.acs.org.

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JM051030L