Contributed Reviews

A Tale of Two Tumor Targets: Topoisomerase I and Tubulin. The Wall and Wani Contribution to Cancer Chemotherapy†

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The seminal discoveries of camptothecin and Taxol by Wall and Wani are discussed in a manner that demonstrates the influence that these two compounds has had on the further development of natural product, natural product-derived, and (some) synthetic entities as potential drug leads that interact either with tubulin or with topoisomerase I. The major categories of tubulin interactive agents in terms of inhibition and promotion of tubulin polymerization are briefly discussed. Likewise, a brief discussion of topoisomerase I inhibitors is presented. Lists of tubulin interactive agents and topoisomerase I inhibitors in preclinical and clinical development are given in Tables 2 and 3, respectively. This review is not meant to be exhaustive, but does illustrate the profound impact that these two plant-derived agents have had on cancer chemotherapy.

Two of the most significant discoveries in the area of cancer chemotherapy originated from the laboratory of Drs. Monroe Wall and Mansukh Wani at Research Triangle Institute, North Carolina. As described by Wall,[1] the discovery of the potent antitumor activity of an extract of the leaves of *Camptotheca acuminata* Decne. (Nyssaceae) in 1958 was somewhat serendipitous and "profoundly changed my career". While screening thousands of plant extracts as a possible source of steroidal precursors for cortisone as part of a program supported by the U.S. Department of Agriculture, 1000 of the extracts were also tested for potential antitumor activity through a collaboration with Dr. Jonathan Hartwell of the National Cancer Institute (NCI) Cancer Chemotherapy National Service Center (CCNSC). The extracts of *C. acuminata* were identified as the only ones showing significant activity in the CA755 (adenocarcinoma) assay. In July 1960, he joined the newly founded Research Triangle Institute as head of the Natural Products Laboratory and, in 1962, was joined by Dr. Mansukh Wani; this was the beginning of a highly productive partnership that would last over four decades.[2]

This also led to a lifelong collaboration with the NCI, and one of the early products of this collaboration was the isolation and structural elucidation of camptothecin (**1**) as the active agent of *C. acuminata* in 1966. The discovery of paclitaxel (Taxol) (**2**) from the bark of the Pacific Yew tree (*Taxus brevifolia* Nutt.), on the other hand, was the result of an exploratory plant screening program sponsored by the NCI with the specific goal of discovering novel agents for the treatment of cancer. Quite apart from the structural novelty and significant antitumor activity of these two agents, research on their modes of action has led to the discovery of unique mechanisms for the selective inhibition of cancer cell proliferation. The interaction of camptothecin with topoisomerase I,[3] and the promotion of polymerization of tubulin and stabilization of the resultant microtubules by paclitaxel,[4] spurred an era of intense research aimed at the discovery and development of new cancer chemotherapeutic agents, which continues to this day.

The aim of this paper is not to present a thorough review of all anticancer agents, either in regular clinical use or in development, acting through interaction with tubulin or topoisomerase I, but rather to highlight the contributions made to the advancement of cancer chemotherapy by these seminal discoveries by the Wall and Wani team.

Tubulin Interactive Agents

Antimitotic agents are compounds that arrest cells in mitosis (the M phase in the cell cycle), and by far the major group of such agents can be classified as tubulin interactive agents (TIAs).[5] These agents may be divided into at least two major categories: those that inhibit the polymerization of tubulin to form microtubules, and those that promote the polymerization of tubulin and stabilize the resultant microtubules.[6] Hamel and Covell[7] use a modified set of subdivisions whereby they identify four well-described modes of interaction. One involves either a covalent cross-linking to the tubulin cysteine residues (predominately by synthetic molecules) or, as found with the natural product ottelione A (RPR112378) (**3**), a specific interaction with CYS-239, which stops tubulin polymerization, in addition to inhibiting colchicine (**4**) binding.[8] The next two modes are defined by agents that bind to the colchicine site or at or close to where vincristine (**5**) binds, the so-called *Vinca* domain. Finally, the fourth mode, which involves preferential binding to polymerized tubulin, is exemplified by Taxol and other natural products such as discodermolide (**6**), the epothilones (**7**−**10**), laulimalide (**11**), eleutherobin (**12**), and sarcodictyin (**13**). Except for the sulfhydryl-specific synthetic agents, the others are discussed below.

The two major classes of natural product-derived antimitotic agents that act through inhibition of tubulin polymerization are those that bind to β-tubulin at the

† Dedicated to the late Dr. Monroe E. Wall and to Dr. Mansukh C. Wani of Research Triangle Institute for their pioneering work on bioactive natural products.

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Table 1. Naturally Derived Microtubule Stabilizing Agents

name	source	status	recent reference
paclitaxel	*Taxus brevifolia* (plant) (made by semisynthesis)	clinical use	He et al.[10] Kingston[13]
docetaxel	semisynthesis from *Taxus* spp.	clinical use	Guenard et al.[31]
discodermolide	*Discodermia dissoluta* (marine) (made synthetically)	phase I in 2002	Gunasekera et al.[32] Longley et al.[33] ter Haar et al.[34] Smith et al.[35] Paterson and Florence[36] Choy et al.[37]
eleutherobin	*Eleutherobia* sp. (marine)	derivatives in preclinical development	Lindel et al.[38] Roberge et al.[39] Taglialatela-Scafati et al.[40]
sarcodictyin	*Sarcodictyon roseum* (marine)	derivatives in preclinical development	Ciomei et al.[41] Nicolaou et al.[42] Hamel et al.[43]
epothilones	*Sorangium* sp. (terrestrial microbe)	naturally occurring compounds and derivatives in early clinical trials	Gerth et al.[44] Hardt et al.[45] Nicolaou et al.[46] Julien et al.[47] Yoshimura et al.[48] O'Connor et al.[49]
laulimalide	*Cacospongia mycofijiensis* (marine)	preclinical development	Mooberry et al.[50] Ahmed et al.[51]
dictyostatin-1	*Spongia* sp. and a deep water Lithistid sponge (marine)	preclinical development	Pettit et al.[15] Isbrucker et al.[16]
jatrophane esters	*Euphorbia semiperfoliata* (plant)	preclinical development	Appendino et al.[52] Miglietta et al.[14]

colchicine site and those that interact with β-tubulin at the *Vinca* domain. The antimitotic activity of colchicine was recognized some 60 years ago,[6] but colchicine itself lacks significant in vivo anticancer activity and is used clinically for the treatment of indications such as gout. Its inhibition of tubulin polymerization is due to binding to β-tubulin at the colchicine binding site, and several antimitotic agents operating by this mechanism, and embracing a variety of structural chemotypes, have been discovered. These include podophyllotoxin, stegnacin, 2-methoxyestradiol, the curacins, heterocyclic ketones (including some flavonoids), benzoylphenylureas, and combretastatin A4 (**14**) and other analogues (cf., Hamel, and Hamel and Covell and references therein).[7,8]

The *Vinca* alkaloids, vinblastine and vincristine (and their later, clinically approved semisynthetic derivatives, vinorelbine and vindesine), were among the earliest antimitotic agents identified as acting through inhibition of tubulin polymerization and act through binding of β-tubulin at the *Vinca* alkaloid site. The *Vinca* binding site is located in the so-called *Vinca* domain, and agents may be classified either as competitive inhibitors of *Vinca* alkaloid binding, which bind directly to the *Vinca* site, or as noncompetitive inhibitors, which bind in close proximity to the site and exert their inhibitory effects due mainly to steric disruption of *Vinca* alkaloid binding to β-tubulin.[7] Agents acting as competitive inhibitors include maytansine and rhizoxin, while noncompetitive inhibitors include two structural classes, the macrocyclic polyethers (the halichondrins and spongistatins), and peptides and depsipeptides. The latter class of compounds has been the subject of two recent extensive reviews[7,8] and include the phomopsins, ustiloxins, dolastatins 10 and 15 (and synthetic analogues), the cryptophycins (and synthetic analogues), the hemiasterlins, the tubulysins, diazonamide A (**15**), and vitilevuamide. These classes of tubulin polymerization inhibitory agents have been discussed in detail in a relatively recent review from the aspects of their activity and use in cancer therapy.[9]

The discovery of Taxol by the Wall and Wani team assumed an added measure of importance through the ground-breaking discovery by Horwitz et al. of its unique mechanism of action, namely, an exclusive interaction with the polymer form of tubulin and "its ability to induce the formation of characteristic microtubule bundles in cells".[10] Later research, elaborating on the details of the stabilization of microtubules by Taxol, has been reviewed by Horwitz et al. and Jordan.[10,11] Although the taxanes are not considered ideal drug entities, they have been explored both therapeutically[12] and chemically[13] for many years while the search for more effective tubulin interactive agents has continued. This search has not been in vain, and a number of novel chemotypes that act by mechanisms similar to that of Taxol have been discovered;[11] these include two compounds reported as cytotoxins earlier, for which their actual biological mechanisms of action were not reported until very recently. The first chemotype is the jatrophane esters,[14] in particular jatrophane 1 (**16**) from samples of the Corsican and Sardinian plant *Euphorbia semiperfoliata*. The second, very interestingly, is a marine-derived macrolide, dictyostatin 1 (**17**), with structural features of discodermolide, originally isolated by Pettit et al. from a Maldivian *Spongia* sp. in low yield;[15] it was later rediscovered, together with its novel mechanism of action, by the Harbor Branch group from a deep water lithistid sponge by following a tubulin interaction assay for isolation rather than relying on simple cytotoxicity determinations.[16]

What is also of import is the fact that the levels of paclitaxel-like drugs and Taxol itself that are required to cause major disruption to the mitotic processes are not at the micromolar levels used in the in vitro studies of interactions of such agents with tubulin, but are frequently 2−3 orders of magnitude lower in cellular assays, particularly in induction of apoptosis studies. The alteration of microtubule dynamics by these interactions, rather than just the stabilization of polymerized tubulin, appears to be a major factor in the activity of these agents at the molecular level.[11]

A list of these agents from natural sources (and modifications thereof), together with their source organisms and stages of development, is given in Table 1.

Recent Preclinical and Clinical Developments of Tubulin Interactive Agents

Of the 2255 cancer clinical trials recorded, as of August 2003, at the website http://clinicaltrials.gov/ct/screen/

AdvancedSearch, 310, or close to 14%, are listed as involving taxane-derived drugs, including 173 with paclitaxel (Taxol), 115 with docetaxel (Taxotere), and 22 with miscellaneous taxanes, either as single agents or in combination with other anticancer agents.

The tubulin interactive agents in clinical and preclinical development, as reported in the Prous *Ensemble* database, are listed in Table 2, with structures **24**−**125** given at the end of the review. Of the 116 agents listed, 35, or 30%, are taxanes and include 12 in clinical trials (see http://cis.nci.nih.gov/fact/7_15.htm; Taxanes in Cancer Treatment). This emphasizes the considerable, continuing interest in enhancing the effectiveness of this class of molecules. Paclitaxel has also attracted attention in the potential treatment of multiple sclerosis, psoriasis, and rheumatoid arthritis (see http://www.phrma.org/newmedicines/newmedsdb/drugs.cfm). In addition, nine paclitaxel mechanistic mimics, including six epothilones, are in various stages of development, with at least two more, jatrophane 1 and dictyostatin 1, identified in the recent literature but not yet included in the Ensemble database as of mid-August 2003.

Of interest is the number of combretastatin (CA4) mimics being developed. Three are in clinical trials, while 13 are in preclinical development. This chemical class has served as a model for the synthesis of a host of analogues containing the essential trimethoxy aryl moiety linked to substituted aromatic moieties through a variety of two or three atom bridges including heterocyclic rings and sulfonamides. This impressive display of the power of a relatively simple natural product structure to spawn a prolific output of medicinal and combinatorial chemistry has been well covered in an excellent review by Li and Sham of Abbott Laboratories.[6] In contrast, in a study of conformational restrictions in some lavendustin A analogues, and their effect upon tubulin, Mu et al. demonstrated that, contrary to expectations, the trimethoxy analogues of lavendustin A were inert as tubulin-interactive agents; the dimethoxy analogues, however, with a free hydroxyl group in the salicylamide ring, were among the most potent (IC_{50} values of 1.4 μM for inhibition of tubulin polymerization). These analogues do not compete well with ^{3}H-colchicine at the colchicine-binding site, in contrast to CA4, and may thus rapidly dissociate from the site. Thus, their mechanism is probably subtly different from that of CA4 and its derivatives.[17]

Topoisomerase I Inhibitors

The two fundamental enzyme complexes involved in DNA winding and unwinding are topoisomerases I and II. We will not be dealing with topoisomerase II, although there are a number of important clinically useful agents, such as the anthracyclines (e.g., doxorubicin), which exert their cytotoxic effects through interaction with this enzyme. Rather, the focus will be on topoisomerase I, whose validation and importance as a molecular target may be directly attributed to the discovery and development of camptothecin.[18] The only topoisomerase I-directed agents currently in regular clinical use are the semisynthetic compounds derived from camptothecin, topotecan (**18**) and irinotecan (**19**) (CPT-11) (Table 3). Although camptothecin itself was originally isolated as a cytotoxin and the topoisomerase I activity was not discovered until later, these semisynthetic derivatives were synthesized in efforts to overcome the instability of the lactone ring and the innate insolubility of the parent compound, while maintaining topoisomerase I inhibitory activity.

Despite significant efforts on the part of many research groups, few structural classes of compounds have demonstrated topoisomerase I inhibitory activity. Over the last 10 or so years, novel lipids such as the ceramide 1-sulfates and other long-chain saturated and unsaturated fatty acids from marine sources have been reported to demonstrate in vitro inhibition against human topoisomerase I, together with microbial products of diverse structures such as the topostins (a collection of lipid-substituted amino acids and short peptides). In addition to these, a few other compounds have been reported to promote topoisomerase I-mediated cleavage of DNA, such as the anthraquinonoid topopyrones, the berberine alkaloid, coralyne, the saintopins, bulgarein, and intoplicine derivatives (see Newman et al. and references therein).[5]

In contrast, however, have been the findings that indolocarbazole derivatives, closely related to the known protein kinase inhibitor staurosporine (**20**) and the cytotoxin rebeccamycin (**21**), are potent topoisomerase I inhibitors.[5] The findings have led to the synthesis of water-soluble analogues, such as NB-506 (**22**) (Table 3), and second-generation products, all of which show excellent preclinical in vivo activity. The topoisomerase I activity of this class of compounds has been extensively studied by Prudhomme and her colleagues.[19] One of the most interesting discoveries in this structural class has been that the simple analogue R-3 (**23**), first reported as a topoisomerase I inhibitor in 1997,[20] not only inhibits topoisomerase I but also completely inhibits the phosphorylation of SF2/ASF, a member of the SR protein family in the absence of DNA. Thus, this compound is the only compound so far reported that inhibits the protein kinase activity of topoisomerase I, an activity first reported in 1996[21] and reviewed in 1997.[22]

Recent Preclinical and Clinical Developments of Topoisomerase I Inhibitors

Of the 2255 cancer clinical trials recorded, as of August 2003, at the website http://clinicaltrials.gov/ct/screen/AdvancedSearch, 121, or approximately 5.3%, are listed as involving camptothecin-derived drugs, including 74 with irinotecan (CPT-11), 32 with topotecan, and 12 with other miscellaneous analogues, either as single agents or in combination with other anticancer agents.

The topoisomerase I-interactive agents in clinical and preclinical development, as reported in the Prous *Ensemble* database, are listed in Table 3. Of the 60 agents listed, 26, or approximately 43%, are camptothecin derivatives and include 10 of the 17 in clinical trials, which, as with the taxanes, emphasizes the considerable, continuing interest in enhancing the effectiveness of this class of molecules. Significant new classes of topoisomerase I inhibitors in preclinical development are the 2-aryl-quinoline derivatives (indenoquinolines), the 3-aryl-isoquinoline derivatives (indeno-isoquinolines), and the naphthyridines which can be traced to the protoberberine alkaloids, such as coralyne and nitidine.[23−25] It is significant in this context that indeno-isoquinolines were recognized as possible topoisomerase I inhibitors by use of the COMPARE analysis of the cytotoxicity profile of NSC 314622 with the profiles of irinotecan, topotecan, and saintopin.[26]

Use of COMPARE in Searching Natural Product Extracts

The COMPARE program referred to above (http://dtp.nci.nih.gov/docs/compare/compare.html) has been applied to crude extracts in order to determine if it was

Table 2. Tubulin Interactive Agents in Clinical and Preclinical Development

structure/ensemble no.	phase	active development[a]	generic name	trade name	source[b]	chemical type
2/101438	launched		paclitaxel	Taxol	N	taxane
24/140605	launched		docetaxel	Taxotere	ND	taxane
5/155597	launched		vincristine		N	vinca
25/274460	launched		noscapine	Narcotussin	N	opium alkaloid
26/267063	phase III	yes	T-138067		S	benzene sulfonamide
[c]/289351	phase III	yes	ABI-007 (suspension)		N	taxane
5/236402	phase II/III	yes	vincristine sulfate TCS	Onco TCS	N	vinca
27/148668	phase II		erbulozole		S	dioxolane-imidazole
28/149507	phase II	yes	dolastatin 10		N	linear peptide
29/185537	phase II	yes	ABT-751		S	sulfonamide
30/203141	phase II	yes	RPR-109881A		ND	taxane
31/222498	phase II		cematodine		ND	linear peptide
8/222557	phase II	yes	epothilone B		N	epothilone
32/225384	phase II	yes	combretastatin A-4 PO_4		ND	CA4 analogue
33/227146	phase II	yes	soblitodin		N	linear peptide
34/261501	phase II		cryptophycin 52		ND	macrolide
35/275943	phase II	yes	BMS-184476		ND	taxane
36/277094	phase II	yes	anhydrovinblastine		ND	vinca
37/277994	phase II	yes	DHA-paclitaxel	Taxoprexin	ND	taxane
38/279172	phase II	yes	2-methoxyestradiol	Panzem	ND	steroid
39/282724	phase II	yes	BMS-188797		ND	taxane
40/287186	phase II	yes	RPR-116258A		ND	taxane
41/287750	phase II	yes	T-900607		S	fluorosulfonamide
42/293356	phase II	yes	ixabepilone		ND	16-azaepothilone B
43/327193	phase II	yes	7-hexanoyltaxol		ND	taxane
44/149879	phase I/II	yes	mivobulin isethionate		S*	deazapteridine
14/229342	phase I/II	yes	combretastatin A4		N	tetramethoxy-stilbene
45/170899	phase I	yes	NSC-639829		S	benzoyl urea
6/171277	phase I	yes	discodermolide		N	linear polyketide
c/222500	phase I	yes	PNU-166945		ND	taxol-HPMA polymer
10/251562	phase I	yes	epothilone D		N	epothilone
46/253902	phase I	yes	LU-223651/ILX-651		S*	linear peptide
47/262290	phase I	yes	AVE−8063A		ND	CA4 analogue
48/262298	phase I	yes	AVE−8062A		ND	CA4 analogue
49/264502	phase I	yes	ortataxel		ND	taxane
50/287199	phase I	yes	E−7389		ND	half-halichondrin B
51/294121	phase I	yes	21-aminoepothilone B		ND	epothilone
52/304277	phase I	yes	DJ-927		ND	taxane
53/308286	phase I	yes	TL-00139		ND	taxane
54/309743	phase I	yes	BMS-275183		ND	taxane
55/317936	phase I	yes	HTI-286		ND	linear tripeptide
56/163519	preclinical		none given		S	diphenylcyclopropane
57/183725	preclinical		NSC-647752		ND	taxane
58/186313	preclinical		none given		ND	taxane
59/212162	preclinical		SB-T-1212		ND	taxane
60/213014	preclinical		BMS-185660		ND	taxane
61/213774	preclinical		altohyrtin A		N	macrolide PKS
62/214156	preclinical		t-BCEU		S	chlorethyl urea
63/215756	preclinical		SB-T-1011		ND	taxane
64/219560	preclinical		ER-34410		S	benzdiazepine
65/234378	preclinical		LS-4559-P		S	biphenyl urea
66/234389	preclinical	yes	LS-4477		S	biphenyl urea
67/234390	preclinical	yes	LS-4559		S	biphenyl urea
c/234623	preclinical		PEG5000-paclitaxel		ND	taxane
68/235935	preclinical		RPR-112378		N	terpene
69/237135	preclinical		DZ-3358		S	pyrimidine-imidazole
12/237965	preclinical		eleutherobin		N	terpene
70/245094	preclinical		FR-182877		N	PKS
71/253755	preclinical		none given		ND	steroid
72/253776	preclinical		KAR-2		ND	vinca
73/255253	preclinical		none given		ND	taxane
74/255254	preclinical		PNU-105298		ND	taxane
75/255761	preclinical		AM-132		ND	propenone
76/257900	preclinical		bromotaxol		ND	taxane
77/260706	preclinical		IDN-5005		ND	colchicine
78/264373	preclinical		vitilevuamide		N	cyclic peptide
79/265677	preclinical		centaureidin		N	chromone
80/269193	preclinical		BTO-956	Oncocidin A1	S*	biphenyl ether
81/270156	preclinical		T-3782		ND	taxane
82/270693	preclinical		none given		ND	CA4 analogue
83/270694	preclinical		none given		ND	CA4 analogue
84/273748	preclinical		DDE−313		S	THF derivative
11/273775	preclinical	yes	laulimalide		N	PKS macrolide
85/274466	preclinical	yes	D-24851		S	indole derivative
86/274502	preclinical		A-105972		ND	CA4 analogue

Table 2 Continued

structure/ensemble no.	phase	active development[a]	generic name	trade name	source[b]	chemical type
87/277447	preclinical		none given		ND	taxane
88/279594	preclinical		none given		S	benzoyl urea
89/282043	preclinical		vanadocene acetylacetonate		S	ferrocene
90/282707	preclinical		T-138026		S	fluorinated sulfonamide
91/282779	preclinical		none given		S	fluorinated sulfonamide
c/283171	preclinical	yes	SDZ-LAV-694		ND	lavendustin A derivative
92/286137	preclinical		none given		ND	taxane
93/287230	preclinical		3-IAABE		S	iodinated benzoic acid
94/291170	preclinical		halichondrin B		N	macrolide
95/291245	preclinical		none given		ND	taxane
96/291247	preclinical		none given		ND	taxane
97/291248	preclinical		none given		ND	taxane
98/295661	preclinical	yes	D-64131		S	aroyl indoles
99/296536	preclinical		desoxyepothilone F		ND	epothilone
15/297883	preclinical		diazonamide A		N	mixed PKS/NRPS
100/298291	preclinical		A-293620		ND	CA4 analogue
101/298297	preclinical		none given		ND	CA4 analogue
102/299805	preclinical		none given		ND	taxane
103/300873	preclinical		none given		ND	CA4 analogue
104/301518	preclinical		2′-palmitoylpaclitaxel		ND	taxane
105/301519	preclinical		2-(2-bromohexadecanoyl)taxol		ND	taxane
106/301741	preclinical		26-fluoroepothilone		ND	epothilone
107/301977	preclinical		none given		ND	CA4 analogue
108/301979	preclinical		A-259745		ND	CA4 analogue
109/302036	preclinical		A-305754		ND	CA4 analogue
110/302054	preclinical	yes	IDN-5390		ND	taxane
111/302098	preclinical		D51−1456		S	piperazine
2/305216	preclinical		none given	Taxosomes	ND	taxane
112/305429	preclinical	yes	halimide		N	diketopiperazine
113/306972	preclinical		none given		S	tripentenones
114/317844	preclinical		A-289099		ND	CA4 analogue
115/317845	preclinical		A-318315		ND	CA4 analogue
116/317881	preclinical		none given		ND	CA4 analogue
[c]/318226	preclinical		DRF-3188		ND	andrographolide derivative
117/318241	preclinical	yes	D-82318		S	acridine derivative
118/318836	preclinical		NSC-12983		ND	steroid
119/319809	preclinical		BPR-0Y-007		S	cyclopentanone
[c]/325421	preclinical	yes	SSR-250411		S	none given
120/326284	preclinical		none given		ND	propenylestradiol
121/331061	preclinical		2′-MPA-paclitaxel		ND	taxane
122/331735	preclinical		none given		ND	taxane
123/333162	preclinical		none given		ND	taxane
76/336823	preclinical	yes	STA-5312		S	none given
124/338268	preclinical		14-dehydro-2-ME		ND	2-methoxyestradiol derivative
125/342740	preclinical		JIMB-01		S	carbamoylbenzamide

[a] Status as August 15, 2003. [b] Source codings as described in Newman et al.[53] [c] No formal structure given (either not available or a suspension and/or polymer adduct).

possible to identify those that might contain compounds that were biologically equivalent to the "seed structure(s)" used. In the case of topoisomerase I inhibitors, using camptothecin as the "seed structure", a series of 25 extracts were initially identified. These extracts were then cross-tested in a selective yeast bioassay for topoisomerase I and in a biochemical assay for stabilization of the topoisomerase I-DNA complex. Removal of all extracts (via taxonomic identity) that were reported to contain camptothecin(s) left an example from the plant *Pyrenacantha klaineana* from which camptothecin and 9-methoxycamptothecin were subsequently isolated and identified. In retrospect, the production of camptothecins by this plant from the family Icacinaceae was not totally unexpected, as there had been reports of camptothecin analogues from *Nothapodytes foetida* (family Icacinaceae) in the past.[27]

With one specific tubulin interactive agent, the COMPARE system was used in a slightly different manner. Thus, the marine metabolite diazonamide A[28] was first reported by Fenical's group in 1991 following bioactivity-driven isolation from an ascidian initially identified as *Diazona chinensis*, but now known to be *Diazona angulata*. Although Fenical's group made repeated efforts to re-collect this organism, they were not successful. In 1996, the NCI's Shallow Water Collection Contractor, Dr. Patrick Colin of the Coral Reef Research Foundation, learned of these efforts and identified some samples from collections that they had made in other parts of the South Pacific as possibly being *Diazona* species from initial taxonomy. These identified samples were rapidly extracted and assayed using the NCI's 60-cell line panel, and then using paclitaxel as the seed, these and other marine-derived extracts were "compared".

From the data, a sample whose Pearson Correlation Coefficient at the GI_{50} level was >0.810 was provided to Fenical's laboratory under a specific materials transfer agreement protecting the rights of the source country. This material yielded diazonamide A in sufficient quantity for

Table 3. Topoisomerase I Inhibitory Agents in Clinical and Preclinical Development

structure/ensemble no.	phase	active development[a]	generic name	trade name	source[b]	chemical type
126/070478	launched		SN-38		ND	camptothecin
19/103766	launched		CPT-11	Irinotecan HCl	ND	camptothecin
18/149556	launched		topotecan	Hycamptin	ND	camptothecin
127/241383	preregistration	yes	rubitecan	Orathecin	ND	camptothecin
128/184764	phase III		9-aminocamptothecin		ND	camptothecin
129/197987	phase III	yes	exatecan mesilate		ND	camptothecin
130/129669	phase II		CRC-88/05		S	acridine
131/196888	phase II	yes	lurtotecan		ND	camptothecin
132/231292	phase II	yes	edotecarin		ND	indolocarbazole
133/231405	phase II		BMS-247615		S	quinoline
134/239803	phase II	yes	CKD-602		ND	camptothecin
135/259669	phase II	yes	BNP-1350	Karenitecin	ND	camptothecin
c/263640	phase II	yes	PEG-camptothecin	Prothecan	N	camptothecin
136/275176	phase II	yes	diflomotecan		ND	camptothecin
c/298601	phase II	yes	PG-camptothecin		N	camptothecin
c/319987	phase I/II	yes	LE-SN38		ND	camptothecin
137/163132	phase I		intoplicine		S	pyridoindole
22/200503	phase I		NB-506		ND	indolocarbazole
138/274523	phase I	yes	XR-5944		S	phenazine
c/280933	phase I	yes	DRF-1042		ND	camptothecin
139/287947	phase I	yes	XR-11576		S	phenazine
c/318228	clinical	yes	DRF-1644		S?	none given
140/171476	preclinical		none given		ND	camptothecin
141/196538	preclinical		Hoechst-33342		S	piperazine
142/250703	preclinical		J-109534		ND	indolocarbazole
143/250906	preclinical		CZ-112		ND	camptothecin
144/251650	preclinical		10-HCPT		ND	camptothecin
145/261782	preclinical		CZ-48		ND	camptothecin
146/264755	preclinical		NU:UB-31		S	anthraquinone
147/271570	preclinical		none given		ND	camptothecin
148/274522	preclinical	yes	F-11782	Tafluposide	ND	epipodophyllotoxin
149/274543	preclinical	yes	DB-67		ND	camptothecin
150/275177	preclinical	yes	BN-80927		ND	camptothecin
151/276066	preclinical		J-109404		ND	indolocarbazole
c/276420	preclinical		F-12167		S	none given
152/280332	preclinical		9-ACG		ND	camptothecin
153/282913	preclinical		DB-174		ND	camptothecin
154/284251	preclinical		amarogentin		N	glycoside
155/284678	preclinical		none given		S*	indenoquinoline
156/284679	preclinical		none given		S*	indenoquinoline
157/290417	preclinical		none given		S*	indenoquinoline
158/290418	preclinical		none given		S*	indenoquinoline
159/294861	preclinical		none given		S*	indenoquinoline
160/295105	preclinical		none given		ND	camptothecin
161/295449	preclinical		DB-202		ND	camptothecin
162/296220	preclinical		DB-148		ND	camptothecin
163/296221	preclinical		DB-158		ND	camptothecin
164/301637	preclinical		BMS-250749		ND	indolocarbazole
165/303076	preclinical		none given		ND	camptothecin
1/305234	preclinical		none given	Camposomes	N	camptothecin
166/306495	preclinical		none given		S	naphthazarine
c/310319	preclinical		NU:UB-199		S?	none given
c/310321	preclinical	yes	XR-11612		S	none given
167/313256	preclinical		none given		S/NM	indenoquinoline
168/314652	preclinical		none given		S?	phenazine
119/319809	preclinical		BPR-0Y-007		S	pyrone
c/327823	preclinical	yes	ALS-559 (or 427?)		S	none given
169/329116	preclinical		S-2526		ND	aclacinomycin
170/329118	preclinical		S-2512		ND	aclacinomycin
171/329119	preclinical		S-2513		ND	aclacinomycin
172/329258	preclinical		S-2521		ND	aclacinomycin
173/330721	preclinical		none given		S	naphthyridine
174/330722	preclinical		none given		S	diazanaphthyridine
175/338753	preclinical		none given		S	naphthyridine

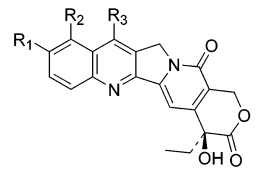
[a] Status as August 15, 2003. [b] Source codings as described in Newman et al.[53] [c] No formal structure given (either not available or a suspension and/or polymer adduct).

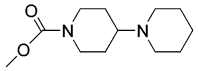
the completion of a Ph.D. thesis in Fenical's laboratory[29] and to provide enough material for some significant comparative work to be commenced in Hamel's group.[30]

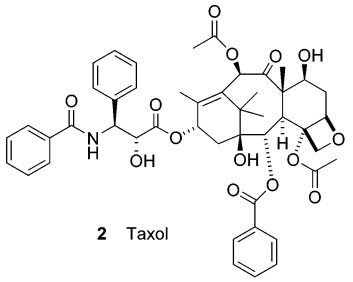
Conclusion

It is obvious that from the initial discoveries of Wall and Wani two completely new areas of cancer chemotherapy have evolved in a short period of time. Their seminal investigations into cytotoxic agents from plants opened up broad new vistas of cancer chemotherapy. It is rare for a chemistry group to discover one completely new chemotype that leads to a clinical drug, let alone discover two completely different ones that have led to four clinically used, commercialized drugs within their lifetimes. There are more in the offing, particularly as their findings have now led to compounds with perhaps similar pharmacophores but different chemotypes from plants, microbes, and marine organisms.

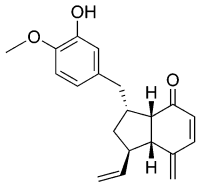
Chart 1. The structures are referenced by both a **bold** number as in the normal Journal style, but then have the Ensemble database accession number in parentheses attached. To minimize the space used, the structures have been grouped into the following general categories. For tubulin interactive agents, the order is as follows: paclitaxel derivatives; docetaxel derivatives; combretastatin A4 derivatives; epothilone derivatives; and then the remaining structural types with similar backbones grouped together as far as possible. Within each group of base structures, as far as is possible, the order is by level of trial. For topoisomerase I interactive agents, the order is as follows: camptothecin derivatives; staurosporine derivatives; and then the remaining structural types with similar backbones grouped together as far as possible. Within each group of base structures, as far as is possible, the order is by level of trial.



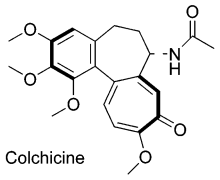
1 Camptothecin $R_1 = R_2 = R_3 = H$
18 Topotecan $R_1 = OH$; $R_2 = CH_2N(CH_3)_2$; $R_3 = H$
19 Irinotecan $R_1 =$  $R_2 = H$ $R_3 = CH_2CH_3$



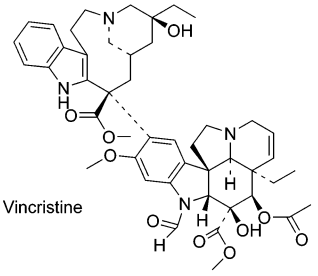
2 Taxol



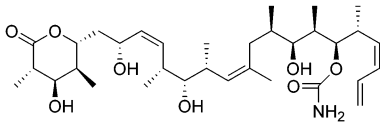
3 Ottelione A



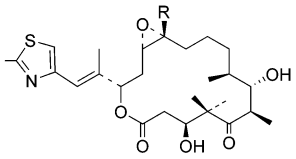
4 Colchicine



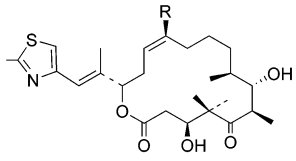
5 Vincristine



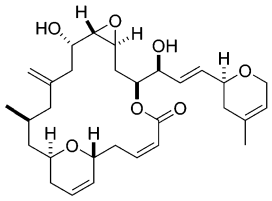
6 Discodermolide



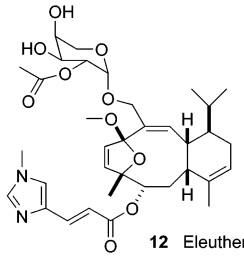
7 Epothilone A; R = H
8 Epothilone B; R = CH_3



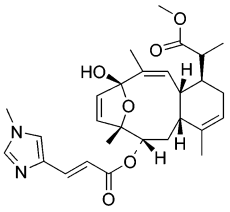
9 Epothilone C; R = H
10 Epothilone D; R = CH_3



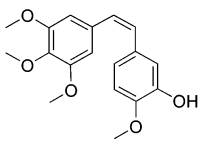
11 Laulimalide



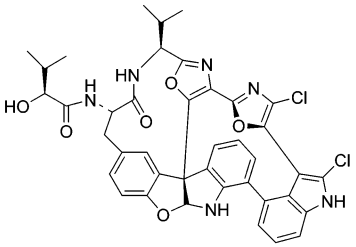
12 Eleutherobin



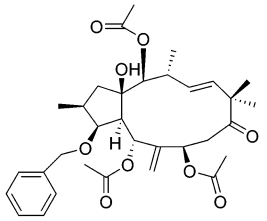
13 Sarcodictyin



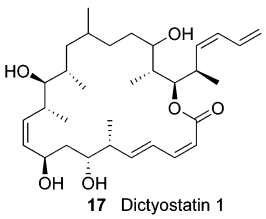
14 Combretastatin A4



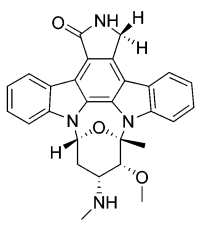
15 Diazonamide A (revised structure)



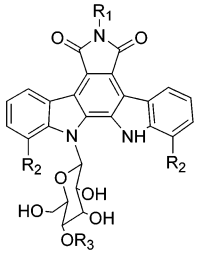
16 Jatrophane 1



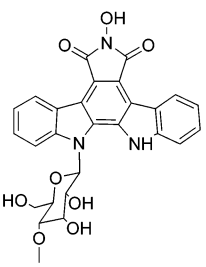
17 Dictyostatin 1



20 Staurosporine

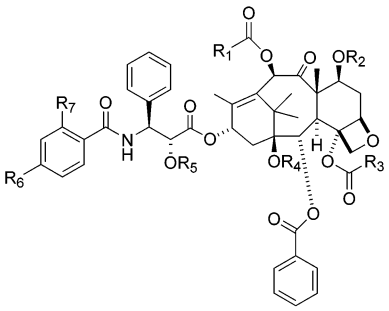


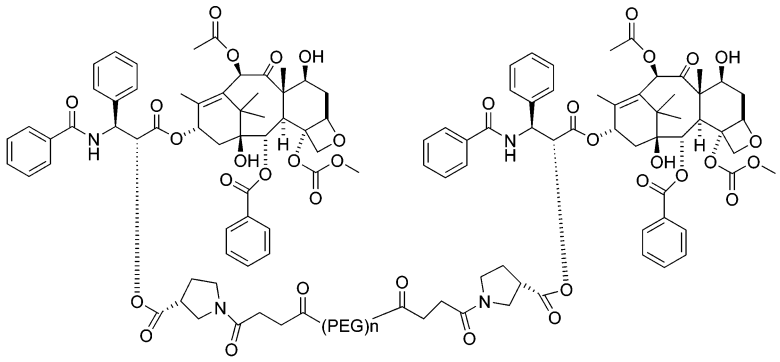
21 Rebeccamycin $R_1 = H$; $R_2 = Cl$; $R_3 = CH_3$
22 NB-506 $R_1 = NHCHO$; $R_2 = OH$; $R_3 = H$



23 R-3

Chart 1 continued





122 (331735)

2 (101438) $R_2 = R_4 = R_5 = R_6 = R_7 = H$; $R_1 = R_3 = CH_3$

35 (275943) $R_4 = R_5 = R_6 = R_7 = H$; $R_1 = R_3 = CH_3$; $R_2 = CH_2SCH_3$

37 (277994) $R_2 = R_4 = R_6 = R_7 = H$; $R_1 = R_3 = CH_3$; $R_5 = COCH_2(CH_2CHCH)_6CH_2CH_3$

39 (282724) $R_2 = R_4 = R_5 = R_6 = R_7 = H$; $R_1 = CH_3$; $R_3 = OCH_3$

43 (327193) $R_4 = R_5 = R_6 = R_7 = H$; $R_1 = R_3 = CH_3$; $R_2 = CO(CH_2)_4CH_3$

57 (183725) $R_2 = R_4 = R_5 = R_7 = H$; $R_1 = R_3 = CH_3$; $R_6 = Cl$

60 (213014) $R_4 = R_6 = R_7 = H$; $R_1 = R_3 = CH_3$; $R_5 = CO_2CH_2CH_3$; $R_2 = CH_2OPO_3$

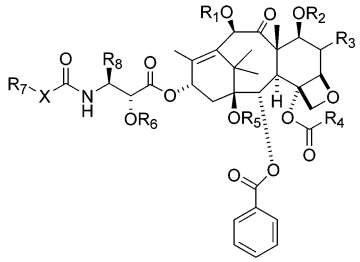
76 (257900) $R_2 = R_4 = R_5 = R_6 = H$; $R_1 = R_3 = CH_3$; $R_7 = Br$

92 (286137) $R_2 = R_4 = R_6 = R_7 = H$; $R_1 = R_3 = CH_3$; $R_5 = CH_2COCH_2CHOHCO_2H$

104 (301518) $R_2 = R_4 = R_6 = R_7 = H$; $R_1 = CH_3$; $R_3 = OCH_3$; $R_5 = CO(CH_2)_{14}CH_3$

105 (301519) $R_2 = R_4 = R_6 = R_7 = H$; $R_1 = CH_3$; $R_3 = OCH_3$; $R_5 = COCHBr(CH_2)_{13}CH_3$

121 (331061) $R_2 = R_4 = R_6 = R_7 = H$; $R_1 = CH_3$; $R_3 = OCH_3$; $R_5 = o\text{-}N(CH_3)Py$



24 (140605) $R_1 = R_2 = R_3 = R_5 = R_6 = H$; $R_4 = CH_3$; $R_7 = C(CH_3)_3$; X = O; R_8 =

40 (287186) $R_3 = R_5 = R_6 = H$; $R_1 = R_2 = R_4 = CH_3$; $R_7 = C(CH_3)_3$; X = O; R_8 =

53 (308286) $R_1 = R_3 = R_5 = R_6 = H$; $R_4 = CH_3$; $R_2 = COCH_2CH_3$; $R_7 = C(CH_3)_3$; X = O; R_8 =

54 (309743) $R_2 = R_3 = R_5 = R_6 = H$; $R_4 = OCH_3$; $R_1 = COCH_3$; X = O, $R_7 = R_8 = C(CH_3)_3$

58 (186313) $R_1 = R_2 = R_3 = R_5 = R_6 = H$; $R_4 = CH_3$; $R_7 = C(CH_3)_3$; X = O; R_8 =

59 (212162) $R_2 = R_3 = R_5 = R_6 = H$; $R_4 = CH_3$; $R_1 = COCH_3$; X = O; $R_7 = C(CH_3)_3$; $R_8 = CHC(CH_3)_2$

81 (270156) $R_1 = R_2 = R_3 = R_5 = H$; $R_4 = CH_3$; $R_6 = CH_2COCH_2OCOCH_2CHNH_2CONH_2$; $R_7 = C(CH_3)_3$; X = O; R_8 =

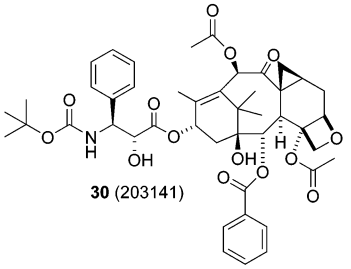
87 (277477) $R_2 = R_5 = R_6 = H$; $R_4 = CH_3$; $R_1 = COCH_3$; $R_3 = F$; X = O; $R_7 = C(CH_3)_3$; R_8 =

95 (291245) $R_2 = R_3 = R_5 = R_6 = H$; $R_4 = CH_3$; $R_1 = COCH_3$; $R_7 = (CH_2)_3CH_3$; X = S; R_8 =

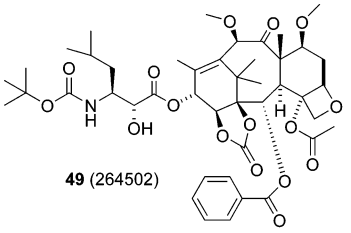
96 (291247) $R_2 = R_3 = R_5 = R_6 = H$; $R_4 = CH_3$; $R_1 = COCH_3$; $R_7 = C(CH_3)_3$; X = S; R_8 =

97 (291248) $R_2 = R_3 = R_5 = R_6 = H$; $R_4 = CH_3$; $R_1 = COCH_3$; $R_7 = CH_2CH(CH_3)_2$; X = S; R_8 =

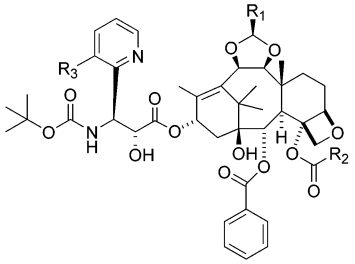
Chart 1 continued



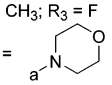
30 (203141)

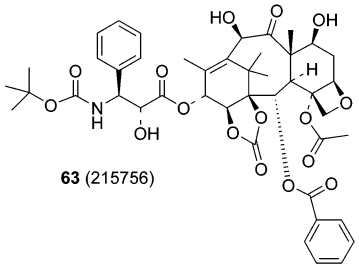


49 (264502)

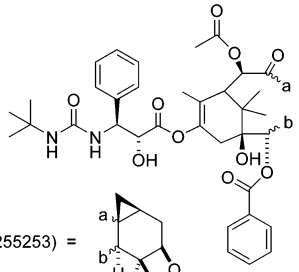


52 (304277) $R_1 = CH_2N(CH_3)_2$; $R_2 = CH_3$; $R_3 = F$

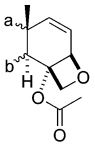
123 (333162) $R_3 = H$; $R_2 = OCH_3$; $R_1 =$ 

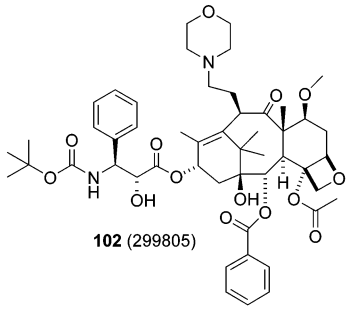


63 (215756)

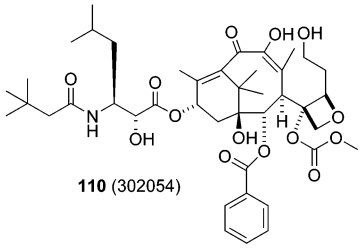


73 (255253) =

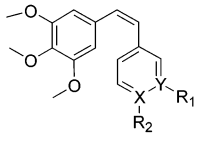
74 (255254) = 



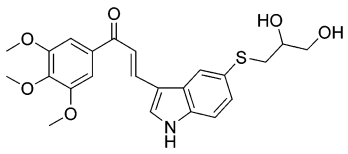
102 (299805)



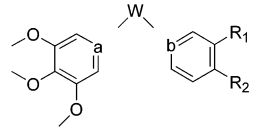
110 (302054)

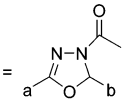


14 (229342) X = Y = C; R_1 = OH; $R_2 = OCH_3$
32 (225384) X = Y = C; $R_1 = PO_4$; $R_2 = OCH_3$
47 (262290) X = Y = C; $R_1 = NH_2$; $R_2 = OCH_3$
48 (262298) X = Y = C; R_1 = NHCO-L-Ser; $R_2 = OCH_3$
82 (270693) X = (C=O); Y = N; $R_1 = CH_3$
83 (270694) X = N; Y = C; $R_1 = OCH_3$



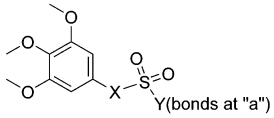
75 (255761)



86 (274502) $R_1 = CH_3$; $R_2 = NH_2$; W = 

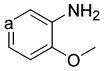
103 (300873) $R_1 = NH_2$; $R_2 = CH_3$; W = 

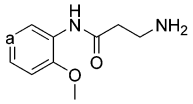
116 (317881) R_1 = $R_2 = (CH)_2NHCH_3$; W = 



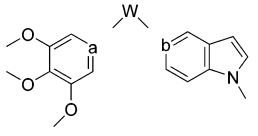
Y(bonds at "a")

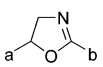
100 (298291) X = NH; Y = 

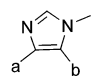
101 (298297) X = O; Y = 

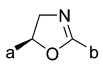
107 (301977) X = O; Y = 

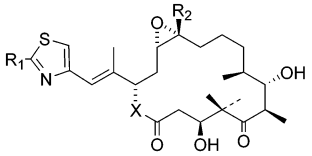
115 (317845) X = $N(CO)CH_2N(CH_3)_2$; Y = 



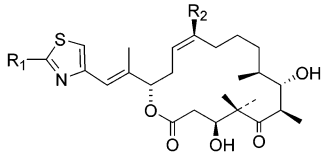
108 (301979) W = 

109 (302036) W = 

114 (317844) W = 



8 (222557) X = O; $R_1 = R_2 = CH_3$
42 (293356) X = NH; $R_1 = R_2 = CH_3$
51 (294121) X = NH; $R_1 = CH_2NH_2$; $R_2 = CH_3$
106 (301741) X = O; $R_1 = CH_3$; $R_2 = CH_2F$



10 (251562) $R_1 = R_2 = CH_3$
99 (296536) $R_1 = CH_2OH$; $R_2 = CH_3$

Chart 1 continued

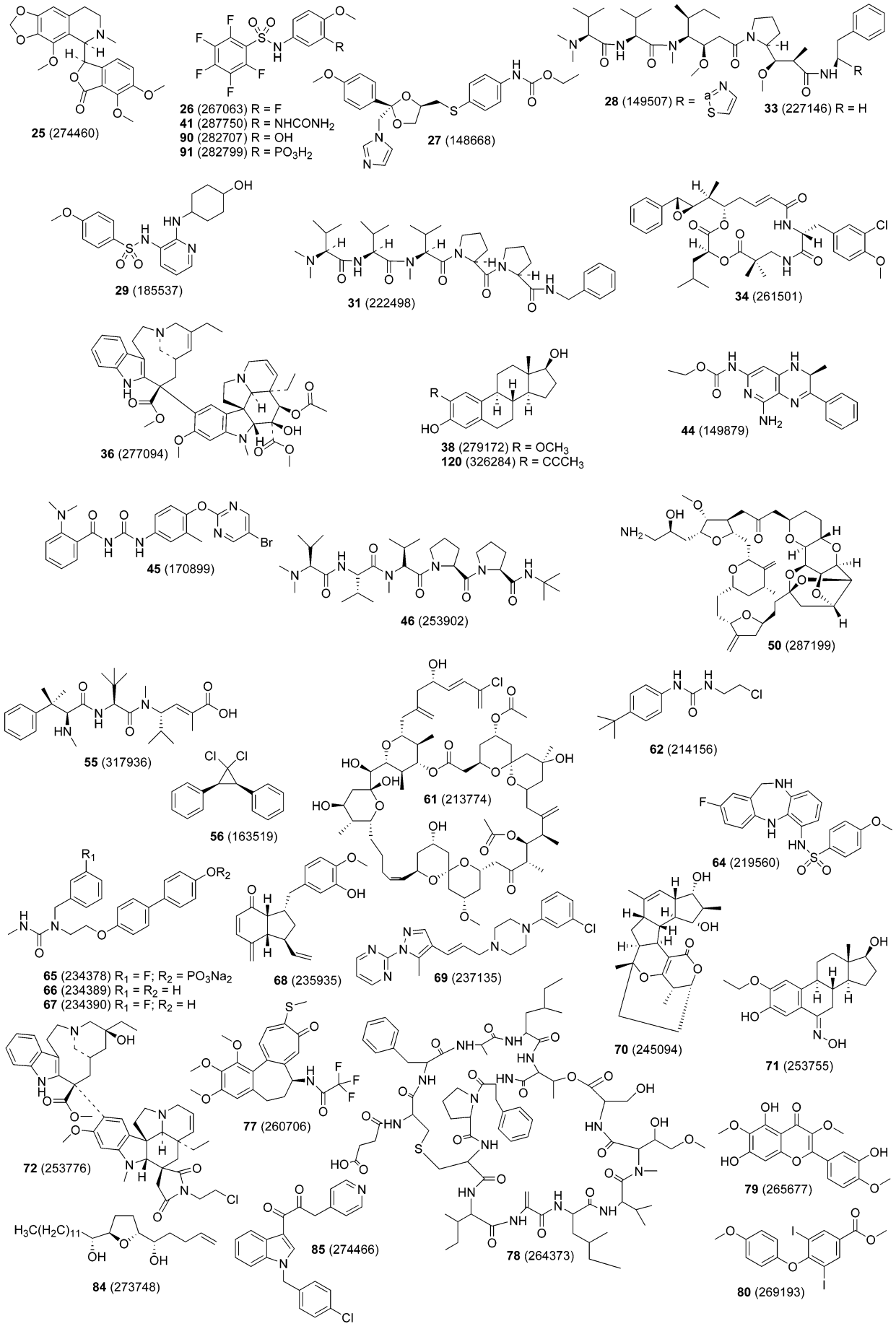
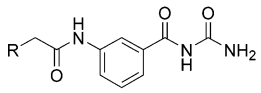
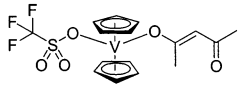


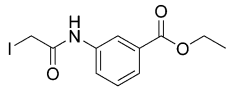
Chart 1 continued



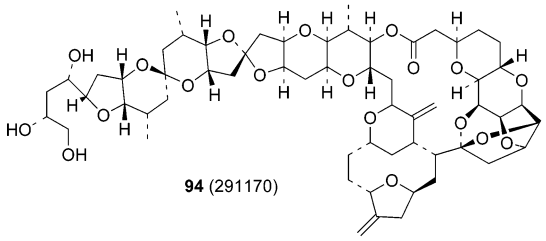
88 (279594) R = Br
125 (342740) R = CH_2Br



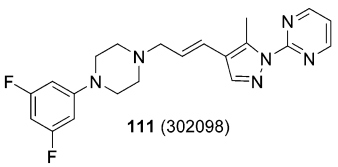
89 (282043)



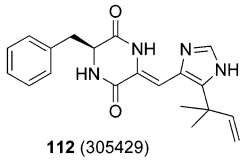
93 (287230)



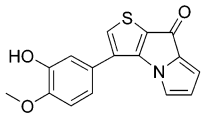
94 (291170)



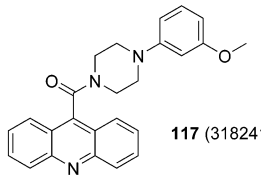
111 (302098)



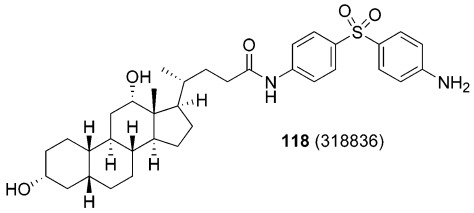
112 (305429)



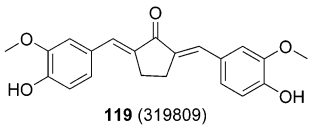
113 (306972)



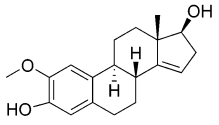
117 (318241)



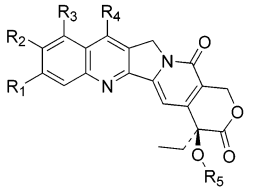
118 (318836)

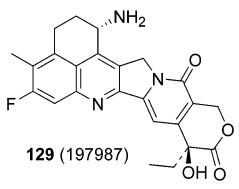


119 (319809)

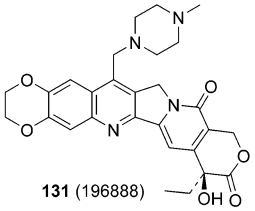


124 (338268)

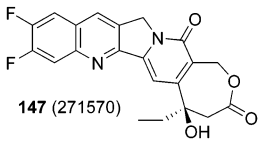




129 (197987)

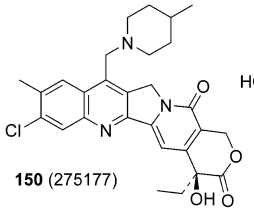


131 (196888)

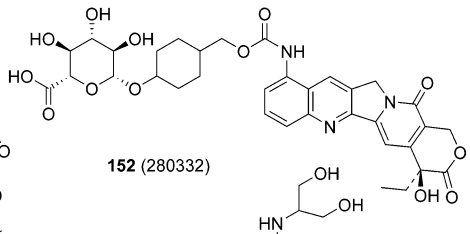


147 (271570)

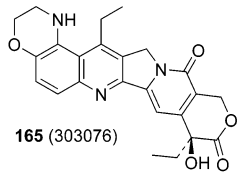
126 (070748) $R_1 = R_3 = R_5 =$ H; R_2 = OH; $R_4 = C_2H_5$
127 (241383) $R_1 = R_2 = R_4 = R_5$= H; $R_3 = NO_2$
128 (184764) $R_1 = R_2 = R_4 = R_5$ = H; $R_3 = NH_2$
134 (239803) $R_1 = R_2 = R_3 = R_5$ = H; $R_4 = CH_2$-CH_2-$N(CH_3)_2$
135 (259669) $R_1 = R_2 = R_3 = R_5$ = H; $R_4 = CH_2$-CH_2-$Si(CH_3)_3$
136 (275176) $R_1 = R_2$ = F; $R_3 = R_4 = R_5$ = H
140 (171476) R_1 - R_2 = O-CH_2-O; $R_3 = NH_2$; $R_4 = R_5$ = H
143 (250906) $R_1 = R_2 = R_4$ = H; $R_3 = NO_2$; $R_5 = CO_2C_2H_5$
144 (251650) $R_1 = R_3 = R_4 = R_5$ = H; R_2 = OH
145 (261782) $R_1 = R_2 = R_3 = R_4$ = H; $R_5 = CO_2C_2H_5$
149 (274543) $R_1 = R_3 = R_5$ = H; R_2 = OH; $R_4 = Si(CH_3)_2C(CH_3)_3$
153 (282913) $R_1 = R_3 = R_5$ = H; R_2 = OH; $R_4 = (CH_2)_2Si(CH_3)_3$
160 (295105) $R_1 = R_2 = R_3 = R_5$ = H; R_4 = CN
161 (295449) $R_1 = R_2 = R_3 = R_5$ = H; $R_4 = Si(CH_3)_2C(CH_3)_3$
162 (296220) $R_1 = R_2 = R_3 = R_5$ = H; $R_4 = Si(CH_3)_2(CH_2)_2CH_2Cl$
163 (296221) $R_1 = R_3 = R_5$ = H; R_2 = OH; $R_4 = Si(CH_3)_2(CH_2)_2CH_2Cl$



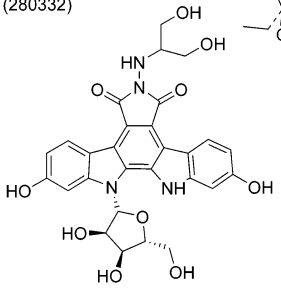
150 (275177)



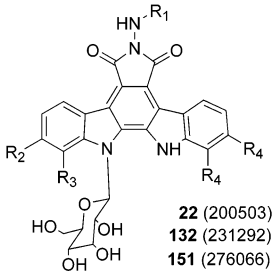
152 (280332)



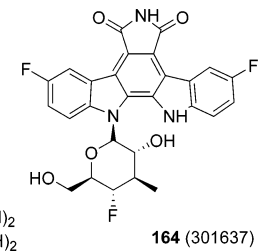
165 (303076)



142 (250703)

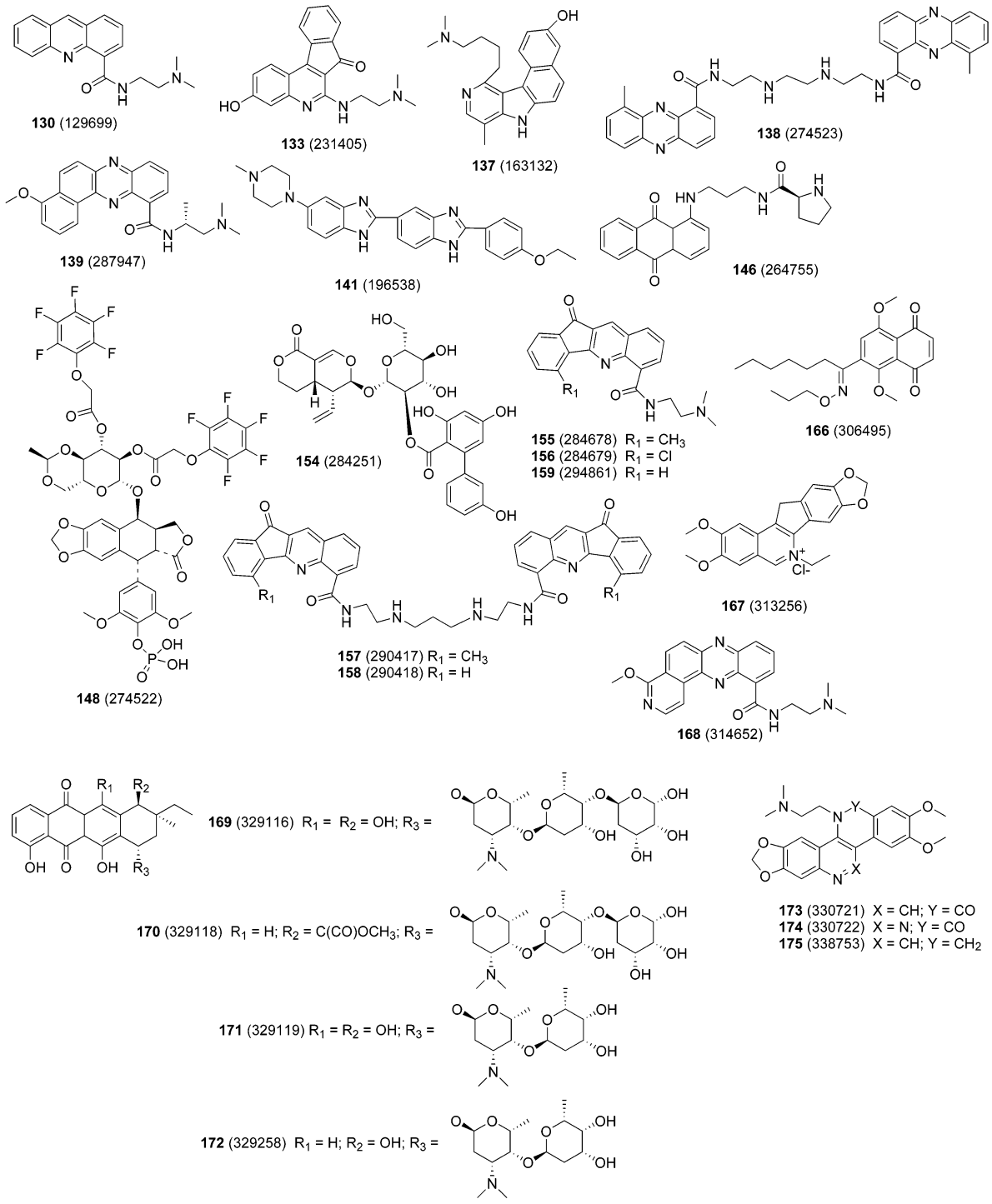


22 (200503) $R_2 = R_4$ = H; $R_3 = R_4$ = OH; R_1 = CHO
132 (231292) $R_2 = R_4$ = OH; $R_3 = R_4$ = H; $R_1 = CH(CH_2OH)_2$
151 (276066) $R_2 = R_4$ = H; $R_3 = R_4$ = OH; $R_1 = CH(CH_2OH)_2$



164 (301637)

Chart 1 continued



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