Published in final edited form as:

J Med Chem. 2011 September 22; 54(18): 6319–6327. doi:10.1021/jm200692n.

Design and Synthesis of (+)-Discodermolide-Paclitaxel Hybrids Leading to Enhanced Biological Activity¹

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Abstract

Potential binding modes of (+)-discodermolide at the paclitaxel binding site of tubulin have been identified by computational studies based on earlier structural and SAR data. Examination of the prospective binding modes reveal that the aromatic pocket occupied by the paclitaxel side-chain is unoccupied by (+)-discodermolide. Based on these findings, a small library of (+)-discodermolide-paclitaxel hybrids have been designed and synthesized. Biological evaluation reveals a two- to eight- fold increase in antiproliferative activity compared to the parent molecule using the A549 and MCF-7 cancer cell lines.

(+)-Discodermolide [(+)-**1**], a potent antitumor polyketide natural product, was first isolated in the early 1990's from extracts of the Caribbean marine sponge *Discodermia dissoluta* by Gunasekera and coworkers.¹ Confirmation of the assigned structure, as well as the absolute stereochemistry, was subsequently achieved by Schreiber and coworkers via total syntheses of the (–) enantiomer (1993), the natural (+) enantiomer (1994) and a number of novel analogs.^{2,3} Initially reported to be a potent immunosuppressive agent, (+)-discodermolide was later recognized to possess significant antiproliferative activity both *in vitro*^{4a} across the NCI panel of human cancer cell lines,⁵ including paclitaxel resistant cell lines,⁶ and *in vivo*.^{4b} Like paclitaxel (**2**), (+)-discodermolide stabilizes tubulin thereby modulating microtubule dynamics that leads to cell arrest at mitosis.

As a result of the observed antitumor activity, (+)-discodermolide generated significant interest in the synthetic community: 13 total syntheses have been reported to date.⁷ Of particular significance, as recently recognized,⁸ our four generation strategies exploited an early example of the Negishi coupling protocol to access the C₁₄–C₁₅ olefin.^{7e,9} Subsequently Novartis employed a similar cross-coupling tactic (i.e. Suzuki) to provide (+)-discodermolide for their Phase I clinical trials.¹⁰ Also early on, we both proposed¹¹ a solution conformation of (+)-discodermolide based on a combination of NMR data and the

¹ABBREVIATIONS USED: ESI-HMRS, electrospray ionization high-resolution mass spectrometry; TMS, tetramethylsilane; TLC, thin layer chromatography; PDB, Protein Data Bank; DISCON: Distribution of Solution Conformations; NCI, National Cancer Institute; SAR, Structure activity relationship; MD, Molecular dynamics; CDI, N,N'-Carbonyl-diimidazole.

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Supporting Information

¹H and ¹³C NMR spectra, experimental methods and synthetic procedures of (+)-**3**-(+)-**20**; HPLC data of (+)-**3**-(+)-**7** used in biological assays; NMR data utilized in solution conformation calculations; SAR data employed in computational studies. This material is available free of charge via the Internet at <http://pubs.acs.org>.

solid state X-ray structure and carried out a comprehensive structure activity relationship (SAR) study with Kosan Biosciences, Inc.^{12,13}

More recently we demonstrated that (+)-discodermolide both causes accelerated cell senescence,¹⁴ and binds to a region on β -tubulin close to the paclitaxel binding site.¹⁵ Of interest here (+)-discodermolide and paclitaxel act synergistically, not only in cell culture¹⁶ but also in an ovarian xenograft tumor model in nude mice.¹⁷ Subsequent studies, utilizing hydrogen/deuterium exchange and mass spectrometry, indicated that paclitaxel and discodermolide induce stability on opposite sides of the microtubule interface, suggesting a possible explanation for the synergy observed between these two drugs.¹⁸ Finally to decrease the pneumotoxicity of (+)-**1**, the presumptive cause leading to the failure of the Novartis Phase I clinical trial,¹⁰ possibly due to metabolites,¹⁹ we extended our analog program, wherein the metabolically less stable lactone portion of the molecule^{13a-c} and C₁₄-C₁₅ olefin,^{13d} were either individually or together, replaced with isosteres. Pleasingly these analogs preserved the cytotoxicity at a level similar to (+)-discodermolide (**1**).

However despite the availability of extensive SAR data,¹² as well as structural,¹ computational and NMR studies,^{11,20} the binding mode of (+)-discodermolide on tubulin remains unclear. Towards this end, we report here two possible binding modes of (+)-discodermolide in conjunction with the design, synthesis and biological evaluation of (+)-discodermolide/paclitaxel hybrid molecules, possessing photoaffinity labels.

In our SAR studies, we demonstrated the following: (1) that changes in the substitution pattern of C(1), C(3) and C(7) centers of (+)-discodermolide are favorable to retention of potent cytotoxicity; (2) that variations at the C(2), C(14) and the carbamate carbons are tolerable; and (3) that changes within the remaining portions of (+)-discodermolide significantly decrease the cytotoxicity.^{12,13} Based on these observations, in conjunction with our earlier photoaffinity labeling study,^{12e,f} we reasoned that the diene terminus of discodermolide resides in a hydrophobic/aromatic pocket, while the lactone moiety either occupies a binding pocket that can accommodate large groups, or more likely, is partially interfaced with the solvent. Further information gained from our earlier photoaffinity labeling study revealed that attachment of a large aromatic system (cf. benzophenone) and/or alkyl groups to the carbamate nitrogen did not significantly alter the observed activity.^{12f} In fact, in two cases, comprising attachment of a *p*-dimethylaniline or *p*-benzophenone substituent at the carbamate nitrogen, a significant increase in tumor cell growth inhibitory activity was observed. We emphasize however that our earlier study was directed specifically at the preparation of photoaffinity labeled (+)-discodermolide analogs, with little or no attention focused on activity changes, except for the possibility of increased hydrophobicity and/or conformational preferences. A closer examination of the earlier carbamate analogs however reveals a strong activity dependency both on the nature of the aromatic rings and linker length. Based on these observations, we have now examined the scope and molecular basis for the observed increase in cytotoxicity of these analogs exploiting detailed computational analysis.

The Solution Conformations of (+)-Discodermolide

In 2001, we reported that the preferred major solution conformation of (+)-discodermolide (**1**) was similar to the solid state structure,¹ notwithstanding the 15 rotatable σ bonds.¹¹ In particular, variable temperature and co-solvent titration NMR studies provided evidence of intramolecular hydrogen bonds between the C(7)-OH and the C(1)-carbonyl, in conjunction with interactions between the carbamate nitrogen and the C(3) and C(7) hydroxyl groups. We postulated that *syn*-pentane interactions²¹ were responsible for the proposed turn geometry of the backbone. Importantly, biological studies of epimers bearing inverted

stereogenicities at the C(7), C(11), C(16) and C(17) revealed significantly lower activities, which led us to suggest that the conformation of the molecular segment between C(7)–C(17) is conserved upon binding. These conclusions were later confirmed by studies from other research laboratories.²⁰

To understand more fully the solution behavior of (+)-discodermolide, we recently initiated computational studies exploiting molecular dynamics (MD) simulations. Monitoring backbone torsional angles over a 1 microsecond simulation period in water revealed the flexible torsional angles. Of considerable interest, the lactone ring and the diene side chain moieties, were found to display more flexibility compared to the rest of the molecule, when the turn geometry [i.e., C(7)–C(16)] was maintained throughout the simulation.

We next turned to NMR experiments to refine the solution conformation. The DISCON (Distribution of Solution Conformations) program developed in our laboratory²² was employed to obtain a distribution of conformers based on NMR data. Earlier studies had revealed that (+)-discodermolide is more flexible in organic solvents.^{20a, 20b} Based on this observation, acetonitrile was selected as the solvent for the NMR experiments, given that the aim of this study was to identify the “flexible” torsional angles and possible diverse conformational families for our pending docking studies. Proton-proton coupling constants and NOE derived distances reported earlier were utilized.¹¹ Deconvolution of the NMR observables over an ensemble of structures obtained by Monte Carlo conformational searches, followed by the clustering analysis that is embedded in DISCON software, led to an ensemble of four principal conformers, that in combination fit the NMR derived distance and torsional angle data better than any single conformation. Two conformers (Figure 2, A and B), similar to the solid-state structure with differences in the lactone ring orientation, were identified as the major solution conformations (**A**: 35% and **B**: 32%), an observation fully consistent with our earlier solution structure.¹¹ Further examination of members of the major conformational families revealed (+)-discodermolide can adopt different conformations by adjusting the torsional angles around C(11)–C(12) and C(7) and C(8) bonds. In addition, the lactone ring can exist in equilibrium between chair, half chair and skew boat conformations. The next most populated conformer (**C**: 26%) comprised an inverted orientation at the diene region, wherein a different gauche orientation is preferred around the C(16)–C(17) and C(18)–C(19) bonds, with the remaining regions of (+)-discodermolide identical to the solid state structure. The remaining major family (7%) comprises both of these conformational changes, and thereby forces the molecule to occupy an extended conformation. The distribution of the conformations can be rationalized by the observed $^3J_{\text{H16-H17}}$ and $^3J_{\text{H11-H12}}$ values of 6.2 Hz and 6.6 Hz respectively, corresponding to torsional angle averaging and by the observed NOE correlation variations from the solid state structure, in particular those between H(7)–H(9), H(8)–H(12), H(9)–H(11), and H(12)–Me(18). Similar analyses reported earlier by the Jiménez-Barbero^{20b} and the Snyder Laboratories^{20c} are in accord with these observations, wherein differences in distributions were observed, presumably due to the differences in the solvents employed in the NMR experiments, as well as to the force fields and clustering methods employed.

In summary, (+)-discodermolide in solution displays considerable flexibility in the orientation of the lactone and the diene/carbamate termini, with conservation of the turn region of the conformation comprising the C(7)–C(16) centers. For docking studies, we reasoned that the solution structures hold considerable importance, since the bioactive conformation is more likely to exist in solution given that a lower energy penalty is paid for ligand reorganization upon binding (i.e., pre-organization).²³ Stated differently, the 3D binding pharmacophore of the ligand is likely to be found in the conformation(s) that exist in solution, thus permitting easy recognition.²⁴

Docking Studies

Having an understanding of the available major ensemble of solution conformations, we turned to define the binding mode of (+)-discodermolide on microtubules. In general, docking studies of microtubule stabilizing agents utilize the heterodimer α - β tubulin structure (PDB code: 1JFF) obtained by high resolution electron microscopy from the Zn induced crystalline sheets of tubulin in the presence of paclitaxel.²⁵ Although the tubulin structure in the crystal was shown to match the microtubule structure, it remains only an approximate model for docking studies for the following reasons: (1) the protein structure is distorted by the bound paclitaxel, and thus does not necessarily represent the unbound microtubule;²⁶ (2) *in vivo* orientations and conformations of tubulin in microtubules and Zn-induced tubulin crystal sheets are different;²⁷ (3) the flexible M-loop region, at the taxane binding site is not well resolved;²⁵ (4) the lateral contacts between protofilaments, which are important for microtubule formation and tubulin dynamics²⁸ are missing; and (5) in humans the tubulin isotypes have different sequences than found in the reported protein structure.²⁹ Equally significant, the majority of reported (+)-discodermolide docking studies employ either the solid state conformation of (+)-discodermolide as a rigid scaffold, or (+)-discodermolide is treated as a fully flexible molecule without regard to the accessible solution conformations.^{15,18,30} Finally, earlier docking studies did not take into account the reported SAR data,¹⁹ in particular the potent carbamate analogs.^{13f}

More recently Carlomagno^{20a} and then Jiménez-Barbero et al.^{20b} concluded, based on NMR transfer NOE studies of (+)-discodermolide and tubulin, that the binding conformation of (+)-discodermolide is identical to the solid state conformation with differences in the lactone ring portion, and that (+)-discodermolide binds at the paclitaxel binding site. Paterson and coworkers in turn, based on these proposals, prepared a series of discodermolide-dictyostatin-paclitaxel hybrids which upon biological evaluation revealed a 20–35 fold *decrease* in tumor cell growth inhibition activity, employing the PANC-1 human cancer cell lines.³¹ Notwithstanding these disappointing observations, we undertook docking studies employing the solution conformations identified by DISCON (*vide infra*).

Our 2009 hydrogen-deuterium exchange experiments also revealed that (+)-discodermolide binds to a region quite close to the paclitaxel site on β -tubulin, however the stabilized microtubule geometries were different as evidenced by the observed solvent exposure of the lateral sites,¹⁸ with the most important interactions observed in the M-loop region of β -tubulin. Based on this information, we also chose to employ the paclitaxel site for docking studies. Beginning with the paclitaxel bound protein structure (PDB Code: 1JFF), paclitaxel was removed and the remaining protein structure was minimized with the OPLS-2005 force field to remove possible steric clashes. The binding site was not altered excessively, as we did not want to dock to a computationally generated binding pocket. Sets of (+)-discodermolide conformations, belonging to the families identified in solution (**A–D**, Figure 2), were then docked, employing the Glide³² and Molegro³³ software packages, treating the (+)-discodermolide conformations as rigid structures and the protein structure as flexible. Docking modes inconsistent with the SAR data were eliminated by visual inspection. We reason that this approach provides more valid criteria for finding a working binding hypothesis than calculated docking scores and energies, due to the uncertainties (*vide infra*) in the protein structure (*cf.* Supporting Information for elimination criteria). Two binding poses for (+)-discodermolide were identified to be consistent with the SAR data.

The first pose (Figure 3B) orients the lactone end of (+)-discodermolide in a region occupied by the baccatin core of paclitaxel. The turn structure of (+)-discodermolide fits tightly in a region under the M-loop (β 212–230) with the C(3) and C(7) hydroxyl groups forming hydrogen bonds to Pro272 and Thr274. In this pose, the carbamate moiety is directed

towards the aromatic pocket that accommodates the paclitaxel tail (Figure 3A). The diene side chain and the lactone carbonyl group are directed towards the solvent, where additional substituents would appear to be tolerated. Rescoring of this pose via the MM/GBSA protocol³⁴ provided a favorable ΔG value of -12 kcal/mol. The second pose (Figure 3C), similar to pose 1, utilizes the (+)-discodermolide turn scaffold to fit in the hydrophobic pocket under the M-loop. This pose however orients the lactone moiety to the region where the paclitaxel C(2) benzoate group binds. This region would also appear to be able to accommodate larger groups, while maintaining the activity of (+)-discodermolide analogs. That is, replacement of the lactone ring with other moieties might prove feasible. In this pose the known decrease in activity of (+)-discodermolide analogs upon acetylation at the C(11) hydroxyl group can be rationalized by the loss of a hydrogen bond to Thr274. This pose also orients the diene side-chain in line with the paclitaxel benzamide group, with the (+)-discodermolide carbamate group directed to the C(3')-phenyl pocket. Rescoring employing the MM/GBSA protocol again provided a favorable ΔG value of -18 kcal/mol. Noteworthy, the Jiménez-binding mode differed significantly from the poses presented here, and was found to be inconsistent with our SAR criteria.

Design of Hybrid Analogs

Based on the (+)-discodermolide docking modes, we designed a series of analogs that we reasoned would hold the promise of not only confirming our binding hypothesis, based initially on enhanced binding to tubulin and in turn enhanced cytotoxicity, but also defining the true binding pose. Importantly, the design criteria were orchestrated to accommodate incorporation of photoaffinity labels for future structural studies.

Utilizing docking poses 1 and 2, we envisioned filling the aromatic pocket in the vicinity of the carbamate group, which in the case of paclitaxel is occupied by C(3')-Ph group. *In silico* docking of our earlier carbamate analogs (*vide infra*), employing the identified (+)-discodermolide poses, revealed that the earlier analogs did not fully occupy the proposed pocket due to the shorter tether lengths. To test the validity of this scenario, analogs having aromatic groups attached to the carbamate group with differing tether lengths were generated. We hypothesized that if the binding pocket is valid, the cytotoxic activities should vary, with the analog possessing the most suitable linker length displaying the highest tumor cell growth inhibition activity. A second amide linkage was incorporated onto the tether to participate in a possible hydrogen bond, with Asp26 and His229 of β -tubulin, similar to the paclitaxel amide linkage. Finally, we envisioned that photoaffinity tags could be appropriately introduced without deleterious effects on the cytotoxicity. To this end (+)-discodermolide/paclitaxel hybrid structures with a *p*-azidophenyl substituent (**3–5**, Figure 4) were generated. Docking studies were then conducted similar to the procedure applied for (+)-discodermolide. In these studies the newly introduced paclitaxel C(3')-phenyl group surrogates were permitted to be flexible, whereas the (+)-discodermolide core was kept rigid. Examination of the docking scores identified hybrid **4** to fill the aromatic pocket. Two additional hybrids **6** and **7** possessing different photoaffinity tags and optimal linker lengths were also designed, to explore the potential effects of the hybrid structure motif, as well as the photoaffinity groups on the cytotoxicity.

Synthesis of the Designed Analogs

The requisite hybrids, carrying the photoaffinity tags, were envisioned to arise via union of known (+)-discodermolide intermediate **19**,^{7e} prepared in connection with our gram-scale synthesis,¹¹ with the photoaffinity units bearing the suitable amine linkers (Scheme 1). One of the attributes of developing preparative scale synthesis of architecturally complex natural

products possessing significant bio-regulatory properties is the subsequent ready availability of advanced intermediates for SAR and photoaffinity labeling studies.

To permit facile formation of carbamate linkage, the secondary alcohol on (+)-discodermolide would be activated as a carbonate ester (cf. **20**). The amine coupling partners were envisioned to arise via condensation reactions of the photoaffinity tags possessing carboxylic acid functionality with the diamine tethers of suitable length. The photoaffinity tags for this study were prepared following known synthetic routes,^{35,36,37} wherein acids **8** and **13** were activated as hydroxysuccinamide esters,³⁸ followed by condensation with the alkyl diamine linkers (Scheme 2). For the aryl-(trifluoromethyl)diazirine component **18**, the mono-Boc protected amine linker was employed with N,N'-Carbonyl-diimidazole (CDI)³⁹ to reduce possible side reactions; the Boc group was subsequently removed under acidic conditions.

In the event, construction of (+)-discodermolide/paclitaxel hybrids **3–7** possessing the phototags entailed a two-step protocol, wherein the secondary alcohol in (+)-**19** was converted to a *p*-nitrophenylcarbonate ester, followed by union with amines **10–12** and **15** and **18**.⁴⁰ Global deprotection under acidic conditions provided (+)-**3–7** in good to excellent yields. Importantly, the synthetic route not only provides facile access to the designed (+)-discodermolide/paclitaxel hybrids possessing the photoaffinity tags, but was also designed to permit ready access to either ³H or ¹⁴C analogs for future labeling experiments.

Biological Evaluations of the (+)-Discodermolide/Paclitaxel Hybrids

The designed (+)-discodermolide/paclitaxel hybrids possessing the photoaffinity labels were tested for antiproliferative activity against human lung (A549) and breast (MCF-7) cancer cell lines. Discodermolide (–1) and Taxol (–2) served as controls. In accord with our design strategy, the analogs revealed improved activity in a trend changing with the tether length (Table 1).

Given the similarity of the analogs, the up to an eight-fold increase in potency is believed to be associated with an interaction with the proposed aromatic pocket, and not simply to an increase in hydrophobicity or other factors. Also of importance, (+)-discodermolide and each of the six analogs increased the amount of microtubule polymerization compared to the control that had no drug added (Fig. 6). Taken together these observations strongly suggest that all of the discodermolide molecules have the same mechanism of action. In summary, we have defined two possible binding modes for (+)-discodermolide in the paclitaxel binding pocket. To test the validity of the proposed modes, we designed and synthesized a series of hybrid molecules, wherein the aromatic ring of paclitaxel was attached to (+)-discodermolide, exploiting a tether of suitable length. Biological testing demonstrated that hybrid congeners possess increased potency compared to (+)-discodermolide. Given the availability of the already incorporated phototags, the discodermolide/paclitaxel hybrids hold the promise of defining the (+)-discodermolide binding site and mode of action, as well as determining whether the drug demonstrates selectivity for any of the 7 β -tubulin isotypes. Since certain isotypes, particularly β III-tubulin, have been associated with paclitaxel resistance,⁴¹ an investigation of the interaction between drugs and specific tubulin isotypes becomes an important goal, and in particular definition of human tubulin isotypes that bind (+)-discodermolide. Such studies in conjunction with radiolabeling experiments are ongoing in our laboratories.

EXPERIMENTAL SECTION

Experimental details for obtaining IC₅₀ values

Cells lines were obtained from the American Type Culture Collection (ATCC) and maintained in RPMI medium supplemental with 10% fetal bovine serum. Cells (800 A549 or 2000 MCF-7) were added to each well of a 96 well plate. Increasing concentrations of the indicated drugs were added 18 h after plating. IC₅₀ values, the concentration of drug that inhibits cell growth by 50%, were determined after 72 h or 96 h incubation at 37 °C for A549 or MCF-7 cells, respectively, using the SRB method (40). The two cell lines were fixed and stained at different times to take into account their different growth rates.

Experimental details for tubulin polymerization studies

Bovine brain tubulin (2.5 μM) from Cytoskeleton Inc. in a buffer containing 0.1 M MES, 1 mM EGTA, 1 mM MgCl₂ was incubated with 3 μM of the indicated drugs, in the presence of 2 mM GTP, at 37 °C for 30 min. Samples were centrifuged at 120,000 × g at 37 °C for 1 h and the pellet that contains the polymerized tubulin was dissolved in SDS sample buffer and analyzed by SDS-polyacrylamide gel electrophoresis. Proteins were then transferred to nitrocellulose and stained with *Ponceau S*.

Experimental details for synthesis and characterization

Except as otherwise indicated, all reactions were run under an argon atmosphere in flame- or oven-dried glassware, and solvents were freshly distilled. The argon was deoxygenated and dried by passage through an OXICLEAR™ filter from Aldrich and Drierite tube, respectively. Diethyl ether (Et₂O), tetrahydrofuran (THF) and dichloromethane (CH₂Cl₂) were purchased from Aldrich (HPLC purity) and further purified by Pure Solve™ PS-400. All other reagents were purchased from Aldrich or Acros and used as received. Reactions were monitored by thin layer chromatography (TLC) either with 0.25 mm Silicycle or 0.25 mm E. Merck (Kieselgel 60F₂₅₄, Merck) pre-coated silica gel plates. Silica gel for flash chromatography (particle size 0.040–0.063 mm) was supplied by Silicycle or Sorbent. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise noted. ¹H and ¹³C spectra were recorded on a Bruker AMX-500 spectrometer. Chemical shifts are reported as δ values relative to internal chloroform (δ 7.26) or benzene (δ 7.15) for ¹H and either chloroform (δ 77.0) or benzene (δ 128.0) for ¹³C. Infrared spectra were recorded on a Jasco FTIR-480plus spectrometer. Optical rotations were measured on a Jasco P-2000 polarimeter in the solvent indicated. High resolution mass spectra were measured at the University of Pennsylvania Mass Spectrometry Center on either a VG Micromass 70/70H or VG ZAB-E spectrometer. Analytical reverse-phased (Sunfire C18; 4.6mm 50mm, 5mL) high-performance liquid chromatography (HPLC) was performed with a Waters binary gradient module 2525 equipped with Waters 2996 PDA and Waters micromass ZQ. All final compounds were analyzed employing a linear gradient from 10% to 90% of acetonitrile in water over 8 min and a flow rate of 1 mL/min, and unless otherwise stated, the purity level was >95%.

Representative Synthetic Procedures

Synthesis of (+)-3a: Compound (+)-20 (80 mg, 0.0725 mmol) was dissolved in CH₂Cl₂ (1 mL) and MeOH (1 mL). N-(4-Azido-benzoyl)-1,2-ethanediamine (**10**) (45 mg, 0.218 mmol) in MeOH (1 mL) and Et₃N (40 μL, 0.290 mmol) were added to the above solution. The resultant solution was stirred at room temperature for 6 days. Water (50 mL) was added to the reaction mixture and extracted with CH₂Cl₂ (3 × 50mL). The combined organic layers were washed with brine (50 mL), dried over MgSO₄, filtered and concentrated. Flash chromatography (11 to 25% EtOAc in hexane) provided compound (+)-3a (75.1 mg, 89%

yield) as a colorless amorphous solid. $[\alpha]^{22}_D = +36.0^\circ$ ($c = 0.65$, CHCl_3). IR (film, NaCl): 3336, 2957, 2929, 2857, 2123, 1718, 1653, 1604, 1539, 1500, 1254, 1044, 836, 775 cm^{-1} . ^1H NMR (500 MHz, CDCl_3) δ : 7.81 (d, $J = 8.3$ Hz, 2H), 7.33 (brs, 1H), 7.03 (d, $J = 8.4$ Hz, 2H), 6.54 (ddd, $J = 16.7, 10.6, 10.6$ Hz, 1H), 5.88 (apparent t, $J = 10.9$ Hz, 1H), 5.35 - 5.13 (m, 5H), 5.11 (d, $J = 10.2$ Hz, 1H), 5.02 (d, $J = 9.9$ Hz, 1H), 4.81 (apparent t, $J = 9.1$ Hz, 1H), 4.74 (apparent t, $J = 5.8$ Hz, 1H), 4.59 (ABq, $J_{AB} = 6.9$ Hz, $\Delta_{AB} = 28.4$ Hz, 2H), 4.50 (apparent t, $J = 10.3$ Hz, 1H), 3.63 (brs, 1H), 3.56 - 3.44 (m, 3H), 3.41 (apparent t, $J = 4.0$ Hz, 1H), 3.39 - 3.30 (m, 1H), 3.34 (s, 3H), 3.06 (apparent t, $J = 5.6$ Hz, 1H), 2.99 - 2.91 (m, 1H), 2.75 - 2.66 (m, 1H), 2.60 (qd, $J = 7.5, 2.8$ Hz, 1H), 2.55 - 2.47 (m, 1H), 2.06 (apparent t, $J = 12.7$ Hz, 1H), 1.91 - 1.76 (m, 3H), 1.75 - 1.66 (m, 2H), 1.63 - 1.54 (m, 1H), 1.57 (s, 3H), 1.23 (d, $J = 7.6$ Hz, 3H), 0.96 (d, $J = 6.7$ Hz, 3H), 0.95 - 0.84 (m, 12H), 0.91 (s, 9H), 0.87 (s, 9H), 0.85 (s, 9H), 0.72 (d, $J = 6.7$ Hz, 3H), 0.08 (s, 3H), 0.06 (s, 3H), 0.054 (s, 3H), 0.048 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ : 173.6, 166.7, 158.8, 143.4, 133.9, 133.4, 132.3, 132.1, 132.0, 131.1, 130.8, 130.0, 129.0, 119.1, 118.5, 97.5, 86.4, 79.5, 77.3, 77.0, 74.9, 64.9, 56.2, 44.2, 42.7, 42.5, 40.5, 37.9, 36.1, 35.8, 35.7, 34.6, 34.4, 34.3, 26.4, 26.1, 25.9, 23.3, 18.7, 18.3, 18.1, 17.7, 16.8, 16.7, 16.5, 14.4, 14.2, 10.2, -3.2, -3.5, -4.2, -4.3, -4.66, -4.68. High resolution mass spectrum (ESI+) m/z 1190.7408 $[(M+\text{Na})^+]$; calcd for $\text{C}_{62}\text{H}_{109}\text{N}_5\text{O}_{10}\text{Si}_3\text{Na}$: 1190.7380].

Synthesis of (+)-3: To a solution of compound (+)-3a (67 mg, 0.0573 mmol) in MeOH (5 mL) was added aqueous hydrochloric acid (4M, 4 mL) in 100 – 250 μL portions over 7 h at a rate which minimized precipitation (ca. 8 to 30 min intervals) and the sides of flask were rinsed with MeOH (1 mL). The reaction mixture was stirred at room temperature for 18 h, diluted with EtOAc (70 mL). The resulting solution was neutralized with NaHCO_3 (1.35 g in H_2O 18 mL) at 0 $^\circ\text{C}$. Phosphate buffer pH7 (1M, 30 mL) and NaCl (17 g) were added and the aqueous layer was extracted with EtOAc (3 $\times$ 70 mL). The combined organic layers were washed with brine (100 mL), dried over MgSO_4 , filtered and concentrated. The residue was purified three times by flash chromatography (80% EtOAc in hexane or 3 – 6% MeOH/ CH_2Cl_2) to afford compound (+)-3 (31.1 mg, 69% yield) as a colorless amorphous solid. $[\alpha]^{22}_D = +17.4^\circ$ ($c = 0.10$, CHCl_3). IR (film, NaCl): 3359, 2968, 2123, 1699, 1639, 1604, 1543, 1501, 1457, 1284, 1120, 1032, 731 cm^{-1} . ^1H NMR (500 MHz, CDCl_3) δ : 7.81 (d, $J = 8.5$ Hz, 2H), 7.26 (br, 1H), 7.06 (d, $J = 8.4$ Hz, 2H), 6.55 (ddd, $J = 16.6, 10.6, 10.6$ Hz, 1H), 5.84 (apparent t, $J = 11.0$ Hz, 1H), 5.54 - 5.41 (m, 3H), 5.26 (t, $J = 10.4$ Hz, 1H), 5.17 - 5.05 (m, 3H), 4.76 - 4.66 (m, 2H), 4.61 (apparent t, $J = 9.3$ Hz, 1H), 3.70 (br m, 1H), 3.57 - 3.44 (m, 2H), 3.43 - 3.31 (m, 2H), 3.29 - 3.16 (m, 3H), 3.00 - 2.91 (m, 1H), 2.83 - 2.74 (m, 1H), 2.68 (qd, $J = 7.3, 4.6$ Hz, 1H), 2.63 - 2.51 (m, 2H), 2.40 - 2.20 (br, 2H), 2.00 - 1.74 (m, 6H), 1.69 - 1.62 (m, 1H), 1.58 (s, 3H), 1.28 (d, $J = 7.3$ Hz, 3H), 1.06 (d, $J = 6.9$ Hz, 3H), 1.01 (d, $J = 6.8$ Hz, 3H), 0.93 (apparent d, $J = 6.8$ Hz, 6H), 0.90 (d, $J = 6.6$ Hz, 3H), 0.82 (d, $J = 6.5$ Hz, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ : 174.6, 167.2, 158.4, 143.6, 134.5, 133.9, 133.6, 133.0, 132.3, 130.6, 130.0, 129.6, 129.1, 119.2, 118.3, 79.4, 78.9, 77.4, 75.2, 73.1, 64.4, 43.3, 42.0, 41.2, 40.5, 37.4, 36.2, 36.0, 35.94, 35.91, 34.9, 33.0, 23.4, 18.8, 17.5, 16.7, 15.7, 14.2, 12.8, 9.3. High resolution mass spectrum (ESI+) m/z 804.4495 $[(M+\text{Na})^+]$; calcd. for $\text{C}_{42}\text{H}_{63}\text{N}_5\text{O}_9\text{Na}$: 804.4523].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Support was provided by the National Institutes of Health through the National Institutes of General Medical Sciences and the National Cancer Institute through grants GM-29028 (ABS) and CA-077263 (SBH), and the Breast Cancer Research Foundation (SBH). We also thank Drs. G. Furst and R. Kohli of the Department of Chemistry NMR and MS Facilities for obtaining the NMR and high resolution mass spectra.

References

1. (a) Gunasekera SP, Gunasekera M, Longley RE, Shulte K. Discodermolide: A new bioactive polyhydroxylated lactone from the marine sponge discodermia dissolute. *J Org Chem.* 1990; 55:4912–4912. Additions and corrections: (b) *J Org Chem.* 1991; 56:1346.
2. Nerenberg JB, Hung DT, Somers PK, Schreiber SL. Total synthesis of the immunosuppressive agent (–)-discodermolide. *J Am Chem Soc.* 1993; 115:12621–12622.
3. Hung DT, Nerenberg JB, Schreiber SL. Distinct binding and cellular properties of synthetic (+)- and (–)-discodermolides. *Chemistry and Biology.* 1994; 1:67–71. [PubMed: 9383372]
4. (a) Longley RE, Caddigan D, Harmody D, Gunasekera M, Gunasekera SP. Discodermolide - A new, marine-derived immunosuppressive compound. I. In vitro studies. *Transplantation.* 1991; 52:650–656. [PubMed: 1833864] (b) Longley RE, Caddigan D, Harmody D, Gunasekera M, Gunasekera SP. Discodermolide - A new, marine-derived immunosuppressive compound. II. In vivo studies. *Transplantation.* 1991; 52:656–661. [PubMed: 1926345]
5. Data is available through Development Therapeutics Program website from NIH: <http://dtp.nci.nih.gov>
6. Kowalski RJ, Giannakakou P, Gunasekera SP, Longley RE, Day BW, Hamel E. The microtubule-stabilizing agent discodermolide competitively inhibits the binding of paclitaxel (taxol) to tubulin polymers, enhances tubulin nucleation reactions more potently than paclitaxel, and inhibits the growth of paclitaxel-resistant cells. *Mol Pharmacol.* 1997; 52:613–622. [PubMed: 9380024]
7. (a) See Ref 2 (b) Smith AB III, Qiu Y, Jones DR, Kobayashi K. Total synthesis of (–)-discodermolide. *J Am Chem Soc.* 1995; 117:12011–12012. (c) Harried SS, Yang G, Strawn MA, Myles DC. Total Synthesis of (–)-Discodermolide: An Application of a Chelation-Controlled Alkylation Reaction. *J Org Chem.* 1997; 62:6098–6099. (d) Marshall JA, Johns BA. Total Synthesis of (+)-Discodermolide. *J Org Chem.* 1998; 63:7885–7892. (e) Smith AB III, Kaufman MD, Beauchamp TJ, LaMarche MJ, Arimoto H. Gram Scale Synthesis of (+)-discodermolide. *Org Lett.* 1999; 1:1823–1826. [PubMed: 10836041] (f) Paterson I, Florence GJ, Gerlach K, Scott JP, Sereinig N. A practical synthesis of (+)-discodermolide and analogues: Fragment union by complex aldol reactions. *J Am Chem Soc.* 2001; 123:9535–9544. [PubMed: 11572673] (g) Smith AB III, Freeze SB, Brouard I, Hirose T. A Practical Improvement, Enhancing the Large-Scale Synthesis of (+)-Discodermolide: A Third-Generation Approach. *Org Lett.* 2003; 5:4405–4408. [PubMed: 14602011] (h) Paterson I, Delgado O, Florence GJ, Lyothier I, Scott JP, Sereinig N. 1,6-Asymmetric induction in boron-mediated aldol reactions: Application to a practical total synthesis of (+)-discodermolide. *Org Lett.* 2003; 5:35–38. [PubMed: 12509884] (i) Mickel SJ, Sedelmeier GH, Niederer D, Daeffler R, Osmani A, Schreiner K, Seeger-Weibel M, Berod B, Schaer K, Gamboni R, Chen S, Chen W, Jagoe CT, Kinder FR, Loo M, Prasad K, Shieh WC, Wang RM, Waykole L, Xu DD, Xue S. Large-Scale Synthesis of the Anti-Cancer Marine Natural Product (+)-Discodermolide. Part 5: Linkage of Fragments C(1)–(6) and C(7)–(24) and Finale. *Org Process Res Dev.* 2004; 8:122–130. (j) Paterson I, Lyothier I. Total synthesis of (+)-discodermolide: An improved endgame exploiting a Still-Gennari-type olefination with a C1–C8 β -ketophosphonate fragment. *Org Lett.* 2004; 6:4933–4936. [PubMed: 15606103] (k) Arefolov A, Panek J. Crotylsilane reagents in the synthesis of complex polyketide natural products: Total synthesis of (+)-discodermolide. *J Am Chem Soc.* 2005; 127:5596–5603. [PubMed: 15826198] (l) Smith AB III, Freeze BS, Xian M, Hirose T. Total synthesis of (+)-discodermolide: A highly convergent fourth-generation approach. *Org Lett.* 2005; 7:1825–1828. [PubMed: 15844916] (m) Lemos E, Porée F-H, Bourin A, Barbion J, Agouridas E, Lannou M-I, Commerçon A, Betzer J-F, Pancrazi A, Ardisson J. Total synthesis of discodermolide: Optimization of the effective synthetic route. *Chemistry-A European Journal.* 2008; 14:11092–11112.
8. Fernholm, A. A Powerful Tool for Chemists. In: Bäckvall, J-E.; Thelander, L., editors. Nobel Prize 2010; Information for public. The Royal Swedish Academy of Sciences; 2010. http://nobelprize.org/nobel_prizes/chemistry/laureates/2010/info_publ_eng_2010.pdf
9. Beauchamp TJ, LaMarche MJ, Kaufman MD, Qiu YP, Arimoto H, Jones DR, Kobayashi K. Evolution of a Gram-Scale Synthesis of (+)-Discodermolide. *J Am Chem Soc.* 2000; 122:8654–8664.
10. Mita A, Lockhart AC, Chen TL, Bochinski K, Curtright J, Cooper W, Hammond L, Rothenberg M, Rowinsky E, Sharma S. A phase I pharmacokinetic (PK) trial of XAA296A (Discodermolide)

- administered every 3 wks to adult patients with advanced solid malignancies. *J Clin Oncol ASCO Annual Meeting Proceedings*. 2004; 14S:2025.
11. Smith AB III, LaMarche MJ, Falcone-Hindley M. Solution Structure of (+)-Discodermolide. *Org Lett*. 2001; 3:695–698. [PubMed: 11259039]
 12. See review article and reference therein: Smith AB III, Freeze BS. (+)-Discodermolide: total synthesis, construction of novel analogues, and biological evaluation. *Tetrahedron*. 2008; 64:261–298. [PubMed: 21113402]
 13. (a) Burlingame MA, Shaw SJ, Sundermann KF, Zhang D, Petryka J, Mendoza E, Liu F, Myles DC, LaMarche MJ, Hirose T, Freeze BS, Smith AB III. A series of 23,24-dihydrodiscodermolide analogues with simplified lactone regions. *Bioorg Med Chem Lett*. 2004; 14:2335–2338. [PubMed: 15081036] (b) Shaw SJ, Sundermann KF, Burlingame MA, Myles DC, Freeze BS, Xian M, Brouard I, Smith AB III. Toward understanding how the lactone moiety of discodermolide affects activity. *J Am Chem Soc*. 2005; 127:6532–6533. [PubMed: 15869264] (c) Shaw SJ, Menzella HG, Myles DC, Xian M, Smith AB. Coumarin-derived discodermolide analogues possessing equivalent antiproliferative activity to the natural product - A further simplification of the lactone region. *Org Biomol Chem*. 2007; 5:2753–2755. [PubMed: 17700839] (d) Smith AB, Freeze SB, LaMarche MJ, Hirose T, Brouard I, Xian M, Sundermann KF, Shaw SJ, Burlingame MA, Horwitz SB, Myles DC. Design, Synthesis, and Evaluation of Analogues of (+)-14-Normethyldiscodermolide. *Org Lett*. 2005; 7:315–318. [PubMed: 15646986] (e) Smith AB, Freeze BS, LaMarche MJ, Hirose T, Brouard I, Rucker PV, Xian M, Sundermann KF, Shaw SJ, Burlingame MA, Horwitz SB, Myles DC. Design, synthesis, and evaluation of carbamate-substituted analogues of (+)-discodermolide. *Org Lett*. 2005; 7:311–314. [PubMed: 15646985] (f) Smith AB, Rucker PV, Brouard I, Freeze BS, Xia SJ, Horwitz SB. Design, synthesis, and biological evaluation of potent discodermolide fluorescent and photoaffinity molecular probes. *Org Lett*. 2005; 7:5199–5202. [PubMed: 16268537]
 14. Klein LE, Freeze BS, Smith AB III, Horwitz SB. The microtubule stabilizing agent discodermolide is a potent inducer of accelerated cell senescence. *Cell Cycle*. 2005; 4:501–507. [PubMed: 15711127]
 15. Xia S, Kenesky CS, Rucker PV, Smith AB III, Orr GA, Horwitz SB. A photoaffinity analogue of discodermolide specifically labels a peptide in β -tubulin. *Biochemistry*. 2006; 45:11762–11775. [PubMed: 17002277]
 16. (a) Martello LA, McDaid HM, Regl DL, Yang CPH, Meng DF, Pettus TRR, Kaufman MD, Arimoto H, Danishefsky SJ, Smith AB, Horwitz SB. Taxol and discodermolide represent a synergistic drug combination in human carcinoma cell lines. *Clin Cancer Res*. 2000; 6:1978–1987. [PubMed: 10815923] (b) Honore S, Kamath K, Braguer D, Horwitz SB, Wilson L, Briand C, Jordan MA. Synergistic suppression of microtubule dynamics by discodermolide and paclitaxel in non-small cell lung carcinoma cells. *Cancer Res*. 2004; 64:4957–4964. [PubMed: 15256469]
 17. Huang GS, Lopez-Barcons L, Freeze BS, Smith AB III, Goldberg GL, Horwitz SB, McDaid HM. Potentiation of taxol efficacy and by discodermolide in ovarian carcinoma xenograft-bearing mice. *Clin Cancer Res*. 2006; 12:298–304. [PubMed: 16397055]
 18. Khrapunovich-Baine M, Menon V, Verdier-Pinard P, Smith AB, Angeletti RH, Fiser A, Horwitz SB, Xiao H. Distinct Pose of Discodermolide in Taxol Binding Pocket Drives a Complementary Mode of Microtubule Stabilization. *Biochemistry*. 2009; 48:11664–11677. [PubMed: 19863156]
 19. Fan Y, Schreiber EM, Day BW. Human Liver Microsomal Metabolism of (+)-Discodermolide. *J Nat Prod*. 2009; 72:1748–1754. [PubMed: 19775091]
 20. (a) Sanchez-Pedregal VM, Kubicek K, Meiler J, Lyothier I, Paterson I, Carlomagno T. The tubulin-bound conformation of discodermolide derived by NMR studies in solution supports a common pharmacophore model for epothilone and discodermolide. *Angew Chem*. 2006; 45:7388–7394. [PubMed: 17036370] (b) Canales A, Matesanz R, Gardner NM, Andreu JM, Paterson I, Diaz JF, Jimenez-Barbero J. The bound conformation of microtubule-stabilizing agents: NMR insights into the bioactive 3D structure of discodermolide and dictyostatin. *Chemistry-a European Journal*. 2008; 14:7557–7569. (c) Jogalekar AS, Kriel FH, Shi Q, Cornett B, Cicero D, Snyder JP. The Discodermolide Hairpin Structure Flows from Conformationally Stable Modular Motifs. *J Med Chem*. 2010; 53:155–165. [PubMed: 19894728]

21. Hoffmann RW, Stenkamp D, Trieselmann T, Göttlich R. Flexible molecules with defined shape XI. - Conformer equilibria in 2,4-disubstituted pentane derivatives. *Eur J Org Chem.* 1999; 11:2915–2927.
22. Information of DISCON software will be provided in a subsequent report; See <http://discon.sourceforge.net/>
23. (a) Cram DJ. Preorganization- From Solvents to Spherands. *Angew Chem Int Ed Engl.* 1986; 25:1039–1057. (b) Houk KN, Leach AG, Kim SP, Zhang X. Binding Affinities of Host-Guest, Protein-Ligand, and Protein-Transition-State Complexes. *Angew Chem Int Ed.* 2003; 42:4872–4897.
24. (a) Perola E, Charifson PS. Conformational Analysis of Drug-Like Molecules Bound to Proteins: An Extensive Study of Ligand Reorganization upon. *Binding J Med Chem.* 2004; 47:2499–2510. (b) Butler KT, Luque FJ, Barril X. Toward Accurate Relative Energy Predictions of the Bioactive Conformation of Drugs. *J Comp Chem.* 2009; 30:601–610. [PubMed: 18711721]
25. Lowe J, Li H, Downing KH, Nogales E. Refined structure of $\alpha\beta$ -tubulin at 3.5 Å resolution. *J Mol Biol.* 2001; 313:1045–1057. [PubMed: 11700061]
26. (a) Arnal I, Wade RH. How does taxol stabilize microtubules? *Curr Biol.* 1995; 5:900–908. [PubMed: 7583148] (b) Elie-Caille C, Severin F, Helenius J, Howard J, Muller DJ, Hyman AA. Straight GDP-tubulin protofilaments form in the presence of taxol. *Curr Biol.* 2007; 17:1765–1770. [PubMed: 17919908]
27. (a) Krebs A, Goldie KN, Hoenger A. Structural rearrangements in tubulin following microtubule formation. *EMBO Rep.* 2005; 6:227–232. [PubMed: 15731766] (b) Li HL, DeRosier DJ, Nicholson WV, Nogales E, Downing KH. Microtubule structure at 8 angstrom Resolution. *Structure.* 2002; 10:1317–1328. [PubMed: 12377118]
28. VanBuren V, Odde DJ, Cassimeris L. Estimates of lateral and longitudinal bond energies within the microtubule lattice. *Proc Natl Acad Sci U S A.* 2002; 99:6035–6040. [PubMed: 11983898]
29. Verdier-Pinard P, Wang F, Martello L, Burd B, Orr GA, Horwitz SB. Analysis of tubulin isotypes and mutations from taxol-resistant cells by combined isoelectrofocusing and mass spectrometry. *Biochemistry.* 2003; 42:5349–5357. [PubMed: 12731876]
30. Martello LA, LaMarche MJ, He LF, Beauchamp TJ, Smith AB III, Horwitz SB. The relationship between Taxol and (+)-discodermolide: Synthetic analogs and modeling studies. *Chem Biol.* 2001; 8:843–855. [PubMed: 11564553]
31. Paterson I, Naylor GJ, Fujita T, Guzman E, Wright AE. Total synthesis of a library of designed hybrids of the microtubule-stabilising anticancer agents taxol, discodermolide and dictyostatin. *Chem Comm.* 2010; 46:261–263. [PubMed: 20024345]
32. (a) Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE, Francis P, Shenkin PS. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J Med Chem.* 2004; 47:1739–1749. [PubMed: 15027865] (b) Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, Banks JL. Glide: A new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. *J Med Chem.* 2004; 47:1750–1759. [PubMed: 15027866]
33. Molegro Virtual Docker software package from Molegro ApS C. F. Moellers Alle 8, Building 1110, DK-8000 Aarhus C, Denmark.
34. Lyne PD, Lamb ML, Saeh JC. Accurate prediction of the relative potencies of members of a series of kinase inhibitors using molecular docking and MM-GBSA scoring. *J Med Chem.* 2006; 49:4805–4808. [PubMed: 16884290]
35. (a) Barral K, Moorhouse AD, Moses JE. Efficient conversion of aromatic amines into azides: A one-pot synthesis of triazole linkages. *Org Lett.* 2007; 9:1809–1811. [PubMed: 17391043] (b) Xiong Y, Bernardi D, Bratton S, Ward MD, Battaylia E, Finel M, Drake RR, Radominska-Pandya A. Phenylalanine 90 and 93 are localized within the phenol binding site of human UDP-glucuronosyltransferase 1A10 as determined by photoaffinity labeling, mass spectrometry, and site-directed mutagenesis. *Biochemistry.* 2006; 45:2322–2332. [PubMed: 16475821] (c) Bernardi D, Ba LA, Kirsch G. First synthesis of N-acylated photoactivatable analogues of glutathione bearing an aryl azide moiety. *Synthesis.* 2007; 1:140–144.

36. (a) Hegyi G, Michel H, Shabanowitz J, Hunt DF, Chatterjee N, Healylouie G, Elzinga M. Gln-41 is Intermolecularly Cross-Linked to Lys-113 in F-Actin by N-(4-Azidobenzoyl)-Putrescine. *Protein Sci.* 1992; 1:132–144. [PubMed: 1363931] (b) Nielsen PE. Photoaffinity Labeling of Chromatin. *Eur J Biochem.* 1982; 122:283–289. [PubMed: 7060576] (c) Borda EJ, Sigurdsson ST. Investigation of Mg²⁺ - and temperature-dependent folding of the hairpin ribozyme by photo-crosslinking: effects of photo-crosslinker tether length and chemistry. *Nuc Acids Res.* 2005; 33:1058–1068.
37. (a) Smith DP, Anderson J, Plante J, Ashcroft AE, Radford SE, Wilson AJ, Parker MJ. Trifluoromethyldiazirine: an effective photo-induced cross-linking probe for exploring amyloid formation. *Chem Comm.* 2008; 11:5728–5730. [PubMed: 19009062] (b) Sammelson RE, Casida JE. Synthesis of a tritium-labeled, fipronil-based, highly potent, photoaffinity probe for the GABA receptor. *J Org Chem.* 2003; 68:8075–8079. [PubMed: 14535785] (c) Ambroise Y, Pillon F, Mioskowski C, Valleix A, Rousseau B. Synthesis and tritium labeling of new aromatic diazirine building blocks for photoaffinity labeling and cross-linking. *Eur J Org Chem.* 2001; 20:3961–3964. (d) Krapcho AP, Kuell CS. Mono-Protected Diamines - N-Tert-Butoxycarbonyl-Alpha, omega-Alkanediamines from Alpha, omega-Alkanediamines. *Syn Comm.* 1990; 20:2559–2564.
38. Anderson GW, Callahan FM, Zimmerman JE. Use of Esters of N-Hydroxysuccinimide in Peptide Synthesis. *J Am Chem Soc.* 1964; 86:1839–1844.
39. Paul R, Anderson GW. N,N'-Carbonyldiimidazole, a new peptide forming Reagent. *J Am Chem Soc.* 1960; 82:4596–4600.
40. Anderson GW, McGregor AC. Tert-Butyloxycarbonylamino Acids and their use in Peptide Synthesis. *J Am Chem Soc.* 1957; 79:6180–6183.
41. Kavallaris M. Microtubules and resistance to tubulin-binding agents. *Nature Reviews Cancer.* 2010; 10:194–204.

Figure 1.
(+)-Discodermolide and Paclitaxel

Figure 2.
Solution conformations of (+)-discodermolide

Figure 3.
Binding mode of **A.** Paclitaxel (PDB: 1JFF) **B.** (+)-Discodermolide/Pose 1 **C.** (+)-Discodermolide/Pose 2

Figure 4.
Designed (+)-discodermolide analogs.

Figure 5.
Docking of **4** in **A**) Pose 1 and **B**) Pose 2.

Figure 6. Tubulin polymerization in the presence of (+)-discodermolide and its analogs. The control has no drug.

Scheme 2.
Preparation of the photoaffinity tags.

Scheme 3.
Syntheses of the designed analogs.

Table 1

Cytotoxicity of (+)-discodermolide and analogs against human lung A549 and breast MCF-7 cancer cell lines.

IC₅₀^a	(+)-1	(+)-2	(+)-3	(+)-4	(+)-5	(+)-6	(+)-7
A549	9.34±0.56	3.14±0.09	5.61±0.15	1.21±0.35	3.76±0.57	1.05±0.15	2.64±0.22
MCF-7	7.98±0.35	0.91±0.11	3.64±0.12	1.90±0.43	2.71±0.55	1.66±0.48	1.92±0.07

^[a] IC₅₀ equals the concentration (nM) of drug that inhibits 50% cell growth.