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Analogue-based drug discovery: Contributions to medicinal chemistry principles and drug design strategies. Microtubule stabilizers as a case in point (Special Topic Article)

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Abstract: The benefits of utilizing marketed drugs as starting points to discover new therapeutic agents have been well documented within the IUPAC series of books that bear the title *Analogue-based Drug Discovery* (ABDD). Not as clearly demonstrated, however, is that ABDD also contributes to the elaboration of new basic principles and alternative drug design strategies that are useful to the field of medicinal chemistry in general. After reviewing the ABDD programs that have evolved around the area of microtubule-stabilizing chemotherapeutic agents, the present article delineates the associated research activities that additionally contributed to general strategies that can be useful for prodrug design, identifying pharmacophores, circumventing multidrug resistance (MDR), and achieving targeted drug distribution.

Keywords: cabazitaxel; docetaxel; drug design strategies; ixabepilone; medicinal chemistry principles; multidrug resistance; paclitaxel; prodrugs; targeted drug delivery.

INTRODUCTION

This report complements the IUPAC book series *Analogue-based Drug Discovery* (ABDD), which presently consists of two issued volumes [1,2] and a third in press. While these books emphasize how ABDD has effectively contributed to the proliferation of marketed drugs, the present report instead conveys how ABDD-associated basic research activities have additionally paved the way for several new avenues of drug design in general. The impact of the latter has the potential to become extremely beneficial as the pharmaceutical enterprise continues to roll into the future.

As described within the first volume of the book series, "ABDD represents a long-standing strategy that can contribute to the generation of new chemical entities (NCEs), as well as play a lead role in the continued chemical and pharmacological evolution of clinically validated therapeutic paradigms [1]" [3]. It was further noted that ABDD can be particularly useful "by virtue of the inherent drug-like properties that already are present when a successfully marketed drug is deployed as a template for elaboration into new compounds [4]" [3]. Not as clearly demonstrated to date, however, is that ABDD also can contribute significantly to the elaboration of new basic medicinal chemistry principles and even to

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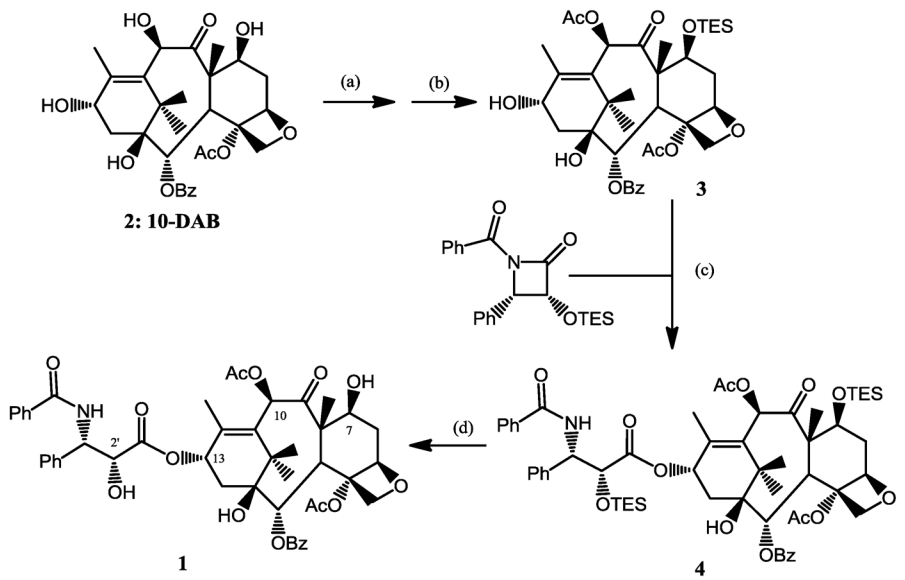
the formalization of new drug design strategies, both of which can then have general utility well beyond the focused applications associated with an initial ABDD campaign. Using the extensive ABDD efforts associated with the microtubule-stabilizing drugs as a case in point, this article will emphasize the types of additional contributions that ABDD is often able to deliver. Strategies relevant to prodrug design, identifying pharmacophores, addressing multiple drug resistance, and achieving targeted drug distribution will be discussed in detail. As background, our review begins with brief descriptions about paclitaxel and docetaxel, both of which served to firmly establish the field of microtubule-stabilizing agents.

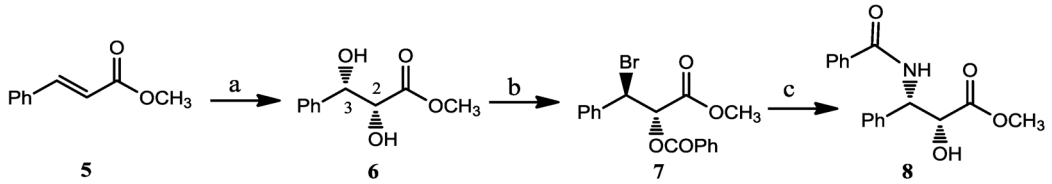
DISCOVERY AND DEVELOPMENT OF PACLITAXEL

As previously suggested [3], within the context of ABDD, paclitaxel (PAC) can be considered to be the *pioneer drug* for the class of agents that display microtubule-stabilizing properties. Several texts and reviews have provided thorough discussions about the historical aspects that eventually led to PAC's introduction into the marketplace (e.g., see refs. [5–10]). The following account pays tribute to some of the notable scientists who played key roles in that story. First obtained from the bark of the Pacific yew nearly 50 years ago by Wall and Wani [11] as part of a natural products screening program being conducted by the U.S. National Cancer Institute, the development of PAC (**1** in Scheme 1) initially was delayed by its low yield after a tedious isolation process coupled with the misconception that its anticancer properties stemmed from a common biological mechanism [3]. It was not until nearly 15 years later, after Horwitz et al. [12] demonstrated that PAC had a unique mechanism of action derived from an overstabilization of microtubules, when excitement about this compound began to mount. The rush to then move PAC into the clinic as rapidly as possible followed by the need to provide huge supplies to accommodate its immediate success, quickly led to considerable concern about its future supply because removal of a yew's bark leads to destruction of the tree. Amid considerable structure–activity relationship (SAR) studies conducted by several laboratories such as that of Kingston et al., extra-



Scheme 1 Semisynthetic route initially used to prepare commercial quantities of PAC shown as **1** from 10-deacetylbaccatin III shown as **10-DAB** [18]. A similar semisynthetic route can be used to prepare DOC (shown later) by deploying the BOC version of the benzyloxy β-lactam, as well as by utilizing other C-13 side-chain synthons. Conditions and yields: (a) Et_3SiCl/pyridine, 86 %; (b) AcCl/pyridine, 86 %; (c) *n*-BuLi/THF then β-lactam/THF, 98 %; (d) HF/pyridine, 98 %.

ordinary chemical efforts were directed toward synthesizing the parent compound [5–10]. Eventually, Potier et al. demonstrated that 10-deacetyl-baccatin III (**2** in Scheme 1), which is obtained from the needles of both the Pacific and European yew in a manner that is not destructive to the source, can be used as a key intermediate for a practical semi-synthesis of PAC [13]. Although many notable chemists, as well as a modest effort in our labs (Scheme 2) [14] using a Sharpless dihydroxylation procedure [15], contributed to this chemical synthesis theme, Holton's patented semi-synthesis that takes advantage of a lithium alkoxide [16,17] eventually was adopted by the industry for converting **2** to PAC via reaction with a β-lactam. For many years, this overall route (Scheme 1) served as the commercial-scale method for supplying the marketplace [18].



5 **6** **7** **8**

Scheme 2 Acyl migration route developed and confirmed in our labs [14] to produce the C-13 side-chain via a Sharpless dihydroxylation procedure [15]. (a) AD-a mixture, *t*-BuOH/H_2O; (b) i. $PhC(OCH_3)_3$, cat. *p*-TsOH; ii. AcBr, –15 °C; (c) i. NaN_3, DMF; ii. H_2, 10 % Pd/C; overall yield = 25 %. Although never progressing to a competitive commercial process, this chemical strategy eventually gained interest in the design of prodrugs having increased selectivity for cancer cells (e.g., ref. [19] and later discussion in text).

Today, the production of PAC can be said to have "gone green" with its principle manufacturer Bristol-Myers Squibb (BMS) recognized by the U.S. Environmental Protection Agency (EPA) for its development of a plant cell fermentation method that has replaced chemical synthesis completely [20]. While not alone in devising such methods (e.g., [21]), the BMS process is remarkably practical. In their process, calluses of a specific taxus cell line are propagated in an aqueous medium in large fermentation tanks under ambient temperature and pressure. The final product PAC is harvested as a crude extract from the plant cell cultures, purified by column chromatography, and isolated by crystallization. Compared to the semisynthetic route across a period of five years of commercialization, the BMS process is estimated to have avoided the concomitant production of 71 000 pounds of hazardous by-product chemicals while eliminating 10 solvents and 6 drying steps, both of which further equate to saving a considerable amount of energy and accumulated costs [20].

CLINICAL SUCCESS AND SHORTCOMINGS OF PACLITAXEL

Initially studied for the treatment of ovarian cancer [22] and shortly thereafter for the treatment of breast tumors [23], PAC quickly became regarded as one of the major breakthroughs in cancer therapy during the 1990s. Since that hugely successful introduction, it has maintained the status of being one of the most important anticancer drugs today wherein its use in other cancers and in combination with other agents is still being optimized [3]. As with many other cancer chemotherapeutic agents, however, PAC does have some shortcomings relative to its deployment in the clinic. The first of these to be noted was its poor aqueous solubility. Because PAC is not orally bioavailable, it is administered by intravenous infusion (iv) wherein its low aqueous solubility becomes problematic [3]. To address this issue, its most widely used formulation contains 50 % Cremophor EL® and 50 % dehydrated ethanol [24]. Unfortunately, the presence of such a high level of Cremophor EL can trigger acute hypersensitivity reactions [25], and it likely contributes to the less than ideal, nonlinear pharmacokinetic (PK) behavior of PAC observed in both mice and humans [26]. A second shortcoming pertains to PAC's inherent toxicity. Like most agents that rely upon rapid cell division to achieve selectivity toward cancer cells, the

therapeutic margin for PAC is not as large as would be desired. Thus, side effects, particularly associated with rapidly dividing normal cells, become dose-limiting [3]. Finally, it was noticed that breast cancer cells can develop resistance to the effects of PAC. When this couples with an already small window for side-effect-free therapy, further treatment at higher doses essentially becomes precluded. One of the predominant mechanisms for resistance is overexpression of the P-glycoprotein efflux transporter (Pgp). The significance of these combined shortcomings is exemplified by the plots displayed in Fig. 1, which conveys data generated from our laboratories [3].

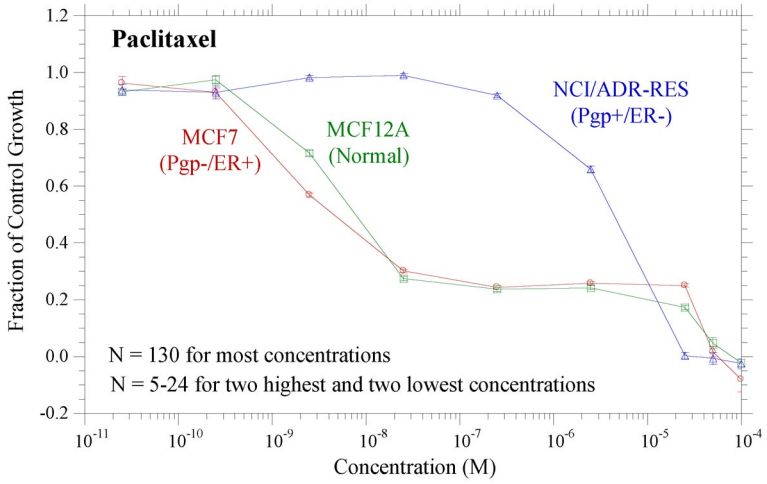


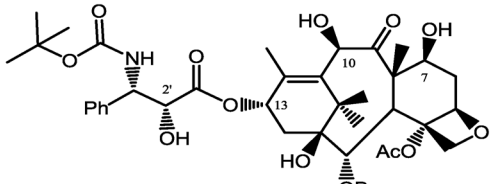
Fig. 1 Log dose–response curves for the action of PAC on three different human cell lines grown in cell culture [3]. Control cultures did not receive drug. MCF7 (red curve with circled data points) is a nonresistant breast cancer. MCF12A (green curve with square data points) is a healthy breast epithelial cell line that is noncancerous and nonresistant. NCI/ADR-RES (blue curve with triangular data points) is a drug-resistant ovarian cancer. Pgp refers to P-glycoprotein, which is either absent (–) or overexpressed (+), and ER refers to estrogen receptor responsiveness (+) or absence (–). Because PAC is used as a standard for all of our cell-based testing studies, we have accumulated a large number of replicates (N = 130 for most concentrations). Thus, the statistical standard deviation bars for the data are so tight that even though they are plotted within this figure, they have become encompassed by the size of the depicted data point symbols in nearly every instance.

The narrow safety margin between PAC's effects on cancer cells vs. its effects on rapidly dividing healthy cells is reflected by the very close proximity of the first two log dose–response curves for causing growth inhibition (GI) of cell cultures. Likewise, the profound effect that Pgp-derived resistance can have upon PAC's effects is illustrated by the third curve, which has shifted to the right to the extent that it now requires nearly three orders of magnitude higher drug concentration to cause 50 % inhibition of cell growth (GI_{50} value). Although PAC and its related taxanes appear to be exceptional substrates for Pgp [27], the far from ideal profile of activity conveyed in Fig. 1 is a common shortcoming that is displayed by many other cancer chemotherapeutic agents. Underscoring this situation is the fact that the Pgp component is so prevalent and is so promiscuous in its substrate structural requirements that its overexpression leads to the broadly applicable phenomenon known as *multidrug resistance* or MDR [3].

ABDD LEADING TO DOCETAXEL

In addition to the compound supply issues that were critical for PAC's development, its huge clinical success, fueled even further by its noted shortcomings, prompted a surge in ABDD-derived basic

research on several fronts. The latter encompassed: (i) Pursuit of a specific binding site in response to the finding that its interaction with microtubules had been shown to be stoichiometric [28]; (ii) elaboration of classical medicinal chemistry SAR including detailed assessments of its conformational behavior in different environments, as well as production of photoaffinity label derivatives for use as receptor probes (e.g., [29]); (iii) numerous efforts directed toward addressing the specific shortcomings present in its clinical profile as discussed in the previous section; and (iv) pursuit of entirely new structures that might still act via the same unique mechanism when it was eventually shown that an over stabilization of microtubules prompts apoptosis at the G2 to M transition stage of the cell cycle during attempted replication [3]. While (i) to (iii) largely represent areas directed toward *structural analogues* that preserve the desired biological effect (so-called "structural and pharmacological analogues"), area (iv) represents a distinct case of pursuing *pharmacological analogues* [1,2] wherein the latter derive from a different molecular scaffold and different 3D display of pertinent functional groups, i.e., the pursuit of *nonobvious* molecular systems even beyond *scaffold-hopping* according to the patent terminology and jargon of today. Aspects of these areas will be delineated in subsequent sections. The remainder of this section will highlight the developments leading to PAC's first clinically successful analogue derived from ABDD, namely, DOC. DOC is shown immediately below as **9**, wherein it should be apparent that it is a very close structural analogue of PAC, as well as being a pharmacological analogue by its retention of the same biological activity.



9 Docetaxel

Responding to the need to produce adequate supplies of PAC, Potier et al. [30–32] had become immersed in the semisynthetic methods that can take advantage of 10-DAB **2** (Scheme 1), including the possibilities that this route additionally affords for the preparation of SAR analogues modified on the C-13 position side-chain. His chemistry initially was explored via the Sharpless hydroxyamination procedure [33] while utilizing *tertiary*-butyl-*N*-chloro-*N*-sodiocarbamate. By this approach, the resulting *t*-butoxycarbonyl (BOC) group can be gently removed from what becomes the 3'-nitrogen atom then poised for further derivatization, including that of acylation with benzoylchloride so as to produce the PAC-form of the C-13 side-chain. Presumably practicing a fundamental strategy of medicinal chemistry to test synthetic intermediates as well as final target compounds [3], Potier et al. found that their BOC version of the C-13 side-chain exhibited nearly twice the potency as PAC's original benzoyl version when it was coupled to 10-DAB and tested in a microtubule stabilization assay [34]. This fortuitous observation occurred whether or not the C-10 position hydroxyl group was acetylated, the latter alteration in itself having little effect on PAC's activity. Further exploration of other types of groups at this position did not show any added benefit over that seen with BOC, although a *para*-fluorobenzoyl seemingly came the closest upon our review of the composite of their data. Capping these fundamental SAR studies, the increase in potency provided by the BOC group was then demonstrated even more convincingly during cell culture, and especially during in vivo studies [35,36]. Together, these results led to the selection of DOC for further study as a preclinical development candidate [37].

As studies progressed, the enhanced potency of DOC compared to PAC was maintained and even greater differences potentially relating to additional mechanisms that could contribute toward overall efficacy were uncovered, such as DOC having about 100-times greater potency than PAC toward phosphorylation of bcl-2 [3]. The latter, in turn, may lead to inactivation of this oncoprotein and a con-

comitant release of its braking effect upon apoptosis [38]. Although these differences in inherent potency are real and apparently served as the main incentive to complete the drive of DOC toward the clinic, they should also be viewed amid the underlying situation that PAC already had more than enough potency to serve quite well [3]. Thus, the more practical attribute of DOC over PAC was yet to be fully realized. It encompasses an important alteration at another site on these molecules, namely, that at the 10-position wherein DOC no longer bears an acetyl ester on the common scaffold's hydroxyl-group [3]. Beyond producing an increase in localized hydrophilicity, it is understandable why this effect was not readily appreciated within the context of the overall molecular system, which remains highly lipophilic and, seemingly just like PAC**,** essentially "water-insoluble" upon gross experimental observation. Ultimately, however, the formulations for DOC during early clinical studies were able to use 100 % polysorbate 80 and thereby avoided the undesirable Cremophor EL altogether [24]. Thus, this initially subtle difference in solubility became a very real and practical improvement in the overall profile that again can be regarded as being highly fortuitous [3]. Not so much for the increased potency but because of these later findings, the present authors agree with an assessment suggested at a much earlier point in the DOC development story by some of the original players, namely, that PAC and DOC are "not simply two of a kind" [24]. The precisely determined solubilities of PAC and DOC in water are 0.25 μg/ml and 6–7 μg/ml, respectively [39]. Some of the other clinically relevant properties for these two drugs are compared in Table 1, which has been collated from several sources [3,10,24,38,40]. To cap the DOC summary, this interesting example of ABDD was initiated by chemical supply issues and then proceeded by practicing the venerable strategy to examine chemical intermediates for both SAR purposes and the distinct possibility of serendipity [3]. In the end, these efforts produced a clinically relevant structural and pharmacological analogue of PAC that has its own unique set of advantages. For in the end, no two pharmacological analogues are ever truly quite the same [3].

Table 1 Clinical comparison of PAC (**1**) and DOC (**9**) [3,10,24,38,40].

Property	**1**	**9**
Formulation	50 % Cremophore EL + 50 % Anhydrous ethanol	100 % Polysorbate 80
Administration[a]		
• Dosing protocol	175 mg/m^2 over 3 h	60–100 mg/m^2 over 1 h
• Frequency	Repeated every 3 weeks	Repeated every 3 weeks
PK profile		
• Disposition	Nonlinear with dose	Linear with dose
• Term. half-life	20 h, highly variable	12 h, highly variable
• Excretion	Hepatic ca. 80 % unchanged	Hepatic ca. 80 % metab.
• Metabolism	C6 and C3'-p-phenyl hydroxylation, saturable	Various metabolites, nonsaturable
Toxicity[b]	>200 mg/m^2	>50 mg/m^2
MDR liability[c]	Yes	Yes

[a]As typically deployed for treating breast carcinoma by iv.
[b]Typically observed as neutropenia.
[c]MDR is multidrug resistance in this case resulting largely from overexpression of Pgp transporter to the extent that treatment becomes compromised.

An intense ABDD effort has continued over the last 15 years using the initial knowledge afforded by the discoveries of both PAC and DOC as a starting point. In addition to enhancing our knowledge about SAR and the conformational details associated with how the taxanes interact with microtubules, numerous structural analogues have been obtained for which some have improved aqueous solubility, others have fewer problems with MDR, and still others exhibit increased selectivity for cancer cells compared to healthy cells. In many cases, these ABDD efforts have encompassed prodrug strategies into their final structural motifs. Several promising compounds have progressed into various stages of clinical study, but as of this writing, only two have made it all the way into the marketplace. Alternatively, the results from many of these efforts have proven to be generally useful toward applications involving other molecules across a broader range of therapeutic indications. The next several sections of this report will review these important types of activities while staying within the context of the marketed microtubule-stabilizing agents, namely, PAC/DOC, one of their new structural analogues, and then the structurally novel EPOs wherein both of the latter constitute more recent additions to the marketplace.

ANALOGUES SPECIFICALLY DIRECTED AT IMPROVING AQUEOUS SOLUBILITY

A considerable amount of attention has been directed toward improving the water solubility of PAC, as well as toward further enhancing that of DOC. Using the phrase broadly for a moment, nearly all of the efforts in this area have focused on prodrug strategies. According to strict definition [41], a prodrug is a compound that is inherently inactive until it is administered and becomes converted to another compound that then displays the desired activity. As such, prodrugs have earned a distinct classification of their own. Within the context of ABDD, prodrugs would thus be designed to be inactive analogues that are then converted back to a pioneer drug (e.g., PAC) or to some other clinically deployed agent (e.g., DOC) upon administration. For the present article, we will consider these prodrug strategies to be a special type of ABDD. Several excellent reviews pertaining to the pursuit of water-soluble prodrugs of PAC and DOC are available (e.g., [29,42]). The following summary has drawn heavily from these reviews with the intent of providing herein a short synopsis about the prodrug-related efforts across the PAC field in general.

Quick inspection reveals that the hydroxyl-groups located at the 2'- and 7-positions in PAC, plus at the 10-position in DOC, represent ready handles for the incorporation of prodrug constructs such as esters, carbonates, or carbamates from which the esters can generally be regarded as being the most amenable for enzymatic hydrolysis back to the parent compounds [41]. All of these approaches have been tried, with the esters not unexpectedly having received the most attention. Likewise, the additional substituents that might be placed on such prodrug adducts when trying to increase aqueous solubility clearly should include groups that can ionize at physiological pH such as amine and acid functionalities, the latter including carboxyl, sulfoxyl, and phosphatyl. A wide range of both of these types of groups, including their various salt forms, have been prepared as well. By way of what should be considered a highly truncated tabulation, just a few of some of the initially promising of such derivatives are shown below in Fig. 2. Figure 2 does not include any 7- or 10-position analogues because these did not show as much promise as those placed at the 2'-position. Similar adducts placed at these positions generally exhibited too much stability such that PAC or DOC was not produced at an adequate rate to elicit strong chemotoxicity. In at least one case when such adducts were extended away from the bulk inherent to the taxane scaffold, adequate release rates were initially demonstrated for certain phosphatyl-containing constructs. However, when plasma proteins were then added during such testing, these rates were significantly attenuated presumably due to very high plasma protein binding [50]. It should also be noted that analogues derived from substitutions at the 7- and/or 10-positions typically retained reduced but significant potency prior to their hydrolysis such that these would not be true prodrugs according to strict definition. Alternatively, analogues derived from substitutions at the 2'-position are completely devoid of activity until they undergo biotransformation to produce PAC or DOC.

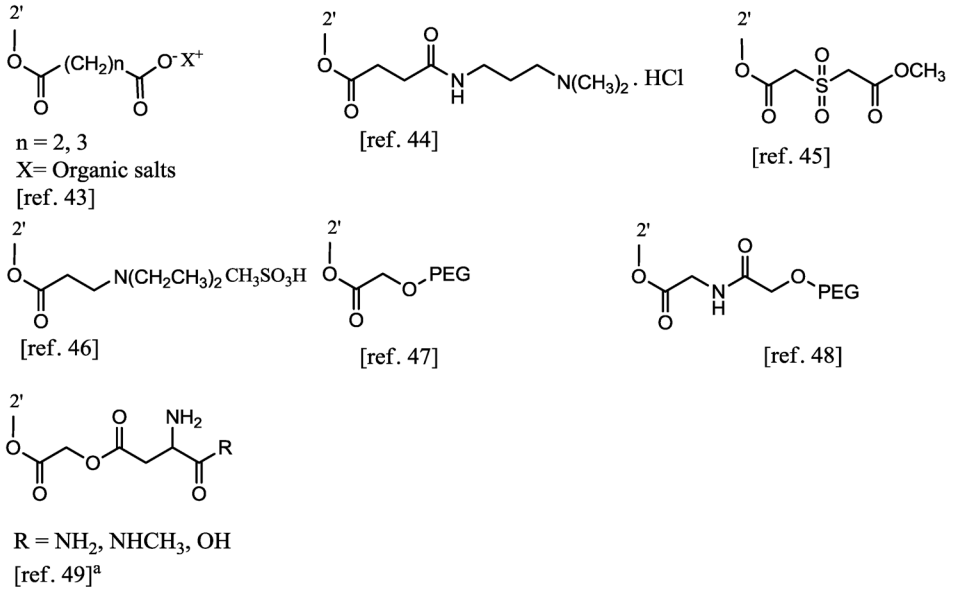


Fig. 2 Representative prodrug constructs prepared in order to increase aqueous solubility. These representatives were selected to convey some of the molecular diversity that was generated at that time and because they initially demonstrated promising properties in terms of stability and release of the parent molecule when studied in vivo. The 2' notation refers to that same position on either PAC or DOC in all cases except for the last entry wherein footnote (a) is intended to indicate that the DOC C-13 side-chain has been further modified by replacing the 3'-position phenyl ring with a cyclopropyl ring.

Likewise, Fig. 2 does not contain any examples of sulfoxyl- or phosphatyl-moieties because these were generally much less promising than the simple carboxyl- and amino-groups as shown. The depicted sulfonyl-system and the amino acid versions also showed some initial promise, as did the highly polar polyethylene glycol (PEG) adducts with, somewhat surprisingly to us, apparently little distinction between molecular weights running from 5 to 40 K. Also note that the last entry in Fig. 2 involving amino acid types of adducts additionally contains a further modification at the 3'-position of the DOC side-chain. Their particular replacement with a cyclopropyl-substituent was done to improve inherent potency and should not be considered to be part of the prodrug design construct.

As prodrug and other design strategies directed toward increasing aqueous solubility continued to be developed, they also began to be hybridized so as to address some of the other clinical shortcomings exhibited by PAC and DOC, such as MDR liability and the narrow window of selectivity. Each of these areas is separately discussed in subsequent sections. In closing this historical segment, two points can be noted. First, the prodrug strategies conveyed herein are applicable in general for therapeutic agents that would benefit by having enhanced water solubility. Since anticancer drugs are often administered in aqueous solution by the iv route, these strategies become particularly useful for promising agents seeking to join this type of chemotherapeutic armamentarium but may otherwise be restricted by their inherent lack of water solubility. Second, some of the most recent trends associated with drug delivery per se, such as combinations with nanoparticle technology, are now being explored within the taxane field as well [51,52]. It seems likely that these technologies will be optimized across a specific range of preferable aqueous solubility for the various components. If that does become the case, the prodrug strategies conveyed herein can be quite useful in general toward modulating the drug component to meet such solubility specifications.

SAR AND RECEPTOR BINDING STUDIES

At this point, the accumulated studies directed toward total and semisynthetic syntheses, plus the efforts directed toward improving aqueous solubility, could be coupled with the ongoing SAR work so as to provide a reasonably detailed composite of the pharmacophoric requirements associated with PAC's unique mechanism of action. Detailed reviews of the excellent work in this area again can be found elsewhere (e.g., [5–10,29,37,42]) such that only an overall summary pertaining to these structural aspects is provided herein. The latter is conveyed within Fig. 3. Alternatively, a more in-depth account of how the key structural features may reside in 3D space is provided herein as a prelude to a discussion about how PAC and DOC are presently thought to bind with microtubules.

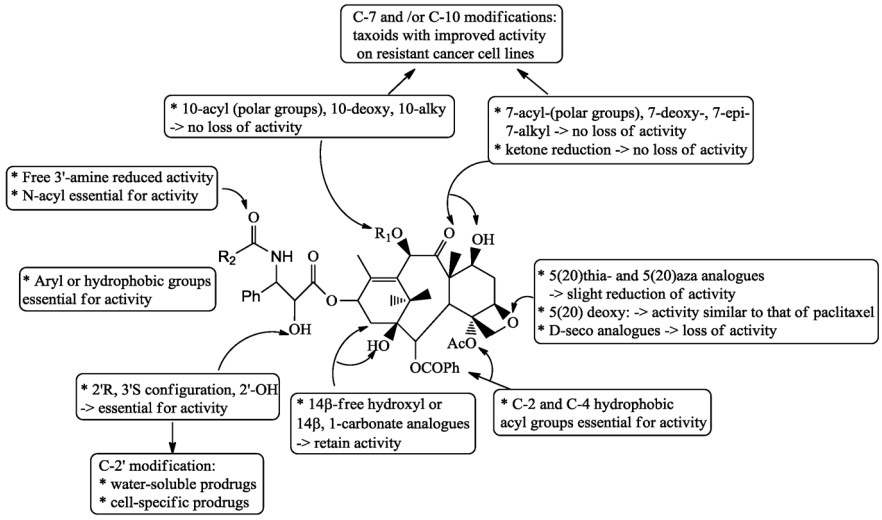


Fig. 3 SAR established during the early days of PAC-related ABDD (reproduced from ref. [53] with permission from Bentham Science Pub.). Note that the 3'-position should be thought of as including both an amide like in PAC or a carbamate like in DOC.

The bioactive conformations of PAC and DOC have been investigated by using NMR spectroscopy, X-ray crystallography, and comparative molecular field analysis (CoMFA) [29]. Owing to the poor crystalline properties of PAC and its analogues, the first X-ray structure of a biologically active taxoid was eventually obtained for DOC in 1990 [54], and finally for PAC itself in 1995 [55]. These results represent the first examples of X-ray analysis of a taxane diterpenoid containing an oxetane ring and a C-13 side-chain, both of which are requisite for biological activity. In the solid state, the overall conformation of the diterpene ring system can be described as "cup-like" or "cage-like". The six-membered ring adopts a boat conformation distorted by the endocyclic olefin and the flagpole interactions between the H-13 and the C-16 methyl group. The eight-membered ring assumes a boat-like conformation while the other six-membered ring adopts an envelope-like conformation distorted by the planar oxetane ring [29,54,56].

The overall conformation of the C-13 side-chain has also been studied by analyzing the X-ray crystal structures of different PAC analogues and comparing the results [56]. While such studies are valuable in their own right, it has been ascertained that the C-13 side-chain is much more flexible than the taxane scaffold system. It can adopt a variety of energetically accessible conformations when attempting to interact with the PAC-binding site on microtubules. Computational studies [57,58] and 2D NMR studies [57,59,60] suggest that in polar solvents, the C-3' phenyl group in PAC resides close to the C-2 benzoyl moiety in a manner that has been likened to *hydrophobic collapse*. That these same

types of interactions are also present in DOC (with its C-3' *t*-butoxy system) wherein they can impact upon the latter's interactions with microtubules, has been pointed out by others as well [61–63]. It is noteworthy that SAR studies have shown that the C-2' hydroxyl group and the C-2 benzoyl group are critical for biological activity. The capability of the C-2' hydroxyl group to act as both a hydrogen-bond donor and acceptor suggests that both of such interactions may be important during binding. Alternatively, the hydrophobic nature of the C-2 benzoate may be important for stabilizing the orientations adopted by the C-3' substituent while itself potentially contributing to binding by interacting with a hydrophobic pocket within the microtubules or by hydrogen-bonding with one of tubulin's more polar residues. Subsequent studies utilizing X-ray structural data for additional analogues have confirmed such collapsed conformations, although subtle differences appear to also exist between the specific compounds [64,65].

Finally, some elegant work has been directed toward defining the solution dynamics of these various conformations by taking advantage of fluorine-containing analogues of PAC and DOC in NMR studies [66], and by 2D NMR wherein both have further used *restrained molecular dynamics* (RMD) methods during concomitant computational studies [66,67]. These results are depicted in Fig. 4, which also serves to summarily capture the most salient features from the preceding discussion.

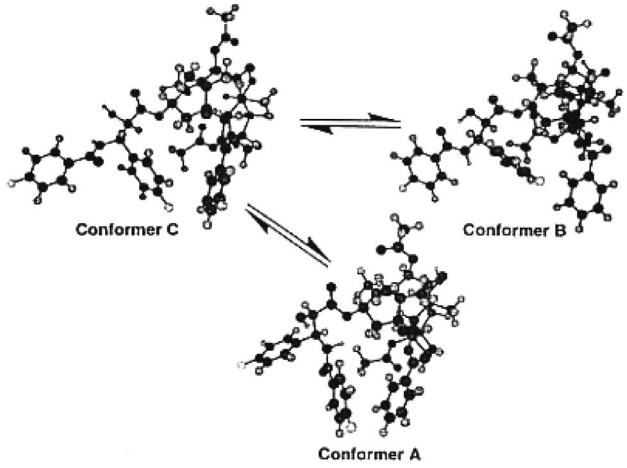


Fig. 4 Conformations of a fluorine-containing PAC analogue in protic and aprotic media (reproduced from ref. [66] with permission from the American Chemical Society). The specific analogue depicted contains a fluorine atom in the para-positions of the phenyl- and benzoyl-rings positioned off the C-3'-position of the C-13 side-chain of PAC.

As shown in Fig. 4, conformers **A** and **B** respectively portray an extended display of the C-13 position side-chain that is preferred in aprotic solvents, vs. a rotated arrangement wherein the C-3' phenyl group has moved further under the taxane cup so as to reside very close to the C-2-benzoyl moiety as a result of the purported hydrophobic collapse. Rotamer **C**, most recently added to this equilibrium, is very similar to **B** and according to certain authors, it "might be the molecular structure that is first recognized by the β-tubulin binding site since the contribution of this conformation at around ambient temperature is substantial in protic solvents" [66]. The importance of the accumulating SAR and conformational studies is further discussed below relative to the work directed toward elucidating how PAC binds with its biological receptors.

Photoaffinity labeling strategies represent the first studies that were conducted to investigate the interaction of PAC with its microtubule target protein. Early studies using [^{3}H]-PAC indicated that it preferentially binds to the β-subunits of microtubules, although the exact site for this interaction could not be ascertained due to insufficient photoincorporation of [^{3}H]-PAC [68]. To more definitively map

binding, analogues bearing photoaffinity groups at the C-2, C-7, and C-3' positions of PAC have been employed (Fig. 5) [69]. Chemical and enzymatic digestion of the microtubules followed by N-terminal amino acid sequencing eventually led to identifying the specific residues that are in close proximity to the PAC binding site. [^{3}H]-3'-(*p*-azidobenzamido)-taxol [70] and [^{3}H]-2-(*m*-azidobenzoyl)-taxol [71] were found to cross-link to the amino acid residues 1–31 and 217–233 of β-tubulin, respectively, while [^{3}H]-7-(dihydrocinnamoyl-benzoyl)-taxol cross-linked with the Arg-282 residue in the β-subunit [72].

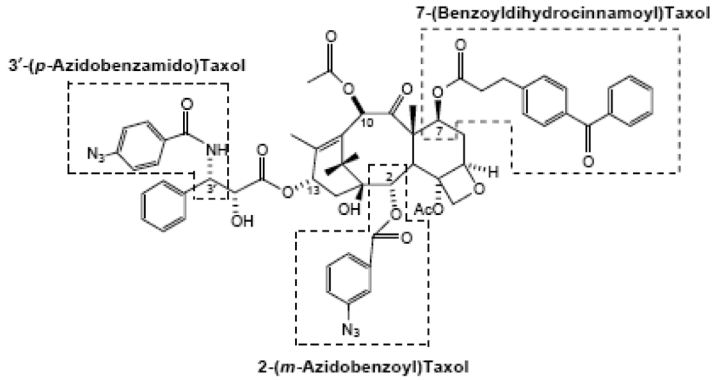


Fig. 5 Photoaffinity labeling analogues of [^{3}H]-PAC used to map PAC's binding site on microtubules (reproduced from ref. [69] with permission from Elsevier Pub.). See text for details.

As discussed above, early studies suggested that the bioactive conformer of PAC would likely be similar to that exhibited by hydrophobic collapse. A polar conformer that juxtaposes the C-2 and C-3'-benzamido side-chains was later proposed to be the preferred bioactive form. However, based on the data obtained from photoaffinity studies and the atomic structure of α,β-tubulin junctions, it is now generally accepted that PAC binds to a deep hydrophobic pocket near the surface of the β-tubulin while adopting a "T-shaped" or "butterfly" conformation [73]. In this conformation, PAC "opens up" and allows for intermolecular hydrophobic association, which is reflected by the irregularly stacked C-3'-benzamido, His-229, and C-2-benzoyl moieties. This arrangement is depicted in Fig. 6 where it can be seen that the C-3'-benzamido group is in close proximity to Val-23, and the C-7-hydroxyl group is close to Thr-274. The C-2-benzoyl group fits into a pocket formed by the imidazole ring of His-227 and the side-chain of Asp-224, both of which are part of the H-7 helix that is in contact with the exchangeable nucleotide binding site of β-tubulin. It is well established that the hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP) in β-tubulin constitutes a key regulatory mechanism that affects microtubule polymerization/depolymerization [74,75]. In this context, the H-7 helix could have a controlling effect on the overall tubulin molecule because its interaction with PAC appears to induce a conformation that mimics the GTP-bound form of β-tubulin, thus promoting microtubule assembly [76,77]. This binding site is close to the M-loop that participates in lateral interactions with the H-3 helix of the adjacent β-tubulin subunit. Therefore, the action of PAC could be understood as a strengthening of the lateral contacts between protofilaments via a conformational change in the M-loop that leads to its increased stability [69,78–80]. It is interesting to note that the position of PAC's binding site on the β-subunit is occupied by an eight amino acid loop associated with an α-subunit, namely, the S-loop A-362 to A-369. It has been suggested that this loop acts as a microtubule-stabilizing factor by promoting lateral contacts between protofilaments. This, in turn, has led to the hypothesis that PAC could act by mimicking the stabilizing effect that the S-loop has on the M-loop [71,76–80]. The dynamics of this binding paradigm are shown in Figs. 6 and 7 with the latter also conveying the change in the binding surface's hydrophobic character.

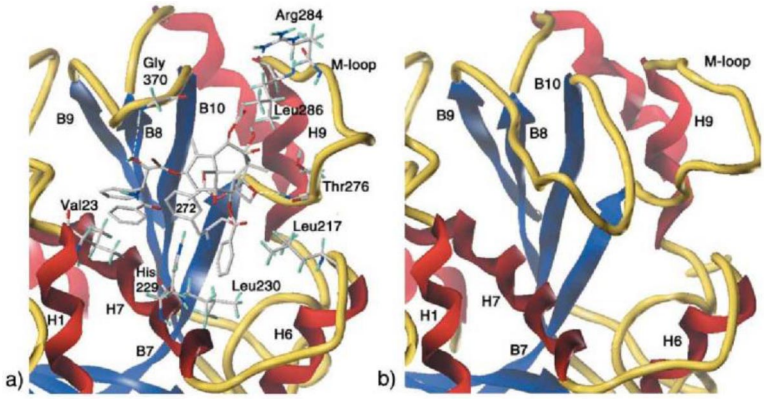


Fig. 6 Binding of PAC with microtubules (reproduced from ref. [73] with permission from PNAS Pubs.). (a) The β-subunit pocket bound with PAC. (b) Same pocket without PAC now showing how the α-B9-B10 loop extends into this area.

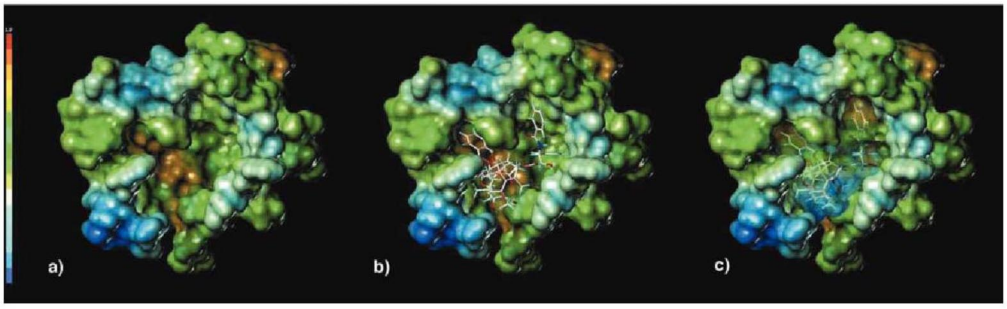


Fig. 7 Solvent accessible surface of PAC's binding pocket on β-tubulin colored according to degree of hydrophobicity (reproduced from ref. [73] with permission from PNAS Pubs.). Color gradient is on the left wherein the maximum hydrophobicity is indicated by red and the lowest by dark blue. (a) Empty binding pocket is burnt orange suggesting that it is highly hydrophobic. (b) Superposition of PAC in its "T-shaped" conformation within the binding pocket. (c) Surface re-coloring that illustrates that the hydrophobic depression has been converted to a more hydrophilic surface upon PAC's binding.

The proposed "T-shaped" bioactive conformation of PAC correlates strongly with the data obtained from SAR studies. As noted by others [73], these key features can be summarized according to the following six points.

(1) The C-2'-hydroxyl group is a hydrogen-bond donor that is crucial for biological activity [63]. In the proposed T-shaped model, there is a hydrogen bond from the C-2'-hydroxyl to the carbonyl of the ARG-369.
(2) There is a notable biological insensitivity or *neutral SAR* [81] of PAC to chemical modifications across the C-7 to C-10 region. This portion of PAC is not involved in binding to the β-subunit and is instead projected outward or away from the surface of the macromolecule.
(3) Replacement of the phenyl rings with cyclohexyl rings in the C-2 and C-13 side-chain positions leads to sustained activity [82]. In the proposed model, both of these phenyl rings are situated in a hydrophobic space.
(4) Extension of the C-4 acetate with longer alkyl chains leads to sustained activity [83]. In the proposed model, this group becomes positioned over a 10 residue hydrophobic basin.

(5) Selected *meta*-substitutions on the C-2 benzoyl can enhance biological activity while *para*-substitutions cause a reduction in potency [84]. The proposed model reveals that the hydrophobic subsite hosting the C-2 ring is reasonably tight on three sides but more open at one of the *meta*-locations.

(6) The biological activity of the C-6 nor-PAC analogues was found to be 10–20 times less than that of PAC. In the proposed model, contraction of the six-membered C-ring of PAC to a five-membered ring pulls the oxetane D-ring away from Thr-276, which reduces the effectiveness of the O-21 to HN-Thr interactions.

Providing additional support to this binding arrangement while also serving as a rather intriguing demonstration of deploying *semi-rigid analogue design* strategies during ABDD, some of the most recent analogues of PAC and DOC have sought to tie the latter's flexible C-13 side-chains into conformations that can take advantage of the aforementioned favorable receptor interactions. A few representatives for these remarkable types of ABDD-driven molecular probes are shown in Fig. 8. Analogue **10** (Fig. 8) is one of the earliest examples that was found to have significant activity, although it had reduced potency when compared side-by-side with its parent compound DOC. Nevertheless and admittedly much to the present authors' surprise, **10**'s activity clearly demonstrates that these types of "carbon tethers do not [necessarily] hamper tubulin binding" [85] when strategically utilized within this critical and otherwise highly sensitive region of PAC's pharmacophore. Analogue **11** and the **12**, **13** pair were then found to have *equipotent* [86] and even "20 to 50-fold more cytotoxic [activity] than PAC" [87], respectively. Analogue **14** "possesses virtually the same potency as that of PAC" [88]. Importantly for the various investigators who still harbored somewhat different versions of the binding scheme up to this point, concomitant computational and docking studies with all of the tethered types of molecular arrangements repeatedly suggest that the more active compounds from these series tend to be the ones that can best accommodate the T-shape motif and can thus enter into binding scenarios either identical or very similar to the paradigm described in the preceding paragraphs.

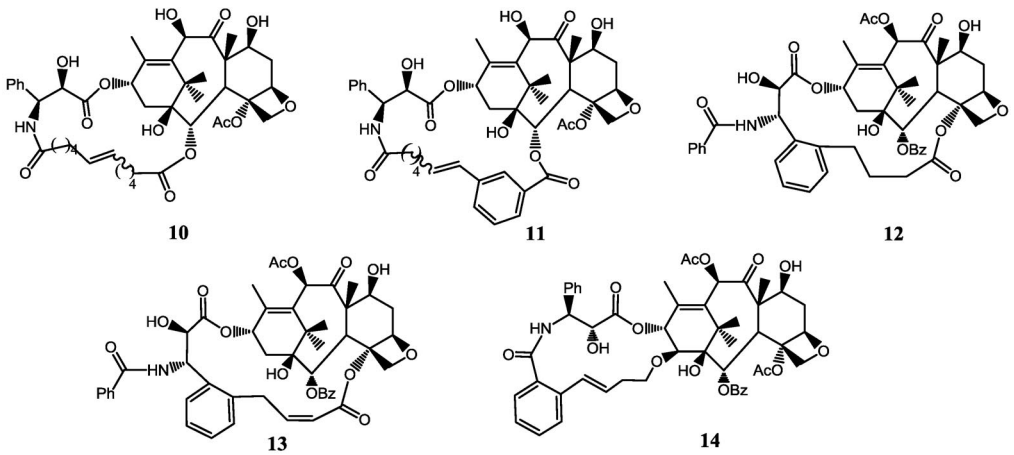


Fig. 8 Structural representatives of semi-rigid analogues having a tether added between some of the key pharmacophoric groups present within PAC and DOC [85–88]. Note that even though the starting bacatin III-related NP from which **14** is derived also bears a C-14-position hydroxyl group, such compounds should still be considered to be analogues of PAC within the context of ABDD.

Finally, it seems fitting to conclude this section by again reviewing some of the earliest observations noted for this class of compounds while keeping the most recent developments fresh in our minds. For example, that PAC was thought to bind reversibly to the microtubule biopolymer in a highly spe-

cific manner with a stoichiometry close to 1:1 relative to an α,β-tubulin heterodimer, would now seem to be definitively characterized at the molecular level as being exactly just such an arrangement [89,90]. Similarly for the early observations that microtubules formed in the presence of PAC exhibit unusual stability against conditions favoring depolymerization such as cold temperature, Ca^{2+} ions, and dilution [12]. Of significant importance in this same regard is the finding that PAC promotes the assembly of tubulin even in the absence of GTP, which under normal conditions is an absolute requirement for microtubule assembly. Finally, at an in vitro concentration of 5 μM of PAC, the critical concentration of microtubule protein necessary for microtubule assembly was previously shown to decrease by a factor of 20 (from 0.2 to less than 0.02 mg/mL) [91]. Thus, when all of the studies to date are taken together, the latest binding paradigm comes full-circle with the very early biological results associated with defining this unique mechanism of action [12] that ultimately became responsible for prompting such a high interest in this fascinating natural product molecule in the first place.

ANALOGUES DIRECTED TOWARD REDUCING MULTIDRUG RESISTANCE

In addition to the fundamental SAR investigations and receptor interaction studies discussed above, and somewhat akin to the applied research that was undertaken to specifically improve water solubility, attention also has been directed toward overcoming the clinical shortcoming exhibited by both PAC and DOC relative to MDR. Within this arena, the substantial efforts contributed by Ojima et al. are particularly noteworthy [88,92–98], as are some of the studies published by Audus and Georg et al. [99,100]. A related effort that pertains to this specific aspect of ABDD within the overall PAC field that derives from some of our own laboratory's experiments will also be conveyed as part of this discussion [27,81,101,102].

As first noted by Ojima et al. [92] during broad SAR studies that led to the replacement of the C-3' phenyl ring with a 2-methyl-1-propenyl or its related saturated *sec*-butyl alkyl system, the promising activity of such substitutions against MDR cancer cell lines "provides significant information for the development of newer antitumor agents effective against drug-resistant cancers." In subsequent work [94], it was further demonstrated that modifications at the C-10 position, such as with an acylcyclopropyl ester rather than the acetyl ester as normally present in PAC, provided compounds "possessing two orders of magnitude better activity than PAC and DOC" when tested in MDR cell lines. Because of the overexpression and prominent role of the Pgp transporter system in MDR, these investigators thus noted that "the C-10 position appears to be crucial for Pgp to recognize and bind taxoid anticancer agents" [95]. Eventually, such compounds were found to function as dual agents in that they appear to both inhibit the Pgp efflux pump while also retaining their desired actions on the microtubules. The promise demonstrated by these types of agents led Ojima et al. to call them "second-generation taxoids", and when they discovered shortly thereafter that replacement of the C-2 benzoate with a 3-methoxybenzoyl substituent was also beneficial, the composite was initially called "advanced second-generation taxoids" [95]. These investigators have also examined the C-3'-N position, which appears to be rather neutral with regard to MDR impact [96]. Alternatively, when studying another set of related taxane natural product compounds analogous to what would be the 14-hydroxy-containing versions of PAC or DOC (e.g., **14** in Fig. 8), they found that a C-1-C-14 carbonate exhibits as much as "50–80 times higher potency than PAC in the apoptosis assay against MCF7-R and CEM VBL-R" human cancer cell lines that display MDR [95]. Given the various combinations of altered functional groups that sometimes work in an additive manner to decrease MDR liability, Ojima appears to have then adopted a definition scheme for these compounds that is more pharmacologically oriented, namely, that the second-generation compounds represent the combinations that show high promise while there is now a third generation that "can virtually circumvent the Pgp-mediated MDR" liability completely [88,96]. An example of a second-generation agent is shown in Fig. 9 as structure **15**. It was regarded to "warrant further preclinical evaluation" because of its "highly promising [profile of] in vivo antitumor

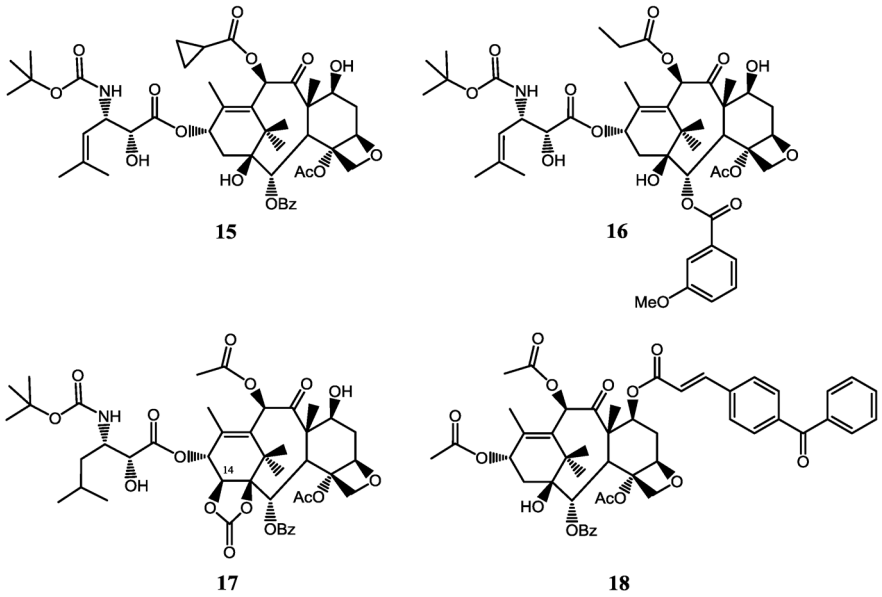


Fig. 9 Promising compounds from ABDD studies directed toward alleviating Pgp-related MDR [88,92–98]. Analogues **15–17** are regarded as dual agents in that they are thought to inhibit Pgp while retaining their desired actions on microtubules. Because the truncated analogue **18** lacks the requisite C-13 side-chain, it does not retain microtubule-binding properties and only acts as an inhibitor of Pgp. Note that **17** is synthesized from the 14-hydroxy DAB natural product rather than from DAB. See text for details.

activity" [96]. Examples of third-generation agents include structures **16** and **17** for which the latter has also progressed into clinical trials [96,95, respectively].

In a different strategy toward addressing MDR, Ojima et al. [97,98] have exploited their Pgp-related SAR findings to generate inhibitors of this transporter that do not retain the microtubule binding properties of PAC and DOC. These truncated analogues could be useful in combination therapy wherein PAC or DOC are then simultaneously administered. Such compounds belong to the general class of MDR reversal agents and to the broader class of chemosensitizer agents. As such, they are also applicable to this type of adjunct usage in conjunction with any other drug that might be otherwise disadvantaged by the actions of Pgp. Their work in this area has focused upon analogues lacking the requisite C-13 side-chain as present in either PAC or DOC, and hence all of such compounds would not be expected to have microtubule binding activity. Both DAB and the 14-hydroxy DAB have been utilized as starting materials. Interestingly, modifications at the C-7 and C-10 positions proved to be most promising for increasing the interaction with Pgp while modifications at the C-2 and C-13 led to compounds with little or no activity. This situation is just the opposite to the regions regarded as being the most important for microtubule binding by intact PAC and DOC. An exemplary compound that exhibits strong MDR reversal activity at 0.1 μM is shown as structure **18** in Fig. 9. From these SAR results coupled with studies using [^{3}H] photoaffinity labeling analogues, these authors have suggested "that there is a specific binding site for PAC on Pgp which has rather strict steric/shape requirements" [97].

Toward a goal of allowing PAC to cross the blood–brain barrier (BBB), Audus and Georg et al. prepared and tested an analogue where a succinic acid group was used to replace the acetyl group normally present at the 10-position of PAC. Their results showed that this substitution resulted in a "reduced interaction with Pgp and, as a consequence, enhanced permeation across the BBB in vitro and in situ" [99]. These investigators have also shown that "simple hydrolysis or epimerization of the C-10 acetate of PAC can reduce interaction with the Pgp transporter" [100]. While these results support the

previous conclusion discussed above about the importance of the C-10 position for PAC's interactions with Pgp, it should be noted that Ojima's compounds are thought to be inhibitors having enhanced interactions rather than reduced interactions with Pgp. These two findings are not incompatible either independently or as a composite of the results, although it should also be recalled that DOC, devoid of an acetyl at C-10, is still very much subject to MDR derived from Pgp, suggesting, in the very least, that different models for making Pgp assessments may vary in both discriminatory sensitivity and predictability for clinical outcomes.

Previous work from our labs during validation of a testing paradigm to assess Pgp interactions associated with MDR indicated that we could clearly discriminate differences among close members of the PAC family when the latter were deployed as standards [27]. However, we did not feel confident in concluding that a specific binding site for PAC resides in either of this transporter's pair of dual pockets, which are otherwise regarded as being quite promiscuous in terms of their overall structural requirements, including the distinct possibilities for simultaneously accepting more than one substrate ligand within a single pocket and for engaging in a dynamic conformational dialogue between the dual sites depending upon their loading conditions. Nevertheless, our work then proceeded to probe this surface with a goal of identifying a functional group that might be incompatible for binding even amid such structural promiscuity and despite the potential for such SAR ambiguity [101]. If able to identify such a *negative SAR* feature within the context of the taxanes, it can then be examined for its generality by placement on different scaffolds, as well as for the possibility of using it to better usher PAC into cancer cells by avoiding Pgp. Because substitutions at PAC's 10-position retain microtubule-binding properties, we utilized this site to attach an initial series of aspartic acid (Asp) derivatives via an ester linkage [81,102]. The fully protected version of Asp (N-BOC and benzyl ester) provided a read-out about the tolerance for steric bulk within this region of Pgp during its association with PAC, this cavity proving to be very tolerant of such. The di-protected derivative also provided a read-out about lipophilicity, which appeared to highly favor binding and cellular export. Similarly, exposure of the basic amine functionality by selectively removing just the BOC-protecting group significantly increased binding and further exacerbated Pgp susceptibility. Alternatively, selective exposure of the acidic carboxyl moiety decreased the susceptibility toward Pgp by about one-third, a result seemingly in accord with the more recent observations noted above for the BBB studies. Finally, complete exposure of Asp as its amino acid form resulted in binding that was again increased when compared to just the carboxylic acid form but was still less than when compared to PAC itself. Subsequent studies deploying Asp and glutamic acid (Glu) appendages positioned at either C-7 or C-10 led to similar findings (details to be published elsewhere).

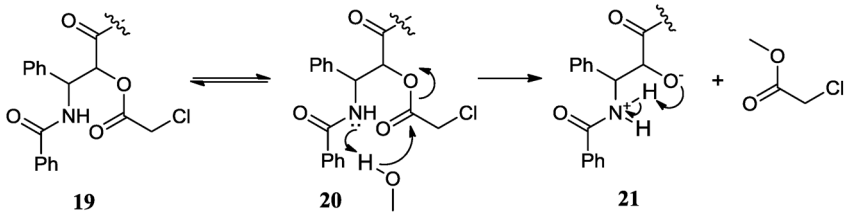
A generally useful note about chemical synthesis merits a quick comment at this point. During all of the aforementioned SAR studies, numerous protecting-group protocols were elaborated by various investigators for selectively manipulating the hydroxyl groups located at the C-2', C-7, and C-10 positions of PAC and DOC. Table 2 lists just a few of the initial versions adopted by some of the more prominent practitioners who were engaged in early SAR studies within the taxane field, such selections often providing an individual signature to their group's efforts in that regard. Table 2 is not meant to be inclusive, and numerous additional protecting groups have become elaborated subsequent to these early, landmark chemical and SAR investigations.

Table 2 Exemplary protecting groups and signature chemical manipulations deployed by some of the more prominent investigators during early SAR studies of PAC and DOC, e.g., refs. [5–10].

Investigator	Protecting group and/or signature chemical manipulation
Wall & Wani	Natural arrangements, e.g., C-10-acetyl
Suffness	TAC[a]
Kingston	Silyl, e.g., TES[b]
Potier	Troc[c] and specifically a C-3'-N-BOC[d]
Holton	TES, Troc, and specifically a β-lactam synthon (see Scheme 1)
Ojima	TES, Troc, and specific MDR-related appendages (see prior discussion)
Georg	Silyl, e.g., TES and TBDMS[e], and C-10 deacetylation

[a]TAC = trichloroacetyl.
[b]TES = triethylsilyl.
[c]Troc = 2,2,2-trichloroethoxycarbonyl.
[d]BOC = tertiarybutoxycarbonyl.
[e]TBDMS = tertiarybutyldimethylsilyl.

Of all of these commonly utilized methods within the taxane field, we instead found a chloroacetyl (CAC) protecting group to be especially useful [103]. A few others had already deployed CAC within the context of the taxanes but, quite accidentally, we discovered that it could be removed from the C-2'-hydroxyl by simply stirring in methanol at room temperature for several hours [104,105]. As shown in Scheme 3, we attributed this facile methanolysis to an intramolecular assistance that can be rather uniquely afforded by the C-3'-amide nitrogen atom in PAC or DOC that is somewhat akin to what we had previously exploited during our synthesis of the C-13-position side-chain (Scheme 2). Thus, deployment of CAC provides for an extremely gentle later removal from the C-2'-position so as to selectively liberate just this particular hydroxyl group. This is useful because only the 2'-hydroxyl is ultimately needed as a requisite pharmacophoric feature, whether exploring SAR in general or specifically pursuing MDR-avoiding molecular constructs. We found CAC to be especially useful when deploying probe structures that may have chemically sensitive features which need to be preserved during liberation, work-up, and purification of the final test compounds. That significant advances in the chemistry associated with the parent molecules undergoing ABDD campaigns can be uncovered, represents another aspect of ABDD that merits additional recognition.



Scheme 3 Proposed mechanism for the facile methanolysis of a CAC protecting group when utilized at the C-2'-position hydroxyl-group in PAC or DOC [103–105]. While shown for clarity as a step-wise process, we feel that a concerted pathway is a more likely mechanism. The latter can take advantage of a pseudo "six-membered ring" arrangement of the participating atoms during the transition state so as to completely avoid the depicted fully charged species.

Encouraged by the biological results from our initial library of just four probe compounds, we decided to prepare a second set of more sophisticated probes. In this regard, we noted that the most promising negative Pgp SAR functionality, namely, a full-blown carboxylate anion, can also be used by virtue of its sheer polarity to enhance PAC's notorious low aqueous solubility. Less obvious, however, we also discerned that this same structural space has a certain degree of molecular similarity overlap with *functional address* systems that can hone to certain types of cancer cells, such as those associated with the folate transporter that contain a Glu moiety attached to pteroic acid via an amide linkage. This situation is depicted in Fig. 10 in a general, strategic design manner. Our ongoing efforts are thus exploring the possibility that from the *northern edge* of PAC, we will be able to construct a single, permanent appendage (and not a prodrug-related cargo) that will: (i) enhance PAC's aqueous solubility by virtue of its simple physicochemical properties; (ii) improve PAC's selectivity for cancer cells compared to rapidly dividing healthy cells by virtue of its folate transportophore *positive SAR*; and, (iii) have a significantly reduced liability toward MDR by virtue of its Pgp transportophore *negative SAR* associated with the presence of a free carboxylate anion.

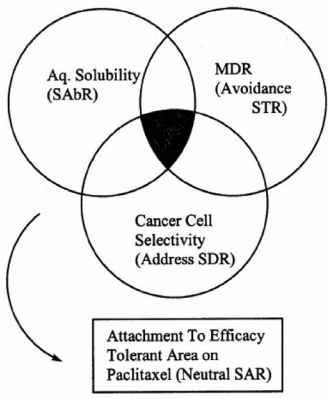


Fig. 10 ABDD strategy for hybrid molecular targets that can address all three of the clinical shortcomings observed for PAC [81,102]. Note that the bull's eye represents that region of overlapping structural space associated with each of the indicated SAR features. Because of the successful results to date, our present strategy aims to capture all three of these features within a single appendage. Thus, as opposed to a prodrug strategy, we have constructed permanent linkages of such appendages to PAC so that avoidance of MDR can be retained throughout the course of the hybrid molecule's residence within the cancer cell.

To quickly summarize this section, it can be emphasized that huge progress has been made from ABDD efforts in the area of alleviating the Pgp-mediated portion of the MDR problem within the specific context of PAC and DOC, as well as within the broader context of their further hybridized molecular targets. However, especially important for the present article's theme, this progress is also potentially useful for various other compounds from the overall arena of chemotherapeutic agents in general. Furthermore, because Pgp often plays a key role in limiting the absorption of xenobiotics from the GI tract and mitigating their distribution into the central nervous system (CNS), many of the basic SAR findings derived from these ABDD studies are directly applicable toward improving the oral bioavailability and brain penetration profiles for numerous drug candidates having shortcomings in either of these other areas. The work of at least one group of investigators already appears to be moving successfully in the direction of the BBB.

ABDD AIMED AT INCREASING SELECTIVITY: TOWARD THE SYNTHESIS OF *SMART TAXOIDS*

As discussed in previous sections, excellent progress has been made toward addressing the issues associated with PAC's low aqueous solubility and MDR liability. Alternatively, PAC and DOC's lack of selectivity for cancer cells compared to normal cells (Fig. 1) constitutes a major shortcoming that still requires considerable attention. This drawback results in systemic toxicity wherein adverse side-effects such as bone marrow depression and neutropenia often limit the dose level that may be needed to completely eradicate a tumor [106]. Similar to the majority of approaches taken to improve water solubility, nearly all of the efforts to increase the selectivity of PAC have focused upon designing prodrug constructs. Several of these prodrug strategies, in turn, have now become popular approaches toward potentially improving anticancer agents in general [107]. Referring to Fig. 10, these strategies pursue structural space that can endow selectivity for cancer cells as an independent investigation for which the results are then linked to the anticancer agent cargo (e.g., PAC, DOC, or their analogues within the taxane history) via a prodrug arrangement. While solubility features also can be incorporated as part of the selectivity component or linker arrangement, structural features intending to address MDR must be relegated to analogues of PAC or DOC if all three improvements are to be accomplished simultaneously within the overall prodrug construct. This triple challenge continues to draw a high level of activity for which the results can have ramifications well beyond their specific uses within the taxane series. After a few comments about general strategies for achieving targeted distribution, a listing of the important ABDD efforts in just the taxoid area constitutes the remainder of this section. In order to convey the enthusiasm surrounding these promising agents, portions of our discussions for several of these cases parallel the dialogue and try to capture the colorful phraseology from the originating investigators, both as then heavily referenced therein.

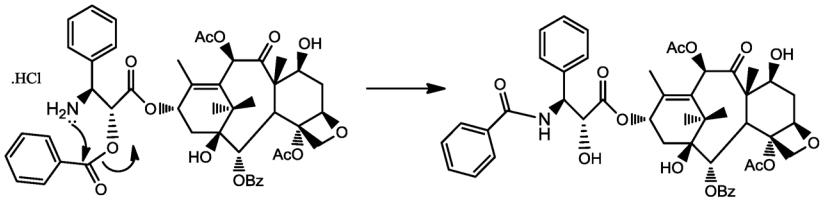
Smart analogues are compounds designed to display their activity in a manner that is selective for the desired target sites. Their design takes advantage of the morphological and physiological differences between cancer and normal cells. For example, rapidly proliferating cancer cells consume more primary metabolites such as fatty acids, peptides, polysaccharides, etc. This increased demand is met by the overexpression of factors that translocate to the cell surface where they serve to attract such metabolites and in some cases contribute to a "leaky vasculature" that allows for the rapid uptake of small molecules [108]. Alternatively, it is known that in certain other cancers, such as those characterized by solid tumors, growth occurs within a unique microenvironment having an abnormal vasculature that leads to an insufficient supply of nutrients and oxygen. Anaerobic metabolism becomes prominent in such hypoxic environments, and this leads to a lower pH in these types of cancer cells [109]. Prodrug approaches can take advantage of such differences so as to deliver the inactive form of the drug and then have it hydrolyze or rearrange to the active form under the unique physiological environment of the cancer cells, or by an enzyme that is selectively present in the tumor [110]. In a drug-targeting protocol, the anticancer agent *cargo* such as PAC or DOC is linked to an *address molecule* [111] that is specifically recognized by receptors overexpressed on cancer cells. The address molecule could be a hormone, peptide, polysaccharide, enzyme, vitamin, etc. [112,113]. Representative targeting-prodrug approaches are discussed below in the next nine subsections. All are done relative to this article's continued review of the PAC/DOC ABDD campaigns.

REPRESENTATIVE *SMART TAXOID* PRODRUGS

An ideal taxoid prodrug should have improved aqueous solubility, prolonged circulation time, reduced toxicity to normal cells, and the ability to readily release the active form of the drug in a selective manner under the unique physiological conditions of the tumor [114]. The majority of taxoid prodrugs or conjugates involve modifications at the C-2'-OH of the active taxoid. These derivatives are more accessible to enzymes in the body that can regenerate the parent drug via processes such as hydrolysis and

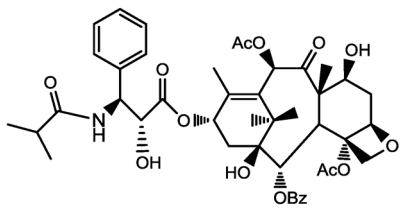
rearrangement to form a free C-2'-OH [42,114,115]. This is a crucial requirement in designing taxoid prodrugs because the latter is essential for bioactivity. Taxoid prodrugs modified at C-7-OH and C-10-Ac are also reported in the literature, although to a lesser extent compared to those at the C-2'-OH position [116–118].

Kiso et al. reported the synthesis of a C-2'-O-benzoyl isoform of PAC that they called "isotaxol" [119]. This compound is shown as structure **22** in Scheme 4. As its hydrochloride salt, this prodrug exhibited improved aqueous solubility, and then generated PAC under physiological conditions via a simple pH-dependent O–N benzoyl migration exactly like that previously reported by us for preparation of the C-13 side-chain (see Scheme 2) [14]. This is also somewhat analogous to the mechanism that we proposed for the ready removal of CAC as a uniquely advantageous protecting group wherein there is neighboring group participation by the amide nitrogen (see Scheme 3) [103–105].



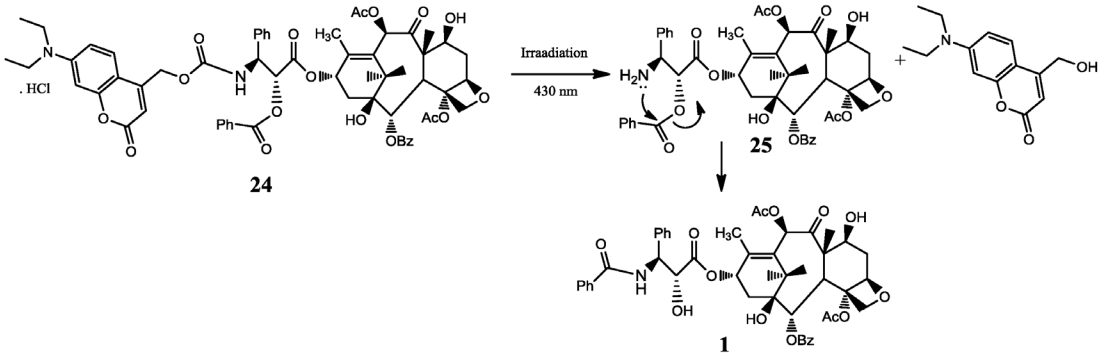
Scheme 4 Conversion of isotaxel shown as **22** to PAC (1) under physiological conditions [119]. The latter were simulated with PBS at pH 7.4 and 37 °C. Also, see precedent for such chemical behavior in prior Schemes 2 [14] and 3 [103–105].

The water solubility of **22** was determined to be nearly 2000 times greater than that of PAC. Kinetic studies of the O–N benzoyl migration indicated complete migration at pH 7.4 with a half-life ($t_{1/2}$) of 15 min, which is almost within the suggested systemic distribution time of the drug. Similar results were obtained when an analogous prodrug having a 2-methyl-propionyl moiety instead of a benzoyl group was deployed [120,121]. This prodrug had even higher water solubility. Its product, called "canadensol" [120,121] and shown below as **23**, represents a taxoid analogue with improved potency against MDR cancer cell lines just as would be anticipated from the discussion in the previous section [122]. **23** is generated spontaneously at pH 7.4 with a $t_{1/2}$ of 4.3 min.



23 Canadensol

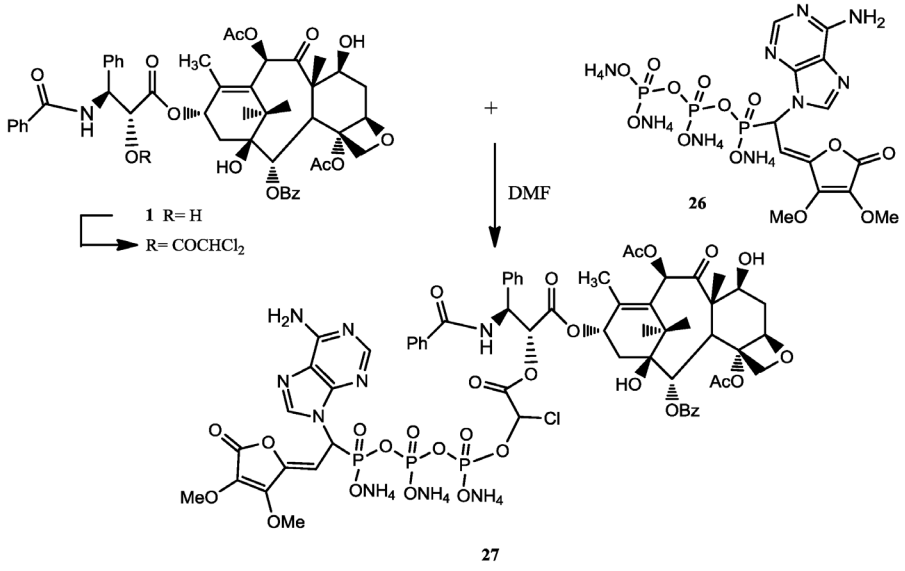
To achieve selective targeting of cancer cells and reduce side-effect toxicity, Kiso et al. synthesized a novel photoresponsive prodrug called "phototaxel" [123,124], which is shown as **24** in Scheme 5. Four different features were combined when designing phototaxel. These incorporate a coumarin derivative at the C-3'-amino group of isotaxel, an O–N intramolecular acyl migration-type prodrug, photodynamic therapy, and *caged compound* chemistry. Irradiation by visible light cleaves the coumarin moiety ($t_{1/2}$ = 4.3 min) and releases isotaxel **25** that undergoes subsequent O–N acyl migra-



Scheme 5 Release of PAC (**1**) from the photoresponsive prodrug **24** [123,124]. See text for details.

tion to generate PAC (Scheme 5). This approach utilizes photoresponsive prodrugs that can be activated at the tumor site by using a selective light-delivery system such as an endoscope.

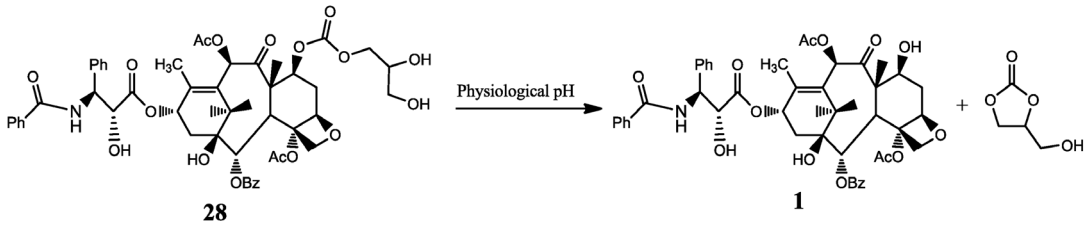
Movahedi et al. [125] synthesized a "pro-dual-drug" **27** by linking a potent anticancer agent **26** to the C-2'-position of PAC using glyoxylic acid as a linker. Triphosphonate **26** itself inhibits ribonucleoside diphosphate reductase (RDPR), which catalyzes the reduction of purine and pyrimidine ribonucleotides to their deoxy forms. Thus, inhibition of RDPR will halt the replication of the genetic material necessary for cell division. However, triphosphonate **26** has high water solubility and low hydrophobicity that prevent it from penetrating to the cell. The rationale behind designing prodrug **27** was to synthesize an anticancer prodrug with better hydrophilic–hydrophobic balance by combining the anticancer analogue **26** to another anticancer agent with high lipophilicity and low water solubility, namely, PAC. This construct is shown in Scheme 6. As expected, prodrug **27** was found to be 1000 times more lipophilic than **26**, and 500 times more water-soluble than PAC. It was completely hydrolyzed to PAC, triphosphonate **26**, and glyoxylic acid in human plasma at 37 °C after 0.4 h. Prodrug **27** was tested for its ability to inhibit *Escherichia coli* RDPR. While **26** inhibited *E. coli* RDPR immediately upon incubation, inactivation by **27** required approximately 2 h, which is consistent with the rate



Scheme 6 Synthesis of "pro-dual-drug" **27** [125].

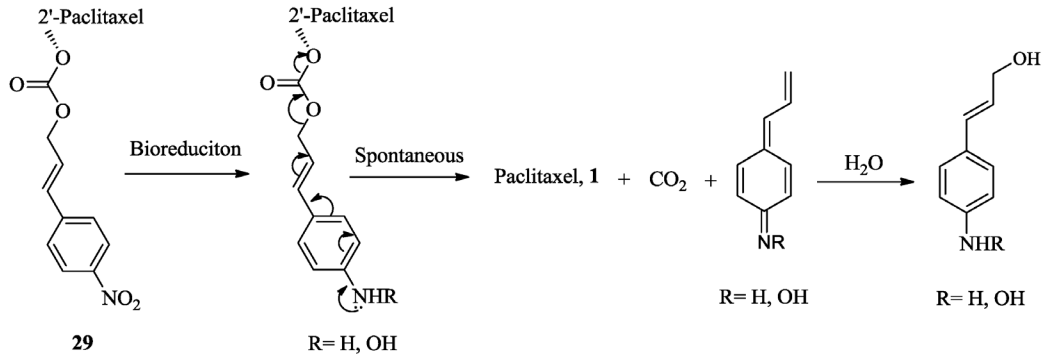
of hydrolysis of **27** to **26** + PAC in the reaction media. Prodrug **27** was also evaluated for its ability to promote microtubule assembly where it proved to be comparable to PAC, although in this case a much higher concentration had been deployed. Thus, the biological profile supports the hypothesis that prodrug **27** hydrolyzes to PAC plus triphosphonate **27**. Interestingly, GI experiments demonstrated a synergistic effect relative to the anticancer activity of the individual compounds. A mixture of PAC, **26**, and glyoxylic acid had an IC_{50} = 2.34 μM, which is about two times more potent than PAC (IC_{50} = 4.5 μM), while prodrug **27** was the most potent with an IC_{50} = 0.54 μM.

"Protaxel", shown as **28** in Scheme 7, is a carbonate derivative of PAC [126]. It is one of the few examples of prodrugs modified at the C-7 position. It has increased hydrophilicity and improved systemic tolerance and the ability to release PAC under physiological conditions [126]. The aqueous solubility of protaxel was ca. 200-fold more than that of PAC with $t_{1/2}$ of about 10 min. While **28** is stable at low pH, a shift to physiological pH causes hydrolysis of the prodrug to PAC and the cyclic carbonate as determined by gas chromatography (Scheme 7). Moreover, the maximum tolerated dose (MTD) of **28** in athymic mice was found to be ca. 2.5- to 3-fold higher than PAC. The therapeutic potential of protaxel was also shown to be superior to that of PAC in human cancer xenografts in athymic mice, and especially so in drug-resistant cell lines [126].



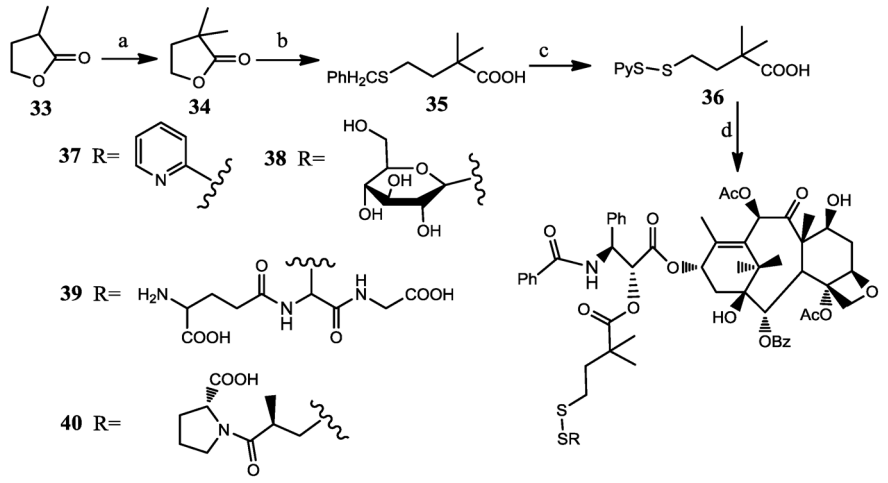
Scheme 7 Release of PAC from protaxel (**28**) via an intramolecular cyclization [126].

Scheeren et al. [127] designed bioreductive prodrugs that take advantage of reductive endogenous enzymes in hypoxic solid tumors to release PAC. Several analogues bearing a nitro group as the bioreductive trigger were synthesized. Prodrug **29**, with a *self-immolative linker*, was of special interest. The release of PAC from **29** was demonstrated chemically by treating **29** with Zn/AcOH/MeOH (Scheme 8). However, activation of the molecule to release PAC under hypoxic conditions was not confirmed. The reduction of the nitro group to an amino or hydroxylamino group followed by 1,6-elimination of the corresponding 4-amino or 4-hydroxylamino benzyloxycarbonyl moiety is believed to be the mechanism that led to the chemical release of PAC.



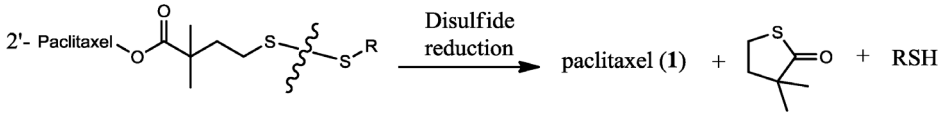
Scheme 8 Bioreductive activation of **29** to release PAC [127]. See text for details.

Appreciating that the cleavage of a disulfide bond is a facile biochemical transformation, Vrudhula et al. [128] alternatively devised disulfide-containing analogues to potentially furnish PAC under hypoxic conditions. Polar moieties were incorporated in the disulfide residue to enhance aqueous solubility, whereas an α-*gem*-dimethyl group was introduced in the linker to offer stability toward serum proteases and to facilitate cyclization of the intermediate thiol by the "gem-effect" [129]. The prodrugs were synthesized as shown in Scheme 9.



Scheme 9 Synthesis of reductively activated disulfide prodrugs **37–40** [128]. (a) MeI, NaH, dioxane; (b) NaH, $PhCH_2SH$, toluene, reflux; (c) i. Na, liq. NH_3; ii. PyS-SPy, MeOH; (d) i. DCC, **PAC**, CH_2Cl_2; ii. RSH, MeOH, Et_3N.

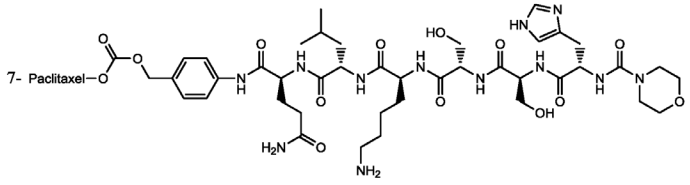
The release of PAC from these prodrugs when treated with dithiothreitol (DTT) (Scheme 10) was investigated by using a high-performance liquid chromatography (HPLC) assay. All analogues behaved as prodrugs with $t_{1/2}$ ranging from 4 min for **37** to >60 min for **39** based upon the steric and electronic differences around the S–S bond. The cytotoxicity of prodrugs **37–40** was evaluated against a lung carcinoma cell line. All prodrugs exhibited reduced toxicity as compared to PAC, with reductions in the order of 30-fold for **37** and 650-fold for the captopril-like prodrug **40**. Interestingly, when pretreated with DTT, the cytotoxicity of **37** and **38** matched that of PAC, while **39** and **40** were slightly less toxic. Prodrugs **37–40** were tested for their anticancer activity in an in vivo assay also having lung carcinoma cells implanted in nude mice. While **37–39** were not as effective as PAC, **40** showed superior activity. At the MTD, **40** demonstrated a 60 % tumor cure rate with only 10–20 % loss of body weight. It is likely that the observed antitumor activity for the dual prodrug **40** results from the combined effects of the antiangiogenic captopril moiety and the cytotoxicity effects of PAC.



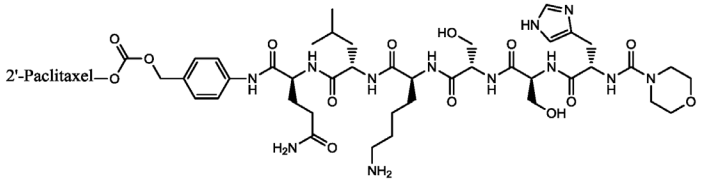
Scheme 10 Release of PAC from the disulfide prodrugs **37–40** [128].

TARGETING SELECTED ENZYMES EXPRESSED BY TUMORS

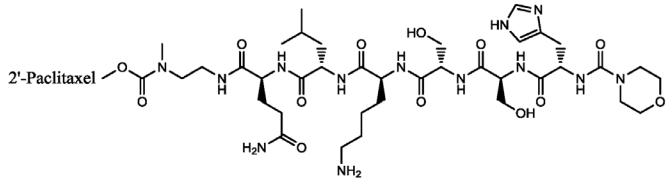
Prostate specific antigen (PSA) is a serine protease that exists in its active form in the immediate extracellular environment and thus allows for targeting of prostate cancer cells [130,131]. It has been reported that PSA selectively hydrolyzes peptides with the amino acid sequence His-Ser-Ser-Lys-Leu-Gln (HSSKLQ). In a recent study, Khan et al. reported the synthesis of four novel peptide-PAC conjugates as potential PSA-activated prodrugs for prostate cancer therapy [132]. The prodrugs consist of a PSA-substrate peptide (HSSKLQ or SSKYQ), a self-immolative linker [*p*-aminobenzyl alcohol (PABA) or ethylene diamine (EDA)], and the cytotoxic drug PAC. These analogues are shown in Fig. 11 [132]. All four exhibit an aqueous solubility superior to that of PAC. While prodrugs **41** and **42** were stable in human plasma with no significant hydrolysis observed, both were readily hydrolyzed in human serum due to the presence of active proteases. To overcome this problem, the authors designed compounds **43** and **44** bearing an EDA linkage. These compounds were stable in both human plasma and serum where less than 20 % degradation was detected. The ability of the prodrugs to release the corresponding peptide and free PAC was evaluated by incubating with PSA in PBS buffer solution (pH 7.8).



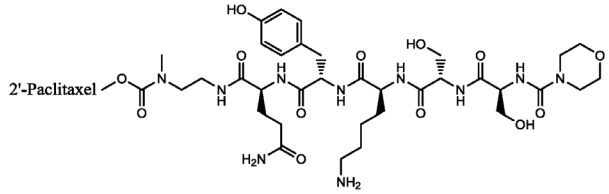
41 7-[MuHis-Ser-Ser-Lys-Leu-Gln-PABC]Paclitaxel



42 2'-[MuHis-Ser-Ser-Lys-Leu-Gln-PABC]Paclitaxel



43 2'-[MuHis-Ser-Ser-Lys-Leu-Gln-NH-$(CH_2)_2$-N(CH_3)]Paclitaxel

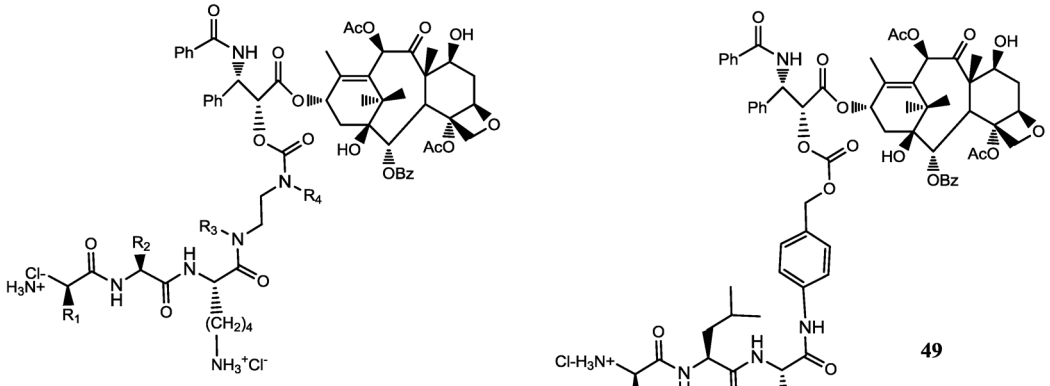


44 2'-[Mu-Ser-Ser-Lys-Tyr-Gln-NH-$(CH_2)_2$-N(CH_3)]Paclitaxel

Fig. 11 Prodrugs relying upon PSA overexpressed by prostate cancer cells to cause release of PAC [132].

The hydrolysis rates of compounds **43** and **44** ($t_{1/2}$ of 10 and 6 h, respectively) with the EDA linker were improved as compared to those having the PABA-containing linker **41** and **42** ($t_{1/2}$ of 14 and 12 h, respectively). Based on the stability studies and hydrolysis profiles of the different analogues, prodrug **44** was chosen to be tested for its activity against PSA-producing and PSA-nonproducing human prostate cancer cell lines, as well as a human bladder cancer cell. As expected, **44** was 3 to 5 times more potent on the PSA-producing cell line.

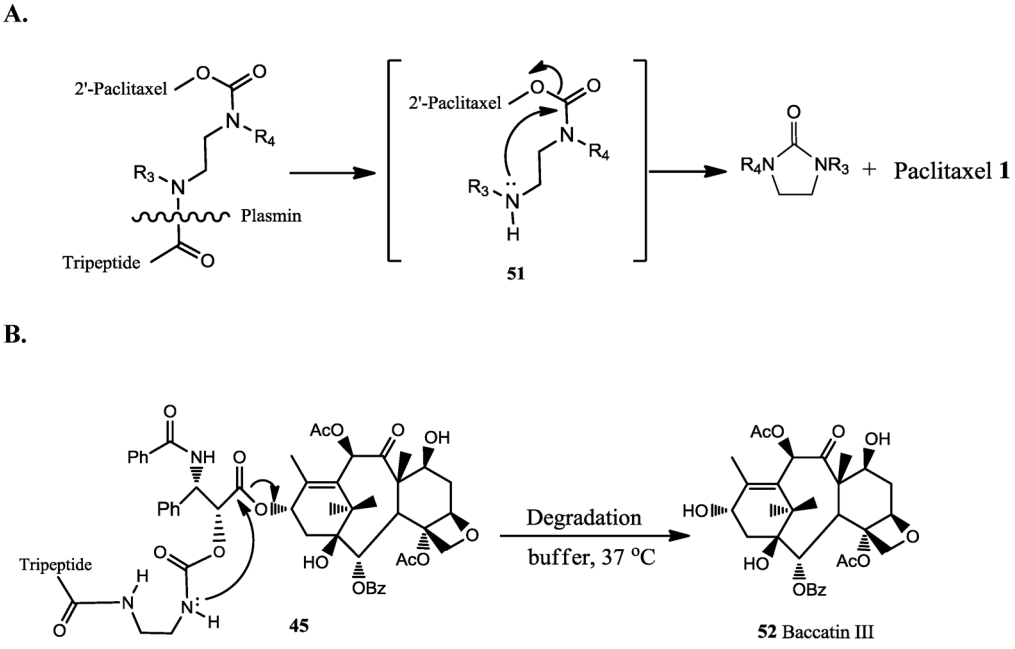
Along similar lines, Scheeren et al. [132] synthesized prodrugs **45–49** (Fig. 12) as substrates for the enzyme plasmin, a serine protease that is overexpressed in tumor cells and plays an important role in tumor invasion and metastasis [134,135]. As shown in Scheme 11A, the strategy behind designing these analogues is that plasmin will cleave the peptide bond to generate the intermediate spacer (shown in brackets as **51**), which then undergoes spontaneous cyclization to eliminate the active drug, namely, PAC. All analogues were evaluated for their stability in Tris buffer (pH 7.3).



45 R_1= CH_3; R_2= CH_2Ph; R_3= R_4= H
46 R_1= CH_3; R_2= CH_2Ph; R_3= R_4= CH_3
47 R_1= $CH(CH_3)_2$, R_2= $CH_2CH(CH_3)_2$; R_3= R_4= CH_3
48 R_1= CH_3; R_2= CH_2Ph; R_3= H; R_4= CH_3

Fig. 12 Carbamate- and carbonate-linked prodrugs of PAC, which are substrates for the enzyme plasmin [133]. Also see Scheme 11.

Prodrugs **45–49** were found to be stable for 3 days while **45** underwent degradation to baccatin III as also shown in Scheme 11. Upon incubation with plasmin, only prodrugs **48** and **49** were hydrolyzed with half-lives of 5 and 42 min, respectively. The spacer of prodrug **49** underwent instantaneous 1,6-elimination to release PAC, whereas $t_{1/2}$ of the spacer for cyclization of **48** after peptide cleavage was 23 h. The results suggest that this may be another highly promising prodrug design strategy.

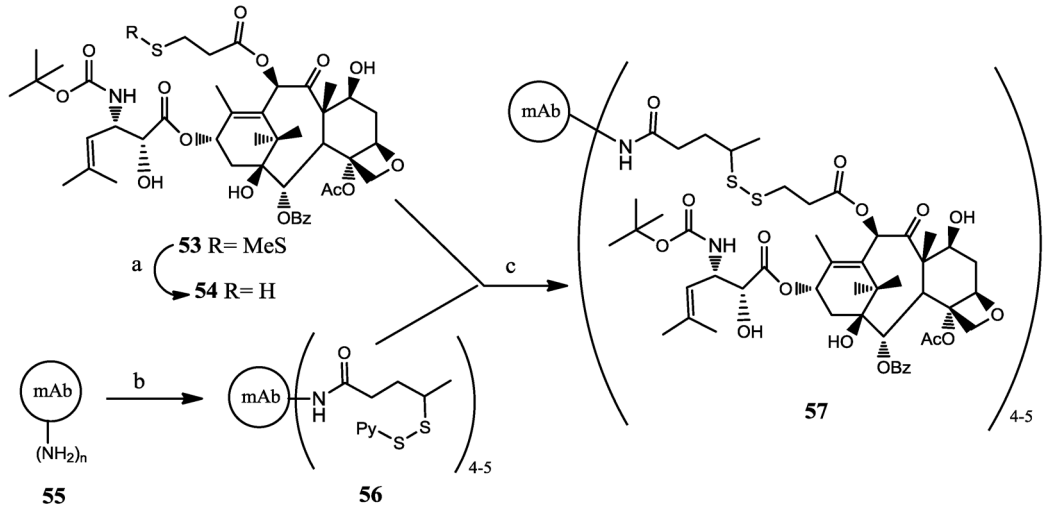


Scheme 11 Selective activation of prodrugs relying upon plasmin to produce PAC [133–135]. A. Depicts the desired pathway. B. Shows an unwanted degradation observed for the first analogue prepared in this series, namely, **45** as shown in accompanying Fig. 12.

TARGETING ANTIGENS EXPRESSED BY TUMORS

Distinguishing malignant tissues from normal ones is possible by conjugating a cytotoxic drug to monoclonal antibodies (mAbs) that have high binding specificity for antigens overexpressed by tumor cells. Once the mAb-drug conjugate specifically binds to the antigens through the mAb component, the entire conjugate can be internalized via endocytosis. In the intracellular matrix, the cytotoxic drug will then be released from the conjugate via chemical or enzymatic processes. Thus, the practical efficacy of such approaches depends on the nature of the cytotoxic agents as well as on the tumor specificity of the mAbs. This type of immunoconjugate strategy has been referred to as "tumor-activated prodrugs" or TAPs [136].

Several reports that describe targeting taxoids using the TAP approach have been published. As just one example, Ojima et al. synthesized several taxoid-immunoconjugates [137]. After evaluating several second-generation (see discussion in prior section) taxoids for their cytotoxic activity on 4 different cancer cell lines, **53** was selected for linking to mAbs to form the proposed taxoid-immunoconjugates. A disulfide linker unit was chosen to connect taxiod **53** to the mAbs, because the latter can be cleaved by an intracellular thiol, such as glutathione, to release the active taxoid. The antigen to be targeted was the epidermal growth factor receptor (EGFR) that is overexpressed in human squamous cancers such as head, lung, and breast cancers. Three murine mABs that belong to the immunoglobulin G family and bind to EGFR were chosen as address molecules. These were KS61 (IgG2a), KS77 (IgG1), and KS78 (IgG2A). The preparation of the mAb-taxoid conjugates is depicted in Scheme 12. The mAb was first functionalized with 4 to 5 4-pyridyldithio (PDT) pentanoyl groups on average. Taxoid **53** was treated with DTT to cleave the disulfide bond and generate taxoid **54** with a free thiol group that was then conjugated to the modified mAb yielding the desired mAb-taxoid conjugates. Preliminary matrix-assisted laser desorption/ionization with time-of-flight (MALDI-TOF) analyses supported the conjugation of 4 to 5 taxoids with the mAb in KS77-**53**. In addition to the three immuno-



Scheme 12 Preparations of mAb-taxoid conjugates [137]. (a) DTT; (b) *N*-succinimidyl 4-(2-pyridyldithio)-pentanoate (SPP, 10 equiv in ethanol), 50 mM potassium phosphate buffer, pH 6.5, NaCl (50 mM), EDTA (2 mM), 90 min; (c) 50 mM potassium phosphate buffer, pH 6.5, NaCl (50 mM), EDTA (2 mM), **54** (1.7 equiv per dithiopyridyl group in EtOH), 24 h.

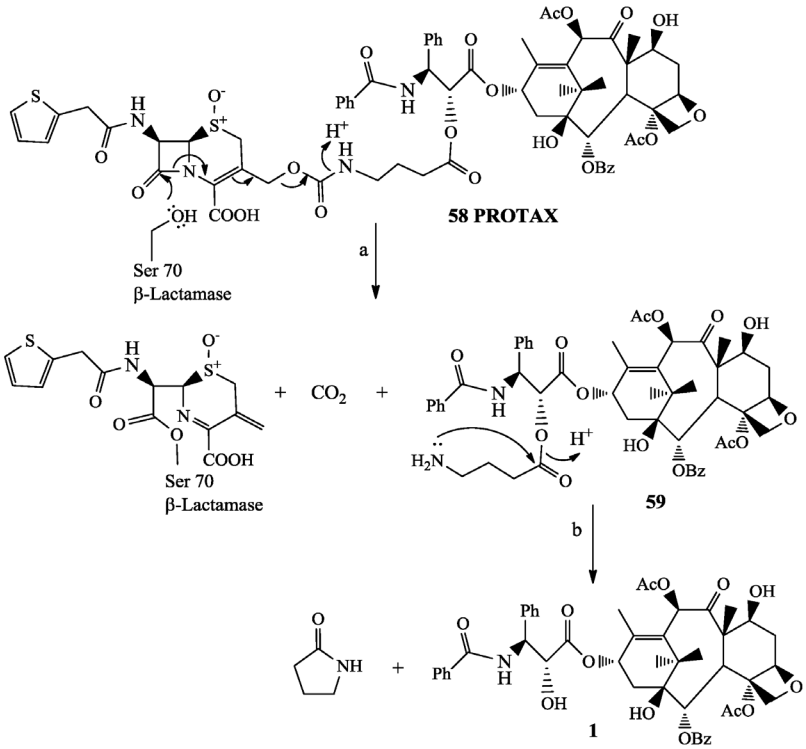
conjugates KS61-**53**, KS77-**53**, and KS78-**53**, a control immunoconjugate mN901-**53** (wherein mN901 has no binding affinity to EGFR) was also prepared. The data collected from analyzing the in vitro cytotoxicity assays was very promising. While the control immunoconjugate mN901-59 had no cytotoxic activity, KS78-**53** showed high potency against the same epidermoid cancer cell line. Adding free anti-EGFR to KS61-**53** eliminated its cytotoxicity, clearly demonstrating that the cytotoxicity caused by the immunoconjugates was dependent on the specific binding of the conjugate to the antigen on these tumor cells. The anticancer activities of KS77-**53** and KS61-**53** were also evaluated against human tumor xenografts in vivo. The free taxoid **53** showed no activity, while both of the tested immunoconjugates inhibited tumor growth in all treated animals. Notably, no systemic toxicity was detected in mice at the administered dose for immunoconjugate KS77-**51**. Although more experiments to study the specific internalization process of the immunoconjugate and the intracellular release of the drug are needed, directed targeting of taxoids toward cancer cells using the TAP approach continue to appear to be extremely promising.

TARGETING TAXOIDS BY ANTIBODY-DIRECTED ENZYME PRODRUG THERAPY (ADEPT)

Although the strategies discussed in the preceding subsections are showing considerable promise, they are not without certain limitations. For example, the enzymes overexpressed in cancer cells are often found at lower concentrations in normal cells where they can cause the release of the active form of the drug and thus prompt systemic toxicity. Similarly, the address-linker-drug conjugate is also subject to premature cleavage by nonspecific enzymes found in the blood. Finally, the success of such approaches is highly dependent upon the concentration of the target enzyme, which may not be sufficient to cause efficient activation of the drug once within the tumor. An alternative approach is to employ the ADEPT strategy [138,139]. In this strategy, the prodrug activating enzyme is first delivered specifically to the tumor by conjugating it to an antibody that binds to antigens overexpressed in the cancer cells. Once the enzyme-antibody conjugate is localized at the tumor and has cleared from the overall systemic cir-

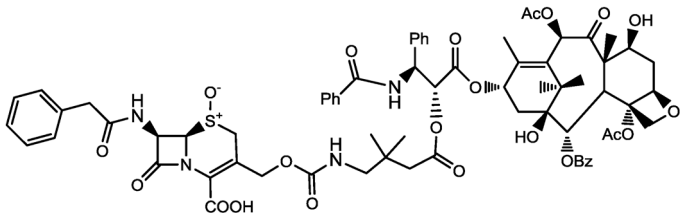
culation, a nontoxic prodrug that is a specific substrate for the antibody-conjugated enzyme is administered.

PROTAX, shown as **58** in Scheme 13, was the first reported enzyme-activated prodrug of PAC designed by the ADEPT strategy [140]. PROTAX was formed by linking PAC to cephalosporin via a self-immolative aminobutyryl linker. The prodrug activating enzyme, β-lactamase, was conjugated to humAb4D5-8, an antibody that binds to $p185^{HER2}$ antigen which is known to be overexpressed in breast and ovarian cancers. PROTAX was 10-fold less toxic than PAC against an SK-BR-3 breast cancer cell line. Analysis after hydrolysis of PROTAX by either free β-lactamase or β-lactamase-humAb4D5-8 revealed that the prodrug was rapidly hydrolyzed to the corresponding taxane-link-intermediate **59** [144]. However, the self-immolative step for **59** to then release free drug was slow, requiring 16 h to completely release PAC. This may allow diffusion of the linker intermediate from the tumor site prior to the complete release of the active agent. These events are depicted in Scheme 13.



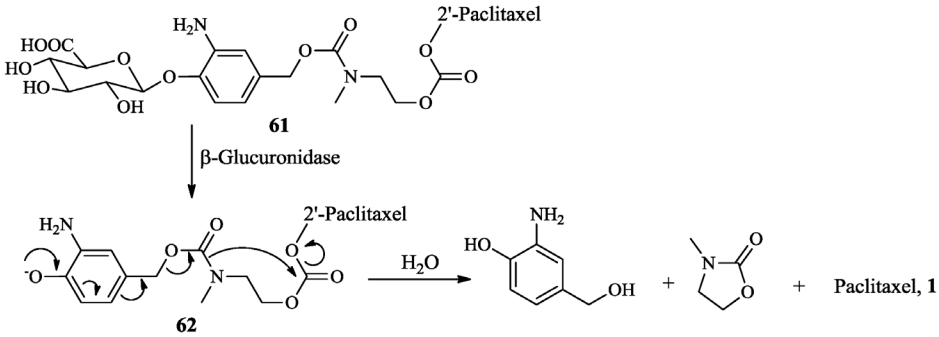
Scheme 13 Activation of PROTAX (**58**) by β-lactamase and release of PAC: (a) β-lactamase or dsFv3-β-lactamase in PBS, pH 7.4; (b) PBS, pH 7.4 [140, 141].

To overcome the slow release issue, Vrudhula et al. designed prodrug **60** where PAC was instead attached to a cephalosporin via 2,2-dimethyl-4-aminobutyric acid as a linker [142]. These authors predicted that the linker would undergo quicker self-immolation based on the *gem*-di-methyl effect. Prodrug **60** was stable in human serum but rapidly released PAC ($t_{1/2}$= 50 min) when treated with β-lactamase.



60

The groups of Scheeren [143,144], Schmidt [145], and Monneret [146] also have investigated several prodrugs of PAC for ADEPT strategies. Taking one of these cases as a last example, human enzyme β-D-glucuronidase was utilized as the prodrug activator to cleave a glycosidic bond liberating the free active drug. Endogenous extracellular β-D-glucuronidase is highly active in necrotic tumors, but has no activity within the blood pH [147,148]. Recently, Schmidt et al. reported the synthesis of prodrug **61** as a potential candidate for the ADEPT strategy [149]. Prodrug **61** consisted of a β-glucuronide moiety, a self-immolative linker, and PAC. The hydrolytic release of PAC from prodrug **61** is shown in Scheme 14. Although the water solubility of **61** has not been reported, it is expected to be several hundred times higher than that of PAC based on the water solubility of similar prodrugs [143–146]. Prodrug **61** was stable over 24 h in a phosphate buffer solution at pH 7.2 or in 10 % FCS (fetal calf serum) in phosphate buffer. The cytotoxicity of **61** in the presence or absence of β-D-glucuronidase was evaluated against a colon cancer cell line. Prodrug **61** was about 70-fold less toxic than PAC. Cytotoxicity similar to that of PAC was obtained when **61** was incubated with the enzyme. These findings are characteristic for an ADEPT approach where a nontoxic prodrug releases a cytotoxic drug when exposed to an enzyme intended to be placed within the system. **61** released PAC with a $t_{1/2}$ of about 35 min.

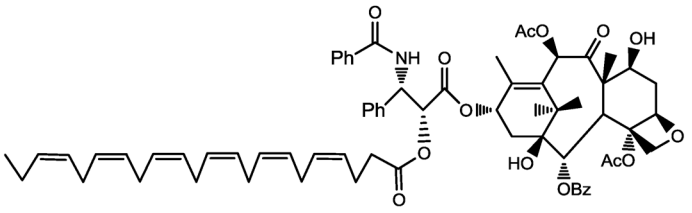


Scheme 14 Hydrolysis of prodrug **61** by β-D-glucuronidase and further collapse to produce PAC [149].

TARGETING TAXOIDS BY USING FATTY ACID AND SUGAR MOLECULES

Polyunsaturated fatty acids (PUFAs), such as linolenic acid (LNA), linoleic acid (LA), and docosahexenoic acid (DHA), are found in vegetable oils, fish, and meat. DHA is found in human milk and is classified as a nutritional additive by the U.S. Food and Drug Administration (US FDA), indicating that DHA and its metabolites are safe to humans [150]. It has been demonstrated that PUFAs are taken up more rapidly by tumor cells than by normal cells, presumably for use as biochemical precursors and energy sources. Thus, like some of the other factors that have been discussed in the prior sections, PUFAs can be used as address molecules to selectively target tumor cells [151]. For example, Bradly et al. developed the DHA-PAC prodrug shown below as **63** [152]. The data collected from the biological

and PK studies of **63** are very promising. One may hypothesize that the increased hydrophobicity of the molecule, due to the incorporation of DHA, would decrease its water solubility and render it a better substrate for Pgp. Surprisingly, **63** was more soluble in 10 % Cremophor EL/10 % ethanol/80 % saline than PAC. An MDR assay suggested that **63** is a relatively weak substrate for Pgp, PAC being ca. 4 times a better substrate in their model. Prodrug **63** was found to be at least 10-fold less active than PAC. The microtubule assembly assay showed that in the absence of GTP, PAC induced assembly at a concentration of 10 μM whereas **63** had no effect. The MTD for **63** was ca. 3 to 4 times higher than that of PAC in mice, rats, and dogs. Although myelosuppression was detected as a dose-limiting toxicity of **63**, no new toxicities other than those known for PAC were reported. The tumor AUC (area under the drug concentration–time curve) of **63** was 61-fold higher than the tumor AUC of PAC at equitoxic doses, and 8-fold higher at equimolar doses. In addition, the amount of PAC derived from **63** remained at a higher concentration in the tumor for a longer time as compared to when PAC was injected, accounting in part for the improved activity of **63**. Thus, **63** could be useful when treating different cancers in general, and particularly so for slow-growing tumors. Owing to its enhanced biochemical properties, **63** entered Phase II clinical study with the potential trade name Taxoprexin® for advanced skin and eye melanoma, and entered Phase III for advanced non-small cell lung cancer.



63 DHA-paclitaxel

Along similar lines, Ojima el al. has developed new PUFA-taxoid prodrugs to target drug-resistant cancer cells [153]. Representative compounds are shown in Fig. 13. An unsaturated fatty acid (DHA, LNA, LA) was conjugated to a second-generation taxoid. The synthesized conjugates were assayed for their antitumor activity against a drug-sensitive human ovarian tumor xenograft and a drug-resistant human colon tumor xenograft in mice. Compound **63** was synthesized for comparison. While **63** remained ineffective against the drug-resistant xenograft, **65** caused complete regression of the tumor in 5/5 mice, and the tumor growth was delayed for more than 187 days. Analogue **64** was similarly active, delaying tumor growth for more than 186 days and causing complete regression of the tumor in all surviving mice (4/5).

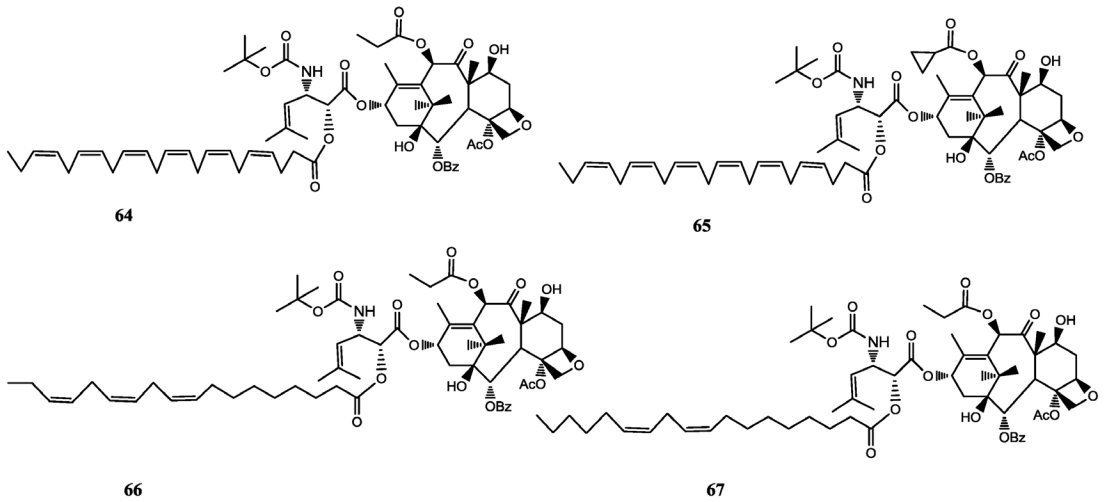
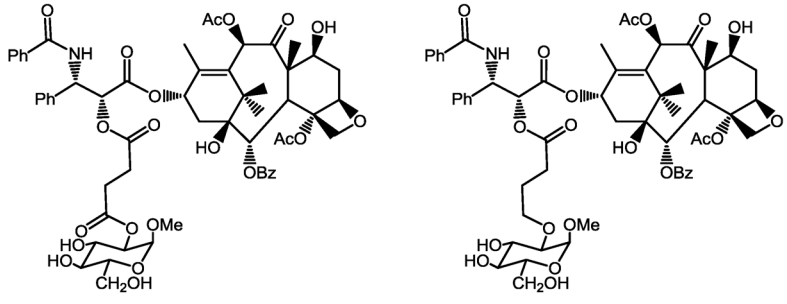


Fig. 13 Polyunsaturated fatty acid (PUFA) address systems deployed with second-generation analogues of PAC [153]. **64** and **65** have a docosahexenoic acid (DHA) adduct, **66** has a linolenic acid (LNA) adduct, and **67** has a linoleic acid (LA) adduct.

More recently, Chen et al. [154] has prepared molecules that can take advantage of the glucose transporter proteins (GLUTs) that become highly expressed in cancer cells. Exemplary structures are shown below as **68** and **69**. The double ester conjugated prodrug showed the most cytotoxicity among such compounds and could be transported into cells via GLUTs. Microscopy demonstrated that targeted cells exhibited morphological changes in tubulin and chromosomal alterations that were similar to those observed with **PAC**. Thus, these glycan-based prodrugs may be an alternative method to enhance the targeted delivery of drugs to cancer cells that overexpress GLUTs.



TAXOID CONJUGATES HAVING ANTIOXIDANT OR OTHER EFFICACIOUS PARTNERS

Dietary antioxidants, such as vitamins and flavonoids, can act as cancer preventive agents by deactivating the reactive oxygen species that damage DNA. There is also evidence that antioxidants can modify the therapeutic response and enhance the antitumor effect of certain drugs [155,156]. In this regard, Lee et al. have reported the synthesis of several antioxidant-taxoid conjugates as potential cytotoxic agents with enhanced tumor selectivity and activity against drug-resistant cell lines [157]. These compounds are shown in Fig. 14 as structures **70–72**. The different conjugates were tested for their antitumor activity against a variety of cancer types. Conjugates **70** and **71** showed the highest activity

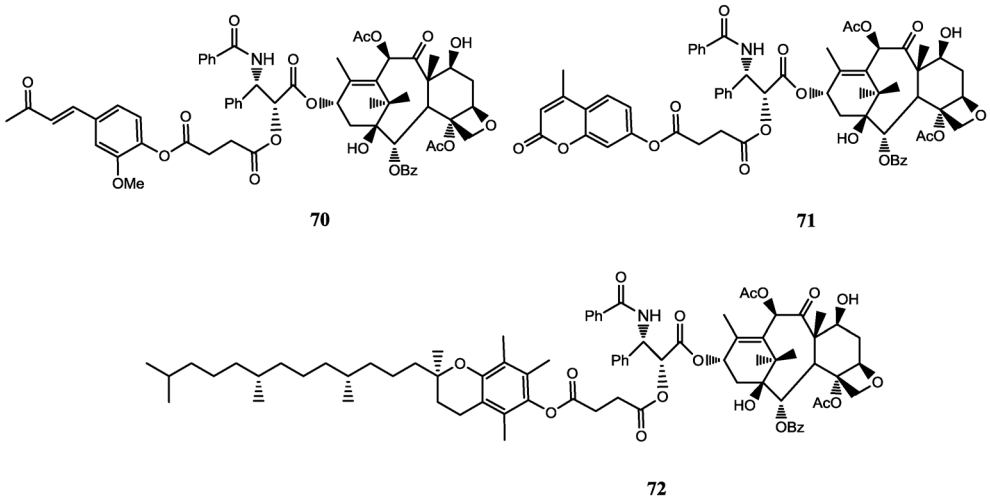
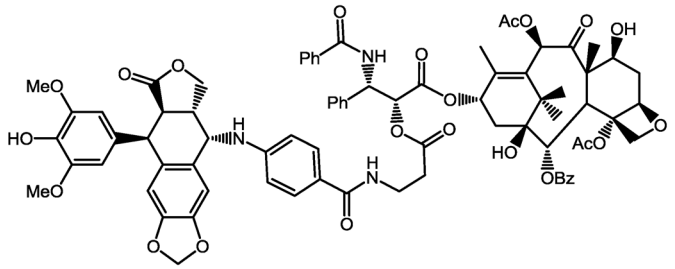


Fig. 14 Representative antioxidant-PAC conjugates [157]. **70** uses dehydrozingerone as an adduct, **71** uses coumarin as an adduct, and **72** uses vitamin E as an adduct.

against ovarian and nasopharyngeal tumor cells, while **72** exhibited strong inhibitory activity against Panc-1, all with less effect on a normal cell line.

Representative of conjugates that utilize other types of efficacious partners, Lee et al. also designed several analogues where PAC was linked to a Topoisomerase II enzyme (Topo II) inhibitor such as 4'-*O*-demethylepipodophyllotoxin (EPT) [158]. An example of an EPT-PAC conjugate is shown below as **73** where the EPT partner is attached to the 2'-hydroxyl-group of PAC. Given the need for PAC to have a free 2'-hydroxy, these compounds likely behave as prodrugs. They showed significant cytotoxicity against several human tumor cell lines at levels which were superior to EPT alone but not for PAC alone. However, the EPT-PAC conjugates also demonstrated sustained activity against several PAC-resistant cell lines. Of all analogues, the EPT-PAC pairs showed the strongest inhibitory effects against the largest variety of tumor cell lines, especially from leukemia, colon, and prostate cancer. In a Topo II inhibition assay, they effectively inhibited Topo II while additionally serving as an intracellular poison.

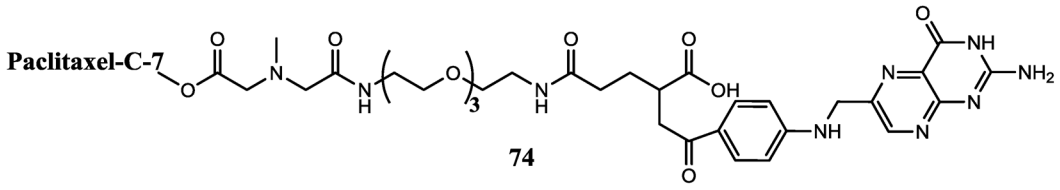


73 EPT-PAC Type of Conjugate

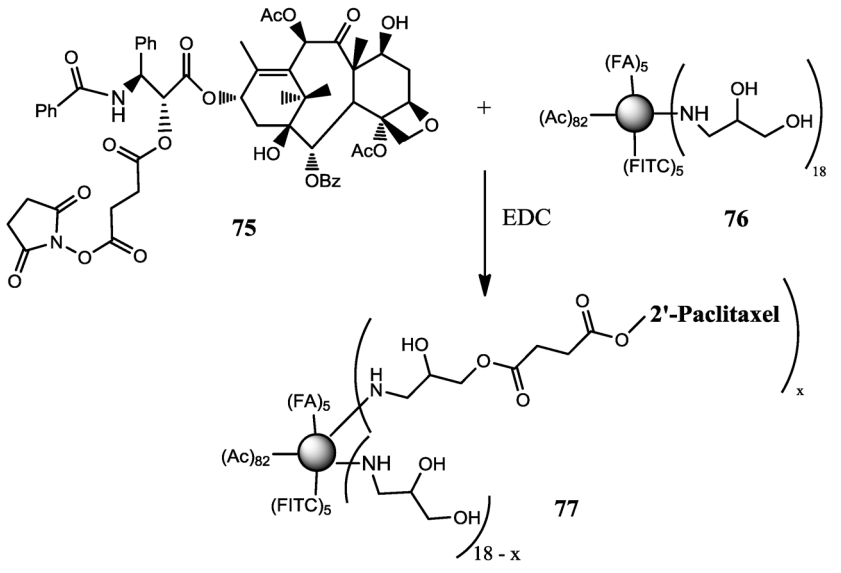
TARGETING TAXOIDS BY USING VITAMINS SUCH AS FOLIC ACID AND BIOTIN

Folic acid receptors (FRs) are overexpressed in various types of cancers and have high binding affinity to folic acid (FA), a molecule essential for the formation of new cells and tissues especially during childhood [159,160]. The high binding affinity between FA and FRs permits designing PAC-FA ana-

logues for selective targeting of FR expressing tumor cells. Once bound to the FRs, the PAC-FA conjugate will be internalized via endocytosis. This process is accompanied by a drop in pH within the endosome which, in turn, has been exploited to release PAC via the cleavage of an acid-sensitive linker [161]. In this strategy, Fuchs et al. developed several PAC-FA conjugates with the FA moiety attached at the C-2' or C-7 positions via its γ-carboxylate [162]. In general, however, the biological data was not encouraging. Of all the analogues, **74** was the most promising, although it was far from being an ideal candidate for further development. These conjugates failed to demonstrate selective killing of FR-expressing cancer cells in vitro, and proved to be less active than PAC when administered in an equimolar quantity formulated in the same injection vehicle. Unpredictably, all the synthesized conjugates hydrolyzed to PAC much faster at pH 7 than at pH 5.



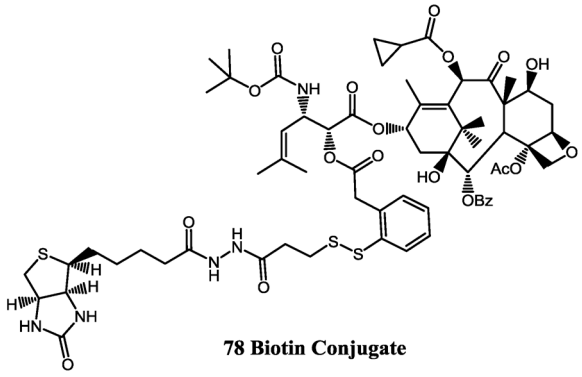
To improve the selective targeting of PAC to FR+ cancer cell lines, Majoros et al. [163] alternatively utilized a poly(amidoamine) (PAMAM) dendrimer as a novel delivery vehicle functionalized with FA molecules for selective targeting, and a fluorescin isothiocyanate moiety (FITC) as a cancer imaging agent. The trifunctional G5-Ac^3-FTIC-FA-OH-Taxol dendrimer **77** was synthesized as shown in Scheme 15. The cytotoxicity and uptake of the PAC-dendrimer conjugate **77** were assayed against folate (+) and folate (–) cell lines. It was found to be cytotoxic against the folate (+) cell line. Both **77** and the drug-free conjugate were internalized by these cells, while no fluorescence was detected inside the folate (–) cells. The control dendrimer, lacking the chemotherapeutic cargo, had no activity. The cytotoxicity of the dendrimer conjugate **77** was further investigated by a proliferation assay. At low concentration, **77** showed selective cytotoxicity against the folate (+) cancer cells. The control dendrimer



Scheme 15 Synthesis of trifunctional dendrimer conjugates **77** [163].

was again completely inactive at high concentrations. The folate system remains of interest as a targeting strategy in conjunction with both small molecule and large molecular systems, and with a variety of chemotherapeutic and diagnostic cargos [164].

Interest in using biotin receptors as an address continues to grow. Rapidly dividing cancer cells overexpress these receptors on their surface to enhance the uptake of vitamins necessary for division and growth. It has been demonstrated that the biotin receptor levels are higher than the folate and/or vitamin B_{12} receptors in many cancer cells such as leukemia, ovarian, and breast. The biotin receptors bind the growth promoter biotin (vitamin H or vitamin B_7) whose content in cancer cells then becomes substantially higher than that in normal cells. Recently, Ojima et al. [165] designed what he likened to a "guided molecular missile" that is recognized by the biotin receptors overexpressed on cancer cells. This molecular construct is shown immediately below as **78**. Ojima's missile incorporates a tumor recognition moiety (biotin) that is connected to the cytotoxic "warhead" (a second-generation taxoid) through a self-immolative disulfide linker. The authors demonstrated that following the receptor-mediated endocytosis of the biotin conjugate, the cytotoxic "warhead" is released via a glutathione-triggered cleavage of the disulfide linker, where it then binds to the microtubules to wreak havoc upon the cancer cell [165].

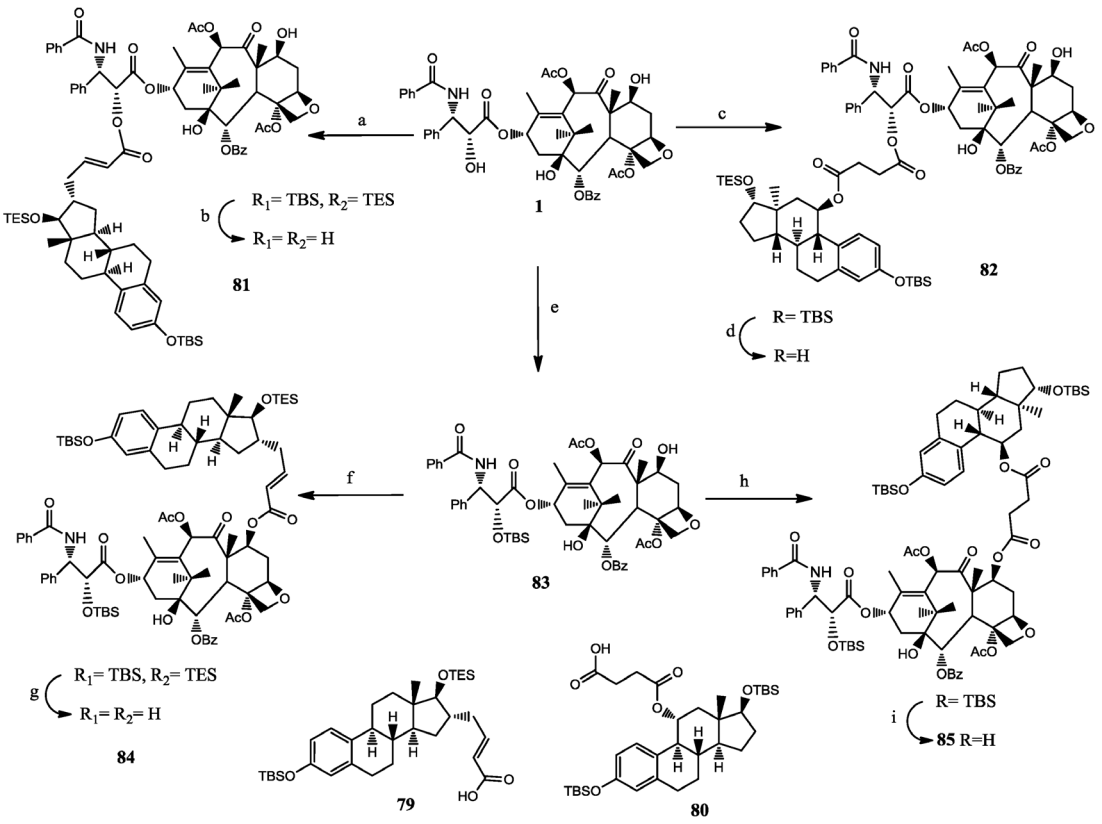


78 Biotin Conjugate

TARGETING TAXOIDS BY USING CONJUGATES RELATED TO GROWTH FACTORS SUCH AS STEROIDS AND BOMBESIN

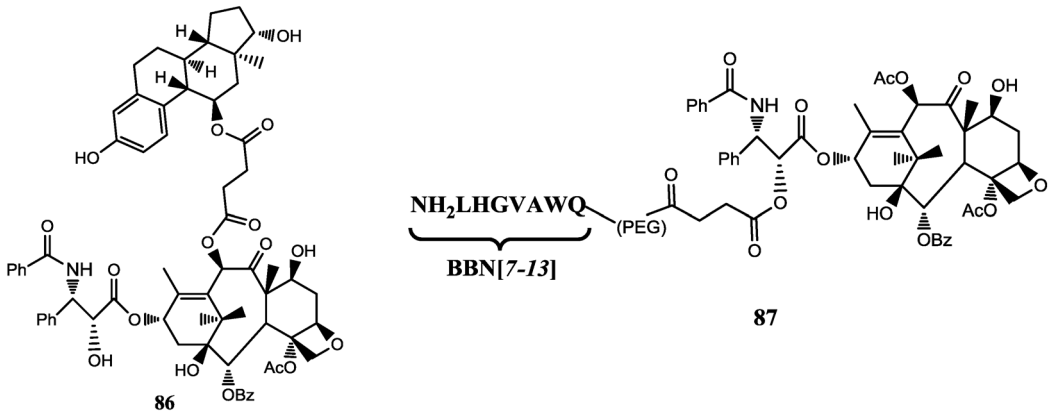
Kingston et al. designed several estradiol-PAC conjugates for the selective targeting of PAC toward estrogen receptor (ER)-positive breast cancer cells, where the interaction occurs between the estradiol component and ERs [166]. The authors relied on the well-established SAR studies for PAC to design the proposed analogues (e.g., see previous section on SAR and receptor binding).

Likewise, SAR studies have shown that modifying estradiol at its 11- and 16- positions does not result in loss of binding affinity to ERs [167,168]. Based on this strategy, Kingston et al. [166] synthesized several steroid-PAC conjugates bearing an estradiol moiety linked to the C-2', C-7, or C-10 positions of PAC, some of which are shown in Scheme 16. Using PAC as a control, the biological activities of the different conjugates were evaluated in a tubulin-assembly assay, and cytotoxicities against ovarian (ER-), prostate, and breast (ER+ and –) cancer cell lines were determined. Most of the estradiol-PAC conjugates were less cytotoxic against the different cancer cell lines than PAC, with the exception of **82**, which had somewhat better activity on a prostate cancer cell line. Moreover, none of the tested conjugates showed significant selectivity for ER+ cell lines. Alternatively, a related C-10-substituted analogue shown below as **86** showed 3-fold selective toxicity for the breast (ER+) cell line, with cytotoxicity comparable to that of PAC against the prostate cell line. The observed losses in cytotoxicity and selectivity can be explained by the inability of the estradiol-PAC conjugate to release PAC, and by the loss of binding affinity of estradiol to ERs upon modification and conjugation to PAC.



Scheme 16 Synthesis of C-2' and C-7 estradiol-PAC conjugates [166]. (a) **79**, EDC/DMAP, toluene, 60 °C, 24 h, 73 %; (b) HF-pyridine, THF, rt, overnight, 97 %; (c) **80**, EDC/DMAP, toluene, 60 °C, 24 h, 78 %; (d) HF-pyridine, THF, rt, overnight, 92 %; (e) TBSCl, imidazole, DMF, 65 °C, 3 h, 95 %; (f) **79**, EDC/DMAP, toluene, 60 °C, 48 h, 65 %; (g) HF-pyridine, THF, rt, overnight, 82 %; (h) **80**, EDC/DMAP, toluene, 60 °C, 48 h, 65 %; (i) HF-pyridine, THF, rt, overnight, 91 %.

In the same context of targeting overexpressed receptors associated with growth and regulation, Safavy et al. [169] designed and synthesized a ternary conjugate consisting of PAC as the cytotoxic drug, poly(ethylene glycol) (PEG) as a solubilizer, and a bombesin (BBN)/gastrin-releasing peptide (GRP) receptor-recognizing heptapeptide (QWAVGHL-NH_2) (BBN[*7-13*]). Shown below as **87**, BBN[*7-13*] is known to have a high binding affinity to GRP that is believed to play a role in tumor growth regulation [170]. Biochemical assays for evaluating **87** were designed to address three main issues: water solubility, binding affinity to BBN/GRP, and drug release kinetics. Conjugate **87** was readily soluble in water. The overall cell binding efficiency of conjugate **87** to the GRP was preserved and was not affected significantly by the presence of either the PEG spacer or PAC. Drug release studies demonstrated that PAC was readily released from the conjugate in PBS buffer at physiological pH with $t_{1/2}$ of 154 min, and in human plasma with $t_{1/2}$ of 113 min. Prodrug **87** demonstrated high potency against a human non small-cell lung cancer cell line. Based on this data, the authors concluded that the improved cytotoxicity is a direct result of specific, receptor-mediated delivery of the drug to tumor cells.



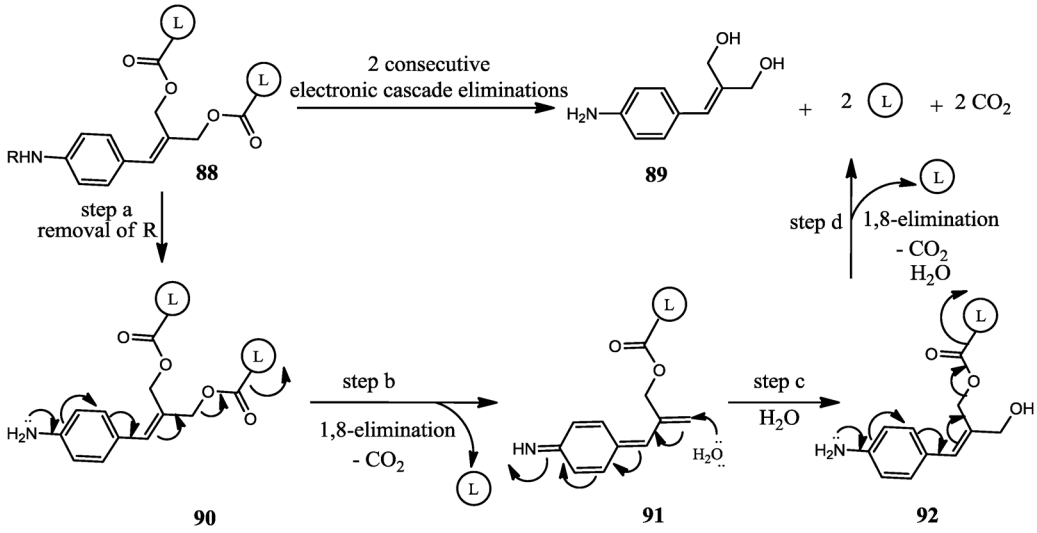
86 87

MACROMOLECULAR TARGETED TAXOID PRODRUGS

The ability of certain macromolecule constructs to accumulate in tumor tissues much more than they do in normal tissues has earned them the reputation of being potentially beneficial drug carriers. Macromolecules are able to penetrate and accumulate in cancer cells due to what has been referred to as an "enhanced permeability and retention" (EPR) effect [171,172]. A general explanation of this phenomenon is that the rapidly dividing cancer cells stimulate the formation of new blood vessels to insure a sufficient supply of oxygen and nutrients, a process known as angiogenesis. The newly formed blood vessels are often abnormal in form and architecture leading to a leaky vasculature with enhanced permeability. In addition, the lack of effective lymphatic drainage likely prevents macromolecules from being removed [173]. These factors lead to abnormal molecular transport dynamics of drug macromolecules, namely, the EPR effect. Excellent studies have been reported utilizing the concept of the EPR effect to selectively deliver PAC to cancer cells using macromolecules as carriers. Among other approaches, these studies involve synthesizing polymer-PAC conjugates such as poly(ethylene glycol)-PAC [174–176] and poly(L-glutamic acid)-PAC [177–179], protein-PAC conjugates such as transferrin-PAC [180] and human serum albumin-PAC [181,182], as well as the dendrimer systems which previously were mentioned and which will be further highlighted below.

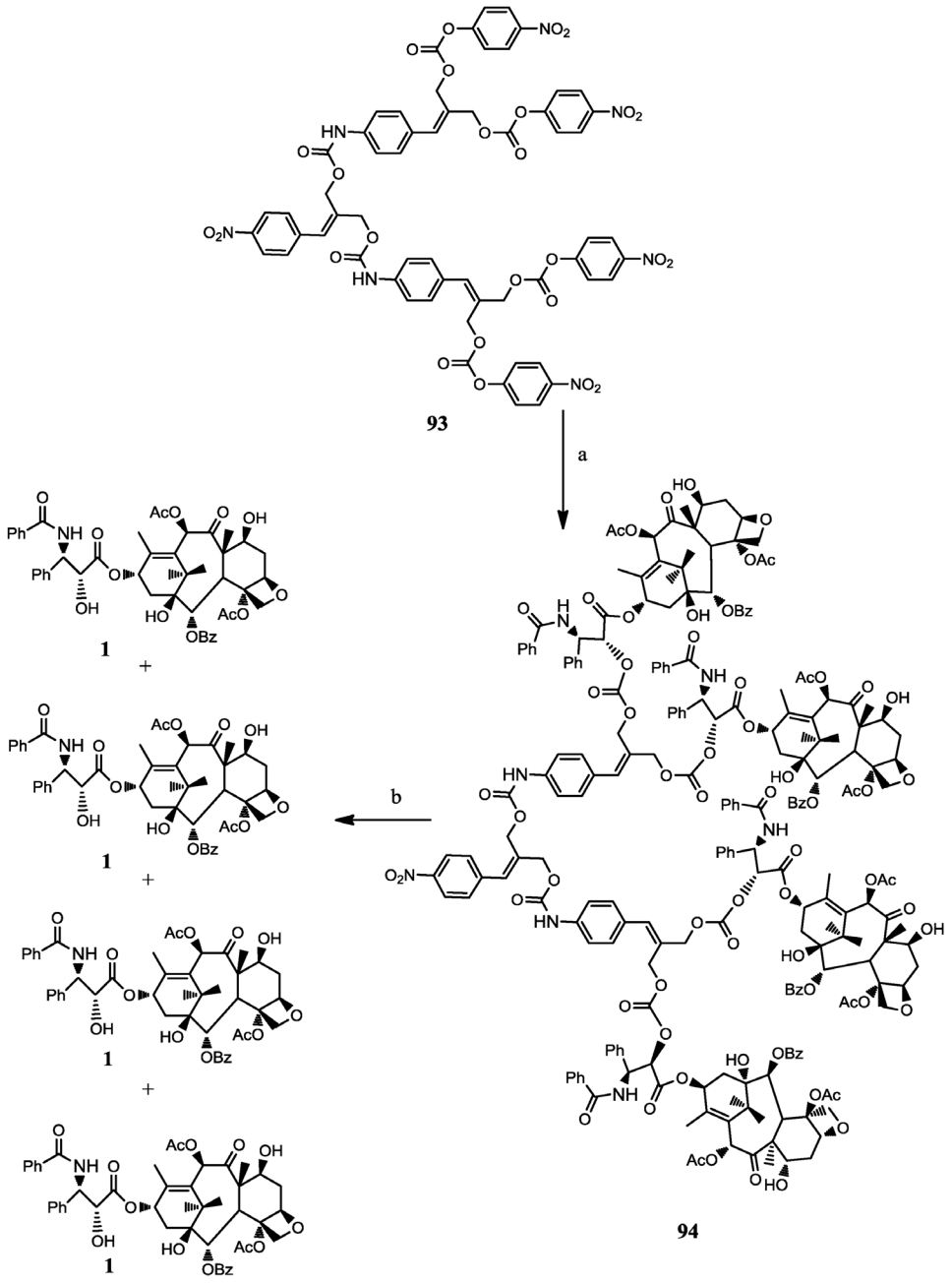
The "cascade-release dendrimer" approach taken by de Groot et al. will be discussed as a representative example of a PAC-carrying macromolecule [183]. Dendrimers are well-defined, branched treelike molecules with multiple end functional groups and physiological properties similar to those of biomolecules [184,185]. Unlike most reported dendrimers where each single drug has to be independently cleaved and released, the dendrimers designed by de Groot el al. collapse into separate monomeric species, including the free active drug, after a single chemical or biological activation step that triggers the "explosion" [183] of the cascade-release dendrimer, thereby releasing multiple drug molecules. Scheme 17 depicts the proposed mechanism for the release of two drug molecules upon de-masking the stable multiple-release system shown as **88**. A divergent route was used to synthesize the cascade-release dendrimer **94** bearing four units of PAC (Scheme 18). This construct was subjected to reduction of the nitro group using Zn/AcOH to validate the utility of the dendritic cascade-release concept. Chromatographic analysis then demonstrated rapid and complete disappearance of **94** and the subsequent formation of PAC. The cytotoxicity and tumor targeting ability of **94** are still being investigated. With this basic research being paved by taxane-related ABDD efforts, these cascade-release dendrimers may become generally useful models for targeted drug delivery, controlled release, and novel diagnostic modalities of the future.

Summarizing this section, it should be emphasized that going all the way back to Ehrlich's "magic bullets" [186], there remains a long-standing goal among medicinal chemists to enhance the selectivity of chemotherapeutic agents across the board. In this regard, the intense ABDD effort directed



Scheme 17 Proposed mechanism for the release of two leaving groups upon activation of **88** [176]. See text for discussion.

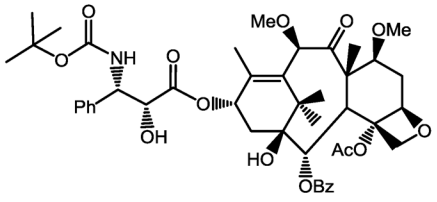
toward PAC and its analogues is also providing basic research findings that may have general applicability to other cytotoxic compounds and even to other therapeutic classes. For example, the strategy of combining a structural component that serves as the *address* for specific biological surfaces, coupled to a *message* or *cargo* component, can be used quite generally in both regards. The address can be designed to bind with selected proteins overexpressed by sick (e.g., inflamed or cancerous) cells, drug transporter systems, or to a vast array of other cell- and tissue-specific proteins. Likewise, in addition to cytotoxic structures intending to be chemotherapeutic agents, cargos can be selected to deliver a wide range of messages such as the use of fluorophores intending to be biomarkers and diagnostic agents, or pharmacophores whose efficacy intends to restore homeostasis to a given pathophysiology. If tolerated at the efficacious site, the address component may be left attached to the cargo component. However, this is generally not the case. Thus, it often becomes necessary to further devise a prodrug arrangement wherein the cargo can be released from the address. This separation is most advantageous when it can be made to occur within the immediate vicinity of the desired efficacy. The targeting strategies exemplified by the enormous amount of work around the taxanes offer a variety of approaches and molecular assemblies that can be directed toward accomplishing these distinct types of objectives. Clearly, some of the most recent trends suggest that the multicomponent design strategies will continue to grow in both their biological and chemical sophistication. As each of the various pieces becomes better understood, the medicinal chemist practicing in this arena will be better able to tailor the structural features needed to optimize a given new chemical entity (NCE), as well as to modify or fine-tune a pioneer drug during the course of an ABDD campaign. Significant progress already can be claimed relative to the work with the taxanes where several candidates are nearing clinical trials and others are already undergoing such validation. Thus, while it still remains to be seen if these encouraging results can be translated just as effectively to the marketplace, the promise afforded by these *smart taxoids* may soon be forthcoming and, furthermore, any definitive successes at this level are likely to have much broader implications.



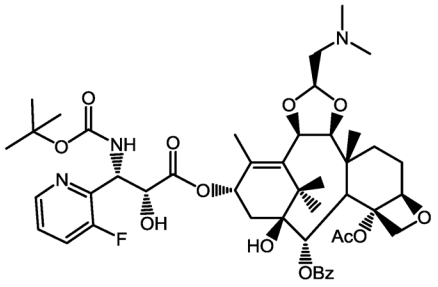
Scheme 18 Synthesis and release properties of a second-generation cascade-release dendrimer **94** [183]. As depicted, this construct releases four PAC molecules upon reduction. (a) PAC, DMAP; (b) Zn/AcOH.

RECENT AND NEAR ADDITIONS TO THE MARKETPLACE

"In June 2010, the U.S. FDA approved cabazitaxel (CAB) in combination with the steroid prednisone for the treatment of metastatic Castration-Resistant Prostate Cancer (mCRPC) for patients who were previously treated with a DOC-containing regimen for late-stage disease" [187]. Clearly resulting from ABDD while also benefiting from the composite of gradually accumulating information elaborated within the several preceding sections, the chemical structure of this latest taxane-like agent to hit the market as Jevtana® is shown immediately below as **95**.



95 Cabazitaxel (CAB)



96 Tesetaxel (TES)

Unlike PAC and DOC, "cabazitaxel has poor affinity for Pgp" [187]. Its mechanism of action is identical to that of PAC and DOC, and its potency is comparable to that of DOC in nonresistant cell lines. However, **95** continues to display antitumor activity in drug-resistant preclinical models that overexpress Pgp and, as a result, it is expected to perform better in clinical situations where MDR can otherwise become problematic during standard therapy [188,189]. Like PAC and DOC, **95** is administered by the intravenous route. It exhibits a linear PK profile with a rather long terminal $t_{1/2}$, namely, about 77 h [190]. **95** is extensively metabolized by CYP enzymes within the liver. It is manufactured by semi-synthesis starting from 10-deacetylbaccatin III (10-DAB in former Scheme 1) [191]. A more detailed review of **95** can be found in Volume III of the ABDD book series [192].

Another taxane derivative that merits mention in passing at this time is tesetaxel. Although still in late-stage clinical testing, it continues to perform well. If and when it is eventually marketed, it will become the first orally administered taxane analogue. Like **95**, "tesetaxel has demonstrated high activity against cell lines that are resistant to PAC and DOC" [193]. Its chemical structure is shown above as **96** wherein its clear link to ABDD can again be readily noted. Also similar to **95**, manufacture of **96** takes advantage of a semi-synthesis route [194].

PURSUIT OF PHARMACOLOGICAL ANALOGUES

ABDD campaigns aimed at discovering completely different structures that have the same mechanism of action as a marketed drug generally involve a random structural survey that deploys a high-throughput screen (HTS) to serve as an effective assay for that mechanism [2]. This approach proceeds in a truly unbiased manner with regard to structural considerations and thus precludes any hint of overlap with structure-driven ABDD. Alternatively, a detailed knowledge about the receptor or enzyme active site associated with the desired mechanism can be used as a blueprint for the ab initio design of another series of distinctly unique structures that can also reside in such a pocket while associating with a different array of contact amino acids. Likewise, it is also possible to engage an allosteric site or some other dynamic aspect inherent to the desired mechanism at the molecular level that is different from the parent drug's interaction, providing that the same consequence then occurs within the system so as to ultimately bestow the same pharmacological endpoint. Note, however, that even in these less frequent

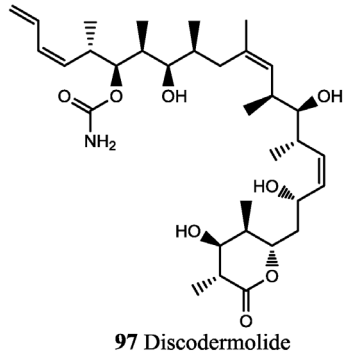
strategies, the pharmacological analogue would still be working within the context of the immediate system, and it would not be working at some separate step that is upstream or downstream along the same signaling pathway. By definition, either of the latter would lead to a new class of drugs rather than to an analogue even if the net pharmacological endpoint was still essentially the same [2].

Turning to the taxanes, we shall see that all of the pharmacological analogues to date have been discovered by the more common, random screening pathway [2]. Whenever an analogue is discovered in this manner, it then becomes a challenge for medicinal chemists to discern how the pharmacological analogue may be overlapping at the molecular level with the original agent that was responsible for prompting the screening campaign. Thus, after reviewing the several pharmacological analogues of PAC discovered to date, this section will discuss the ongoing attempts being made to discern their common structural features. Again, it should be noted that in order to capture the continuing enthusiasm expressed at this juncture by researchers working in the field of microtubule-stabilizing agents, portions of our discussions for some of these examples parallel the dialogue from the originating investigators or prior reviewers, all of such then being referenced herein.

The chemotherapeutic success of PAC coupled with its unique mechanism of action prompted researchers around the world to discover new cytotoxic natural products that can promote microtubule assembly and stabilization leading to apoptosis. Recent reviews reflect the high interest in both microtubule inhibitors and stabilizers [195–198], although we will focus herein upon only stabilizers [199]. In 1993, Reichenbach and Höfle isolated a new class of cytotoxic compounds as secondary metabolites from the fermentations of myxobacterium *Sorangium cellubosum* [200]. Based on their molecular structure, they named these molecules "epothilones". In 1995, a group at Merck Research Laboratories confirmed that the newly discovered class of molecules possesses a PAC-like mechanism of action [201]. Thereafter, the rush was on and several other natural compounds were subsequently found to stabilize microtubules such as discodermolide, eleutherobin, laulimalide, and peloruside A. Each of these agents is discussed separately within the subsections below, with the EPOs being reserved until the end so that they can be used as a closer lead-in for the article's next major section, which will provide a comparative summary for all of the microtubule-stabilizing agents marketed to date.

Discodermolide

Discodermolide, **97**, is a polyhydroxylated alkatetraene lactone marine natural product first isolated from the Caribbean sponge *Discodermia dissoluta* by Gunasekena et al. in 1990 [202]. Although **97** also demonstrated immunosuppressive activity in initial biology testing [203–205], the potent antimitotic activity was of special interest with a reported IC_{50} value in the low nM range against certain cancer cell lines [206].



97 Discodermolide

Eventually, **97** was found to be more effective than PAC in inducing microtubule assembly [207]. It caused major rearrangement of cellular microtubules in breast carcinoma cells where filamentous and bundled microtubules were observed to be retracted and concentrated around the nucleus. Likewise, a tubulin assembly assay indicated that in the presence of GTP and microtubule-associated proteins (MAPs), **97** could induce rapid and nearly complete polymerization at 0 °C. Under similar conditions, PAC induced significant assembly only when the temperature was raised to 10 °C. The **97**-induced biopolymer was completely stable at 0 °C in the presence of high concentrations of Ca^{2+} ions, in contrast to PAC-induced biopolymer that dissociated under similar conditions. In addition, **57** demonstrated potent activity against an MDR carcinoma cell line presumably because it is a poor substrate for the Pgp efflux pump. **97** is ca. 160-fold more water-soluble than PAC, making it more easily formulated. A Phase I clinical trial of **97** was started in 2002, but was suspended when three patients receiving 14.4–19.2 mg/m^2 q3w suffered interstitial pneumonitis after the fourth cycle of therapy [196]. Due to the low yield of **97** obtained from its natural source (7 mg from 434 g of frozen sponge), total synthesis constitutes the most reliable source of the natural product [208]. For this purpose, several total syntheses have been reported describing the synthesis of the (+)-natural and (–)-unnatural enantiomers. In addition, several analogues have been synthesized to establish SAR. The status of the latter is depicted in Fig. 15 [209–211].

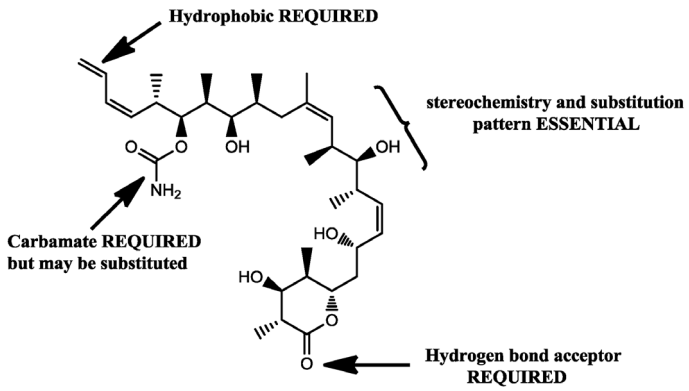
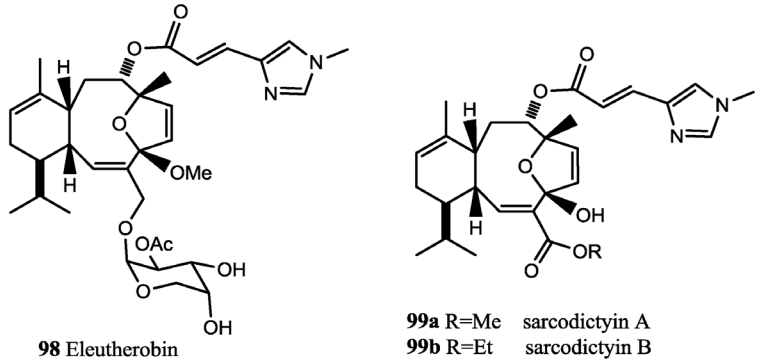


Fig. 15 Discodermolide, **97**, SAR (adapted from ref. [196]).

Eleutherobin/sarcodictyins

Eleutherobin, **98**, is a marine cytotoxin isolated from the soft coral *Eleutherobia* sp. collected from the waters off Western Australia [212]. The structurally and biologically related sarcodictyins, **99**, were discovered in the Mediterranean stoloiferan coral *Sarcodictyon roseum* [213] and are 100-fold less cytotoxic than **98**. **98** was found to be slightly less potent than PAC. It was able to induce tubulin polymerization, and microtubules formed at 37 °C were resistant to depolymerization by cold temperature and Ca^{2+} ions [214]. **98** competitively displaced [^{3}H]PAC, indicating that these compounds share a common binding site. However, like PAC, **98** showed dramatically reduced cytotoxicity against a drug-resistant colon cell line, suggesting that **98** is also a good substrate for Pgp.



Nicolaou et al. [215] designed a combinatorial sarcodictyins library to develop adequate SARs for **99a,b** and the related compounds including **98**. The corresponding SARs are summarized in Fig. 16 [215]. In brief, the urocanic acid side-chain appeared to be crucial for activity, and substitutions on the acetal and ester functionalities were well tolerated. Despite the nature of its antimitotic activity, it is doubtful that this class of taxol-like compounds will yield an efficient lead compound due to the reduced cytotoxicity against resistant tumor cell lines.

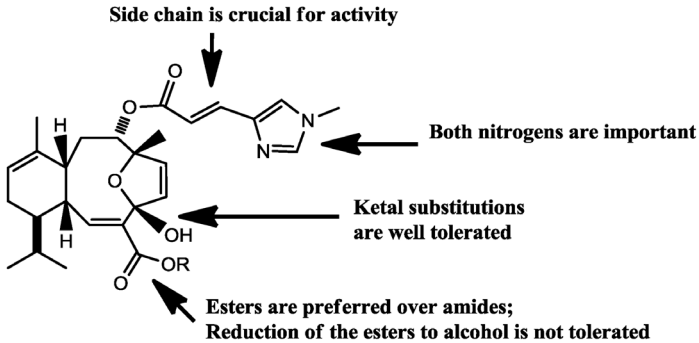
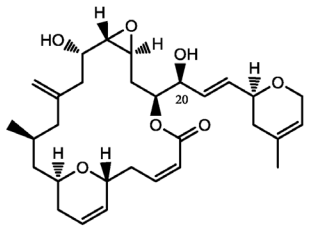


Fig. 16 Eleutherobin (**98**) and sarcodictyins (**99**) SAR (adapted from ref. [215]).

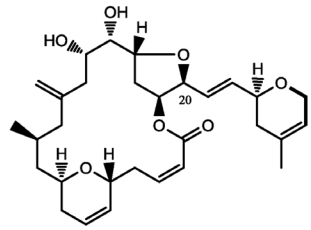
Laulimalide and isolaulimalide

The 18-membered marcocylic lactones laulimalide **100** and isolaulimalide **101** were isolated from the marine sponges *Hyattela* sp. (Indonesia) [216], *Spongia mycofigiensis* (Vanuatu) [217], *Fasciospongia rimosa* (Okinawa) [218], and *Cacospongia mycofigiensis* (Marshall Islands) [219]. Although first isolated in 1988, the laulimalides were not classified as PAC-like stabilizers of microtubules until 1999. Isolaulimalide **101** is the rearrangement product of laulimalide **100** formed through an acid-catalyzed attack of the C-20 hydroxyl group on the epoxide ring.

100 is a potent cellular proliferation inhibitor with IC_{50} values in the low nanomolar range, while its congener **101** is less active. In a tubulin assembly assay, **100** resulted in a dose-dependent reorganization of microtubules along with microtubule bundles and micronuclei formation. Both compounds inhibited the proliferation of a PAC-resistant cancer cell line overexpressing Pgp, suggesting that these compounds are poor substrates for transport by Pgp [219]. Interestingly, **100** was unable to inhibit the binding of [^{3}H]PAC to the tubulin polymer [220], indicating that it is apparently binding to a site on microtubules that is distinct from that of PAC. The in vivo evaluation of the macrocyclic lactone **100** has not been described, probably due to the lack of enough material for such experiments. The total syn-



100 Laulimalide

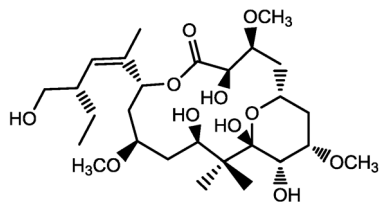


101 Isolaulimalide

thesis of **100** has been reported by three different groups. Such efforts will help in providing the compound quantities needed for further in vivo investigation [221–223].

Peloruside A

Peloruside A, **102**, is another natural product 16-membered macrolide with PAC-like microtubule activity. It is a secondary metabolite isolated from the New Zealand marine sponge, *Mycale hentscheli* [224]. **102** was cytotoxic at nanomolar concentrations against human myeloid leukemic cells. **102** caused tubulin to polymerize into microtubules that were stable to cold temperatures and to the presence of Ca^{2+} ions. Treatment of a human lung adenocarcinoma epithelial cell culture with **102** led to multiple micronuclei, microasters, microtubule bundle formation, and cell cycle arrest at the G2/M phase, a behavior typically observed in PAC-exposed cells. In addition, **102** was found to be less lipophilic than PAC. This property may allow for better formulation of **102** and its analogues, thus minimizing the side-effects associated with PAC's formulation [225].



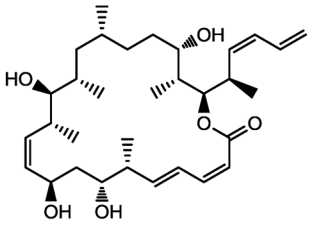
102 Peloruside A

OTHER MICROTUBULE-STABILIZING NATURAL PRODUCTS

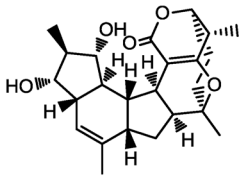
Dictyostatin 1 (**103**) is a 22-membered macrocyclic lactone first isolated in trace amounts from *Spongia* sp. collected in the Republic of Maldives in 1986 [226]. **103** stabilized microtubules and exhibited nanomolar potency against MDR cancer cell lines. These features render the compound an interesting lead for further biological investigation, a process currently limited by the short supply of the material.

WS9885B (**104**) [227] is another potential chemotherapeutic agent for the treatment of cancer with a demonstrated microtubule-stabilizing activity similar to that of PAC. WS9885B was isolated from the fermentation broth of *Streptomyces* sp. No. 9885. In in vitro assays, **104** showed anticancer activity as potent as PAC, exhibited no antimicrobial activities, promoted microtubule assembly, and arrested the cell cycle at the G2/M phase. These properties have drawn the attention of synthetic chemists to synthesize the hexacyclic compound [228].

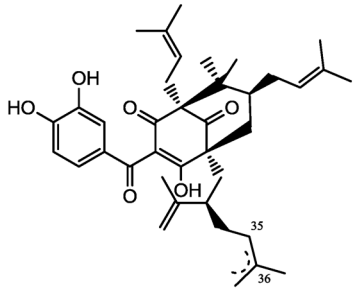
The polyisoprenylated benzophenones xanthochymol (Δ^{36}) (**105**) and guttiferone E (Δ^{35}) (**106**) were isolated from the fruits of *Garcinia pyrifera* collected in Malaysia [229]. Despite showing microtubule disassembly inhibitory activity, these compounds were not able to promote microtubule assem-



103 Dictyostatin 1



104 WS9885B

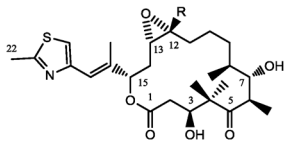


105 Xanthochymol (Δ^{36})
106 Guttiferone E (Δ^{35})

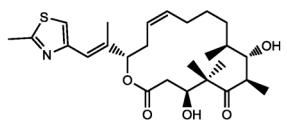
bly at 0 °C or in the absence of GTP. Somewhat higher IC_{50} values were reported for xanthochymol and guttiferone E compared to PAC in a microtubule disassembly inhibition assay.

Epothilones

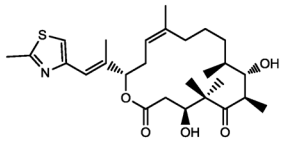
Of all the new PAC-like microtubule stabilizers, the EPO family has excited the most interest and is, by far, at the most advanced clinical testing stage [2] (also see discussion in later section pertaining to marketed agents). EPOs A (**107**) and B (**108**) were isolated in 1993 by Reichenbach and Höfle as the major products from the fermentations of myxobacterium *Sorangium cellulosum* Sc90. They were initially described as antifungal agents. A group at Merck Research Laboratories later demonstrated their PAC-like mechanism of action. These 16-membered macrolides mimicked all the biological effects of PAC both biochemically and in cell culture. A variety of other EPO-related structures, such as EPOs C **109**, D **110**, E **111**, and F **112** were also isolated from the fermentations as minor components [230–234]. In general, these compounds are reported to be 30–50 times more water-soluble than PAC [235]. **107** and **108** promoted microtubule polymerization, hyperstabilized polymerized microtubules, and induced microtubule bundling. Competitive studies revealed that the macrolides acted as competitive inhibitors for [^{3}H]PAC binding to microtubules. This is consistent with the interpretation that **107** and **108** compete for the same binding site as PAC. These pharmacological analogues were roughly one order of magnitude more potent in cell culture models than was PAC, with IC_{50} values in the sub- and low nanomolar range. The cytotoxicities of EPOs A, B, and D, and PAC in human cancer cell lines are summarized in Table 3. In key contrast to PAC, **107** and **108** were active on MDR cell lines and on PAC-resistant cell lines [236,237].



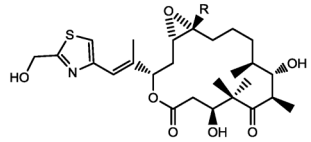
107 R= H Epothilone A
108 R= CH_3 Epothilone B



109 Epothilone C



110 Epothilone D



111 R= H Epothilone E
112 R= CH_3 Epothilone F

Table 3 Cytotoxicity (IC_{50}) in nM of EPOs in comparison to PAC in human tumor cell lines (adapted from ref. [231]).

Cell line	EPO A	EPO B	EPO D	PAC
HCT116 (colon)	2.51	0.32	NA	2.79
SW620 (colon)	NA	0.1	NA	0.2
SW620AD (PAC-resistant colon carcinoma subline)	NA	0.3	NA	250
PC-3M (prostate)	4.27	0.52	NA	4.77
A549 (lung)	2.67	0.23	NA	3.19
MCF-7 (breast)	1.49	0.18	2.90	1.80
MCF-7/ADR (breast)	27.50	2.92	NA	9105
KB-31 (epidermoid)	2.10	0.19	2.70	2.31
CCRF-CEM (leukemia)	NA	0.35	9.5	NA

NA: Data not available.

The promising anticancer activity of the EPOs prompted medicinal chemists to design and synthesize hundreds of EPO analogues with improved cytotoxic and pharmacologic properties. Such syntheses led to the SAR summarized in Fig. 17. SAR studies showed that the C-1-C-8 region is highly sensitive to structural modifications where even modest changes led to diminished activity. In contrast, the C-9-C-17 portion tolerated modifications and offered greater degrees of flexibility. The C-13 and C-15 stereochemistry must be 'S', while that of C-12 can be 'R' or 'S'. Binding affinity was enhanced when the epoxide ring was replaced by a cyclopropane ring or by a double bond, and when the C-21 methyl was replaced with a thiomethyl moiety. Based on extensive studies of the SAR of EPOs, a remarkable seven analogues, both natural and synthetic, have made their way to human clinical trials. Some of these analogues are still in various stages of clinical trials, while others have advanced to Phase III and one has made it all the way to the marketplace (see discussion in later section). Figure 18 depicts some of the structures undergoing clinical study [228].

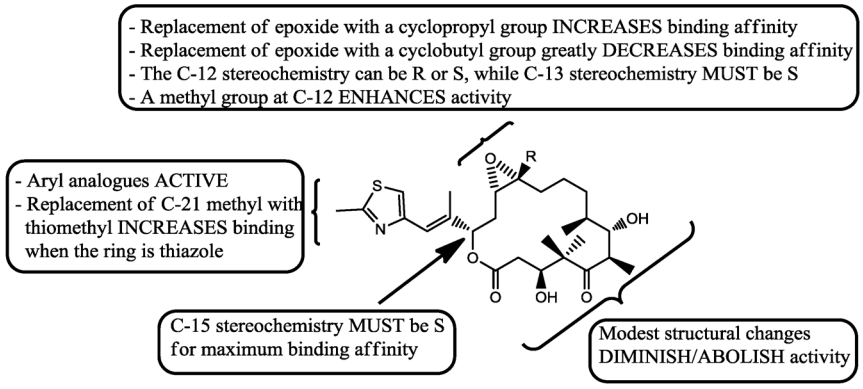


Fig. 17 EPOs SAR (adapted from refs. [196,239]).

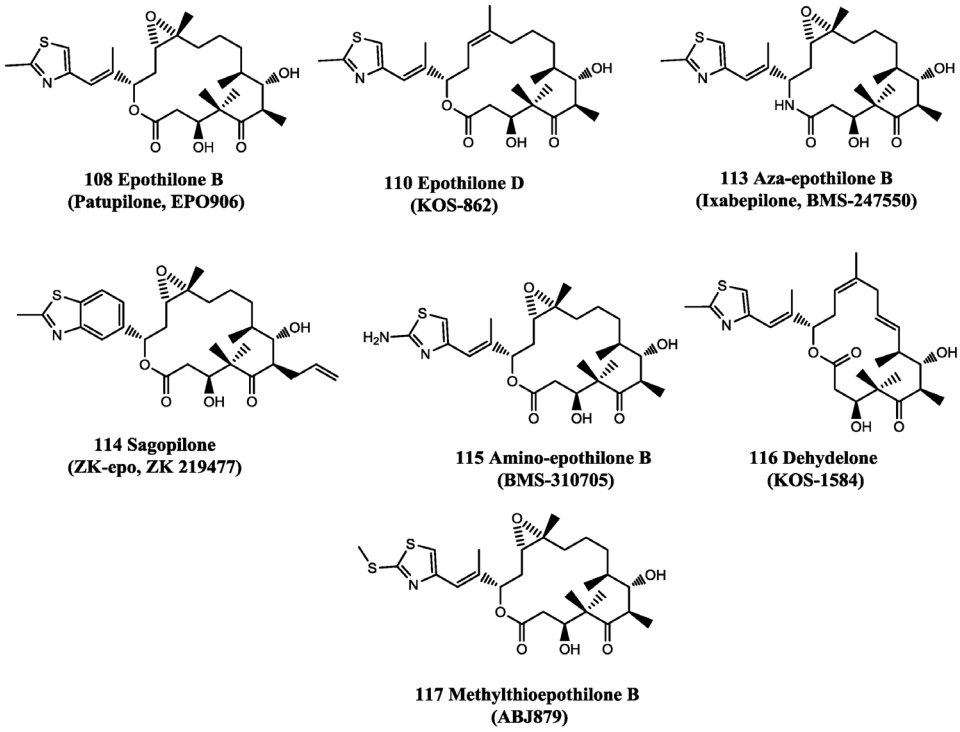


Fig. 18 EPO analogues that were entered into clinical trials (adapted from ref. [238]). Note that aza-EPO B **113** has already progressed to a market launch (also see further discussion in later section on marketed agents).

IN PURSUIT OF A COMMON PHARMACOPHORE FOR PAC AND THE EPOTHILONES

As mentioned above, the EPOs are competitive inhibitors of PAC in terms of binding to microtubules. Several investigators have tried to identify structural overlap with PAC by developing a common pharmacophore, but different conclusions were drawn [240]. One of the first attempts to find a common pharmacophore was reported by Winkler and Axelsen in 1996 when no structural information about the drug binding site was yet available [241]. In their attempt to overlap both structures, the authors relied on the SAR available for PAC and the EPOs, and used molecular mechanics software to find regions of steric and functional similarities in the conformational space of the two molecules. They developed a 3D model (Fig. 19) that superimposed 13 of the 15 ring carbon atoms of EPO A and most of the side-chain atoms onto corresponding atoms in PAC. The C-1-C-3 and C-8-C-12 fragments, and the thiazole ring in EPO A were superimposed with the C-1'-C-3', C-2 benzoyl, and C-10 acetate of PAC, respectively. The authors warned that other plausible conformations of both molecules could be superimposed, and that the steric complementarity between PAC and EPO was not ideal. In addition, they failed to address several crucial SARs associated with the activity of both PAC and the EPOs.

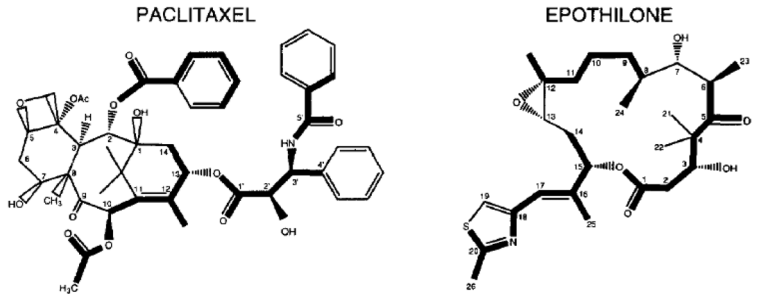


Fig. 19 Schematic 2D representation of the model proposed by Winkler and Axelsen to define the structural similarity between PAC and the EPOs (reproduced from ref. [241] with permission from the American Chemical Society).

Ojima et al. proposed a pharmacophore common for PAC, nonataxel, the EPOs, eleutherobin, and discodermolide [242]. Nonataxel (**118** in Fig. 20) is a PAC analogue that exhibits 2- to 8-fold higher activity against various cancer cell lines. The authors expected that the nonaromatic groups in nonataxel would allow for better mapping to the rings of EPOs. Comparing the 3D structure of nonataxel derived from limited 2D NMR studies and RMD, with that of the template-fitted EPO B, revealed excellent topological homology between the two structures. The C-1-C-6 portion of EPO B corresponded to the southern hydrophobic surface of nonataxel, while the thiazole side-chain overlapped with the t-BOC group at the C-3'-N of nonataxel. These relationships are depicted in Fig. 20.

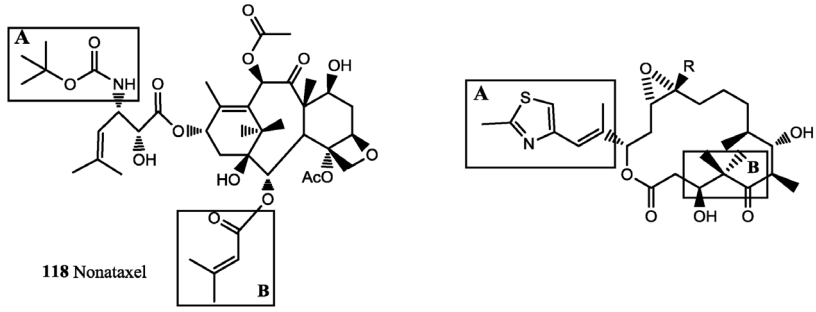
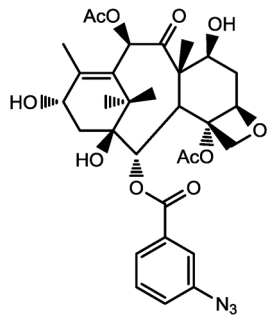


Fig. 20 Labeled boxed regions represent areas of common overlap between nonataxel and the EPOs (adapted from ref. [242]).

The authors claimed that the proposed model accounts for a vast SAR data associated with the structural modifications of EPO B such as the diminished/abolished activity observed upon epimerization at the C-3 position, reduction of the C-5 carbonyl group, or deletion of any moiety in the C-3-C-8 region. Moreover, the SAR studies indicated that the C-12-C-13 epoxide is not crucial for EPO binding to microtubules based on the fact that analogues such as 12,13-desoxyepothilone B (*E* and *Z* isomers) retained excellent tubulin binding activity. In the model proposed by Ojima et al., the epoxide oxygen pointed away from the overlapping structural terrain and is thus not essential for binding.

He et al. proposed an alternative common pharmacophore for PAC and the EPOs based on SAR indicating that 2-*m*-azidotaxol had greater activity than PAC, and that even 2-*m*-azidobaccatin III (**119**) surprisingly exhibited PAC-like activity, while Baccatin III and 2-*p*-azidobaccatin III did not [243]. These observations highlight the specificity and significance of the *m*-azido substitution in enhancing

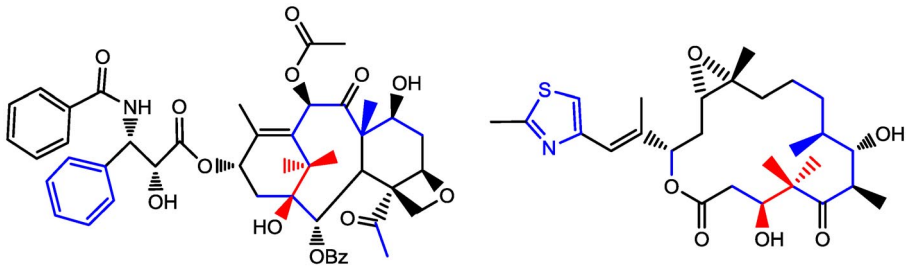


119 2-*m*-Azidobaccatin III

biological activity. 2-*m*-Azidobaccatin III was cytotoxic to different cancer cell lines although the cytotoxicity was 25- to 45-fold less than that of PAC.

119 induced all the morphological changes in microtubules typical for PAC. It promoted tubulin polymerization in the absence of GTP, stabilized the polymerized microtubules against depolymerization by cold treatments, induced microtubule bundle formation in cultured cells, and caused cell cycle arrest at mitosis. Neither baccatin III nor 2-*p*-azidobaccatin III demonstrated such activity. More important, **119** competitively inhibited the binding of [^{3}H]PAC to microtubules. This observation demonstrated that **119** and PAC bind to the same or overlapping sites on the microtubules, although the binding affinity of **119** was reduced compared to that of PAC. The authors conducted molecular modeling studies to rationalize the significance of the *m*-azido substitution. In the proposed model, the C-2 benzoyl ring was positioned into a pocket formed by His-227 and Asp-224. Placing the azido group at the *meta*-position kept it in close proximity to the carboxylate of Asp-224, thus forming a new salt bridge as a consequence of the electrostatic interactions, and resulting in enhanced binding of the molecule to the β-tubulin. Although the C-13 side-chain is normally thought to be requisite for the binding of PAC, the presence of the *m*-azido group on the C-2 benzoyl ring of baccatin III apparently can compensate for the loss of the C-13 side-chain and reestablish the binding affinity of the molecule. In contrast, a repositioning of the taxane ring would be required to fit the *p*-azido into the binding pocket formed by His-227 and Asp-224, thus inhibiting the interaction of PAC with the microtubules. Based on the observation that the C-13 side-chain is not an absolute requirement for biological activity, the authors proposed a common pharmacophore for PAC and the EPOs in which the thiazole chain of the EPOs corresponded to the C-2 side-chain of PAC and the macrocyclic ring of the EPOs overlaid with the taxane core. The proposed model is distinctly different from the one proposed by Ojima et al. where the thiazole side-chain of the EPOs was superimposed with the C-13 side-chain of PAC.

Finally, Giannakakou et al. have proposed a common pharmacophore for PAC and the EPOs based on the data collected from molecular modeling, mutations, and cytotoxicity assays [244]. To identify tubulin residues important for EPO binding, two EPO-resistant human ovarian carcinoma cell lines were isolated, each with a different point mutation: 1A9/A8 ($\beta274^{Thr\rightarrow Ile}$) and 1A9/B10 ($\beta282^{Arg\rightarrow Gln}$). Of two originally proposed pharmacophores, the one in which the methylthiazole side-chain of EPO B **108** superimposed with the C-3'-phenyl ring of PAC was more clearly discernable. This result is highlighted in Fig. 21. Mutating Thr-β-274 to Ile in clone 1A/A8 had a greater impact on the binding of EPO than PAC. The hydrogen bond that existed between the C-7 OH of EPO B and the Thr-β-274 was disrupted, while the hydrogen-bond donors or acceptors at the C-7, C-10, and C-19 positions of PAC can form alternate hydrogen bonds to compensate to the disrupted hydrogen bond. This rationale is in agreement with the cross-resistance data and the in vivo polymerization studies. In addition, the $\beta282^{Arg\rightarrow Gln}$ mutation, sitting on the M-loop, directly affected the binding of taxanes and EPOs, and disrupted the lateral contacts between protofilaments. The data collected from drug sensitivity assays against a resistant PAC-selected cell line containing a $\beta270^{Phe\rightarrow Val}$ mutation provided evidence



1 **108**

Fig. 21 Common overlap of PAC and EPO B as proposed by Giannakakou el al. (adapted from ref. [244]).

for the preference of the proposed model. In this model, the thiazole side-chain was placed in close proximity to Phe-270. As expected, the pyridine-containing EPO experienced a 10-fold change in sensitivity compared to a 3-fold change observed with thiazole-containing EPO B. The proposed pharmacophore accounted for a significant amount of SAR developed for EPOs and taxanes. The oxygen atoms of the oxetane ring of PAC and the epoxide of EPOs overlapped, and are located near a cluster of polar tubulin residues (273, 275, and 276) with the Thr-274 hydroxyl group in this hydrophilic area. It is known that EPO B, **108**, is 14-fold more potent that EPO A, **107**, with the only structural difference between the two molecules being the presence of a methyl group at C-12 of EPO B. The proposed model positioned this methyl group in the vicinity of the hydrophobic side-chains of Leu-273, Leu-215, Leu-228, and Phe-270, thus stabilizing favorable hydrophobic interactions and accounting for the enhanced potency. Such hydrophobic interactions also accounted for the fact that replacing the EPOs' epoxide ring by a double bond enhanced in vitro tubulin polymerization because the electron-rich π cloud of the double bond also can act as a hydrogen-bond acceptor for a nearby water molecule.

Capping this effort to equate PAC and the EPOs, however, the most recent report by Snyder, Downing et al. [245] ultimately concludes "that the longstanding expectation of a common pharmacophore is not met, because each ligand exploits the tubulin-binding pocket in a unique and independent manner" [245]. These investigators combined NMR, electron crystallography, and molecular modeling across a series of analogues. In the resulting model, the EPOs occupy the same binding site as PAC but this pocket is quite expansive and displays promiscuous binding with various ligands by exploiting contacts with different residues. For example, of the five oxygen-containing polar groups present on the EPO macrocycle, only the C7-OH falls near the similar C7-OH of PAC, "making this center the only notable common nonbonded contact for the two molecules" [245]. These authors further suggest that the observed promiscuity afforded by this pocket will likely apply to the binding of other ligands that occupy the taxane site on microtubules such as the discodermolide, eleutherobin, and sarcodictyin natural products.

SYNTHETIC MICROTUBULE STABILIZING AGENTS

A few synthetic compounds have been reported to promote microtubule polymerization and stabilization. Although less active than PAC or other natural microtubule-stabilizing agents, the synthetic agents provide a scaffold that is highly amenable toward developing more potent and effective analogues due to their structural simplicity and synthetic accessibility. Examples of such synthetic microtubule stabilizers are shown in Fig. 22 and include the 2-ethoxyestradiol analogues **120** and **121** [246], the syntab A **122** system [247], a borneol ester **123** [248], and GS-164 **124** [249].

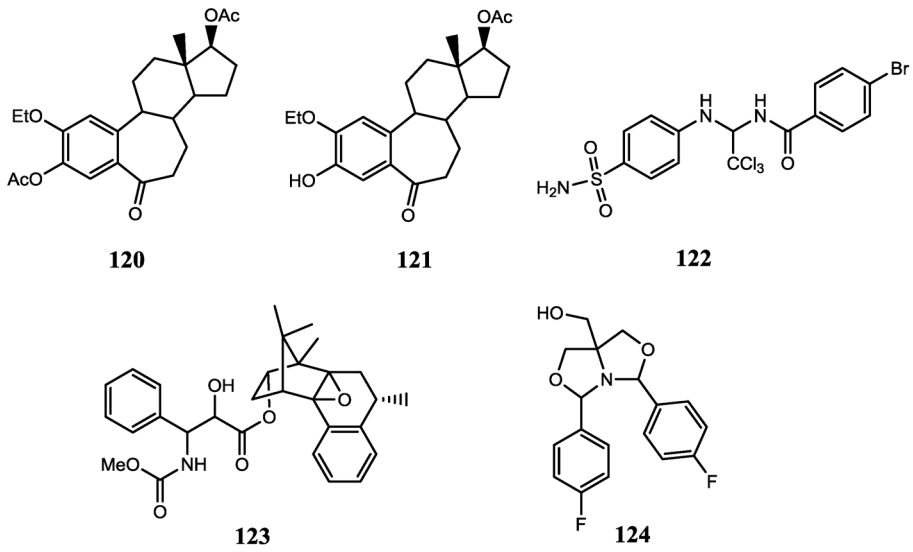
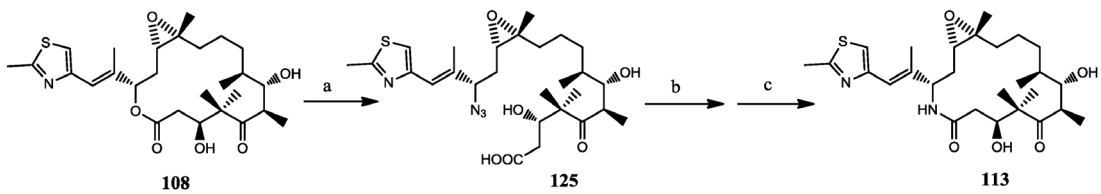


Fig. 22 Synthetic microtubule-stabilizing agents (composite of refs. [246–249]).

ABDD OF IXABEPILONE

As discussed above, EPOs A and B are novel cytotoxic macrolides obtained from bacterial fermentation that represent pharmacological analogues of PAC by virtue of having deployed an assay for the latter's microtubule-stabilizing properties to identify their interesting biological properties. While their mechanism is essentially the same as that of PAC, they are thought to interact with microtubules at somewhat different contact sites, and they exhibit different drug-induced resistance mechanisms including sensitivity to β-tubulin mutations. They exhibit potent in vitro activity including action against MDR cancer cells. However, their in vivo activity was disappointing because of PK-related issues, namely, poor metabolic stability and a short $t_{1/2}$ due in part to the presence of the lactone moiety within the parent scaffold. Ixabepilone, **113**, is an analogue of EPO B, **108**, wherein the lactone has been replaced by a lactam. As shown in Scheme 19, this ABDD-related transformation can be accomplished via a semi-synthesis in three steps [250].



Scheme 19 Synthesis of ixabepilone (**113**) from EPO B (**108**) [250]. (a) $Pd(PPh_3)_4$, 10 mol %, NaN_3, degassed THF-H_2O, 45 °C, 1 h, 65–70 %; (b) PPh_3, THF, 45 °C for 14 h, then 28 % NH_4OH, H_2O, 45 °C for 4 h, or H_2, EtOH, PtO_2, 50 wt %, 10 h, then an additional 25 wt % of PtO_2, 10 h, or PMe_3, THF-H_2O, 25 °C, 2 h, 53–89 %; (c) DPPA, $NaHCO_3$, DMF (2.5 mM), 4 °C, 24 h or EDCl, HOBt, MeCN (0.03 M), 25–65 %.

113 is comparable to **108** in all biochemical and in vitro tests including activity against three different MDR cancer cell lines. Like PAC and DOC (Table 1), it is a parenterally administered drug with a recommended dosage protocol of 40 mg/m^2 administered iv over 3 h every 3 weeks [251]. Despite its considerable improvement in the PK profile, **113** is still subject to extensive metabolism by CYP3A,

which can potentially lead to drug–drug interactions. It is primarily eliminated as various metabolites in the feces (65 %) and urine (21 %) after a terminal elimination $t_{1/2}$ of 52 h [251]. **113** is used to treat metastatic or locally advanced breast cancer. It is specifically indicated for use in combination with capecitabine in patients who have failed treatment with an anthracycline such as doxorubicin and a taxane such as PAC or DOC; or for use as a monotherapy in patients whose tumors are refractory (resistant) to anthracyclines, taxanes, and capecitabine [252]. The most common adverse reactions (20 % or higher) are peripheral neuropathy, myalgia, alopecia, and related conditions. The most common hematologic abnormalities (40 % or higher) are neutropenia, anemia, etc. Thus, the side-effect toxicity profile is not too dissimilar from that of PAC and DOC (Table 1).

"TO MARKET, TO MARKET"

While we have tried to capture the enthusiasm of the practicing investigators and the promise of their numerous analogues displayed throughout our review by conveying much of their colorful phraseology and sometimes quoting them verbatim, this section and the subsequent concluding section of our article take a "hard look" at the number of compounds that have actually made it to the marketplace followed by a comparative review of their biological and clinical profiles. In this regard, a survey across *Annual Reports in Medicinal Chemistry* [187,251,253,254], from which the phrase for this section's title is borrowed, starting with the market launch of PAC in 1993 by BMS [253], until the end of 2011 (with the additional aid of *Drug Facts and Comparisons* for the last couple of years) reveals that only three additional analogues have been marketed during this nearly 20-year period, and one of those analogues of course being DOC in 1995 by Rhone-Poulenc Rorer [254]. Extrapolating from the tightly reproducible periodicity of "one or more new analogues every 10 years" uncovered in our previous review for consecutive market launches of numerous ABDD-evolved β-adrenergic receptor blocking agents [4,255], one would have expected that at least two and perhaps even three or four different new versions of PAC would have appeared by about 2003, and that another wave of different new analogues should be closing in for launch well before 2015. Instead, the only additional entries revealed by this survey beyond PAC and DOC turn out to be a pharmacological analogue, namely, ixabepilone marketed in 2007 by BMS [251], and a very close structural analogue, namely, CAB just recently marketed in 2010 by Sanofi-Aventis [187].

Given that a number of the previously discussed ABDD structural analogues have entered into clinical trials during the last several years, however, we remain optimistic that at least a few of these will proceed to market launch in the very near future. Importantly, that valuable medicinal chemistry principles can already be claimed from many of these efforts is a distinctly positive benefit from ABDD no matter what happens to the development candidates upon entry into the clinic.

CONCLUSION

Figure 23 attempts to capture the breadth of ABDD activities and their associated contributions that have stemmed from the initial discovery of PAC. Starting with the U.S. National Cancer Institute (NCI) Plant Program's survey, wherein *Taxus brevifolia* was first collected in 1962 and from which PAC was isolated by Wall et al. at the Research Triangle Institute (RTI) in 1966, it was Horwitz et al.'s later demonstration in 1979 showing PAC to possess a unique mechanism of action involving stabilization of microtubules that finally ignited the preclinical development of this compound which, in turn, led to an approved U.S. Investigational New Drug (IND) application/Phase I clinical study in 1984 and an eventual market launch in 1993. Contributing to this flow of events was Potier et al.'s key finding that 10-deacetyl-baccatan III (10-DAB) could be isolated from natural sources without the latter's depletion, and then used in an effective semi-synthesis strategy so as to meet the critical supply issues associated with the taxane compounds. A similar contribution came from Holton et al. for coupling a β-lactam version of the C-13-position side-chain to 10-DAB in high yield via deploying a specific lithium salt form

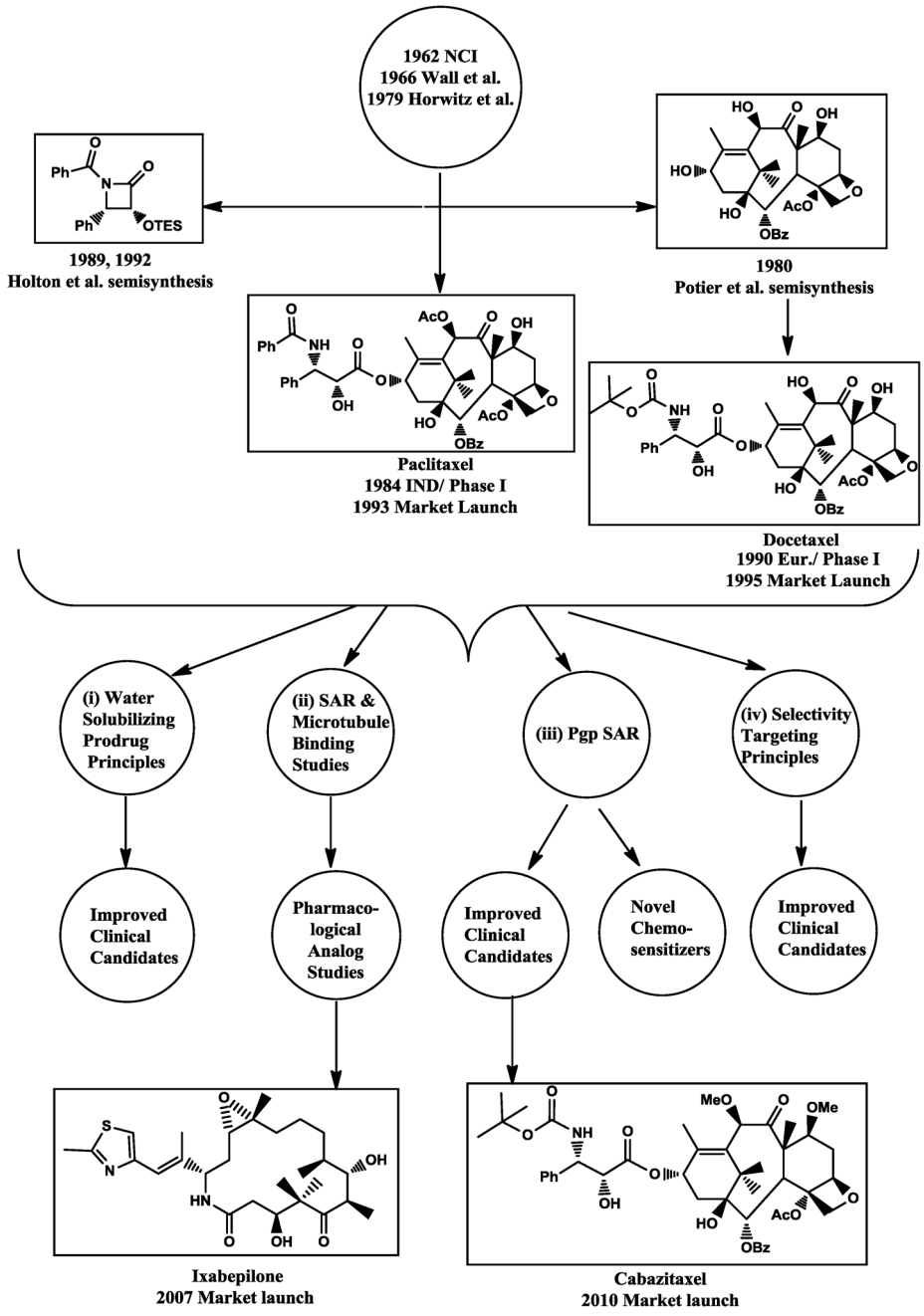


Fig. 23 Summary of significant events leading to the discovery of PAC and for the subsequent ABDD activities that led to the immediate discovery of DOC and eventual discovery of ixabepilone and CAB. Also highlighted are the significant contributions from the ABDD efforts toward establishing general principles associated with: (i) designing water-soluble prodrug moieties; (ii) understanding SAR and ligand binding that causes stabilization of microtubules; (iii) understanding SAR and substrate binding that causes inhibition or avoidance of Pgp; and (iv) designing simple and increasingly complex prodrug systems that can be used to achieve selectivity. See text for details.

of the latter. Potier's contributions also went on to produce the first ABDD-derived analogue of PAC to reach the market, namely, DOC, after a European Phase I study in 1990 that quickly led to a successful launch in 1995. Although DOC essentially has the same pharmacological profile as PAC with a bit more potency, it does have enough of an increase in aqueous solubility so that a less toxic formulation can be advantageously deployed in the clinic.

Importantly and as also shown in Fig. 23 across the entire middle of the panel, all of the aforementioned events came together during the 1990s to inspire a barrage of ABDD campaigns which, even today, are still contributing very significantly to the evolution of basic medicinal chemistry principles across several drug design constructs. The latter encompass contemporary topics like: (i) altering a drug candidate's lipophilicity so as to provide for a better PK profile or formulation partner, particularly by taking advantage of highly polar prodrug strategies; (ii) the interdisciplinary pursuit of efficacy-related SAR and ligand-receptor (active site) binding details using a composite of medicinal chemistry, molecular biology, computational chemistry, and X-ray and NMR spectroscopy approaches; (iii) delineation of these same types of details for Pgp using the same composite of interdisciplinary approaches, particularly with practical endpoints that can be directed toward improving a drug candidate's oral bioavailability and distribution profile wherein the latter includes avoidance of Pgp-derived MDR during chemotherapy and modulation for or against passage across the BBB depending upon what is desired; and (iv) enhancing a drug candidate's selectivity of actions and reducing side-effect toxicity by again using a composite of interdisciplinary approaches for which the derived molecular constructs are rapidly growing in sophistication and complexity as a workable knowledge about these areas is expanded, and again by particularly taking advantage of prodrug (conjugate) strategies. Perhaps, in the end, it should not be a surprise that ABDD has also made such very significant contributions toward basic medicinal chemistry principles, the evolution of new drug design strategies, and finally in "full circle" fashion, toward the elaboration of novel approaches for discovering new drugs. This is because there is no better way to critically assess the merits of new principles and new methods other than to compare their derived products to standard agents that already have a proven track record and history of successful performance within the marketplace, i.e., the very starting point and perpetuated context for ABDD campaigns according to the latter's strict definition.

Alternatively, it remains somewhat surprising that given the enormous amount of impressive efforts in basic research activities that have resulted in numerous clinical candidates, as well as in ongoing clinical trials for several selected agents, there is an already significant and still growing gap in time for the next big wave of second-generation taxoids to truly arrive. We reserve this phrase to mean the next round of compounds that actually become launched into the marketplace after the arrival of the first-generation compounds, rather than using it for what we instead refer to as still being "drug wannabes" [256] wherein no matter how impressive their perceived attributes may be, the latter are only on route to the market. In this same context, we prefer to consider both PAC and DOC to represent a pair of first-generation agents with PAC constituting the *true pioneer* drug and *first-in-its-class*. It is worthwhile to note that the emphasis placed upon screening for microtubule-stabilizing agents as an integral part of the ABDD activities directly stemming from PAC, successfully led to yet another, structurally novel natural product that has indeed been entered into the marketplace. In our view, the latter is shown appropriately within Fig. 23 as flowing directly from the ABDD-driven receptor binding studies (ii) so as to lead to what can be called a "pharmacological analogue". From such a prestigious lineage, this effort ultimately took the form of **113** and then went on to achieve a market launch in 2007. Considering **113** to represent a second-generation microtubule stabilizer, we still look forward to seeing the arrival of the second-structural-generation taxoids into the marketplace. In this regard, perhaps CAB's recent market launch in 2010 can be thought of as representing just the beginning of the swell that immediately precedes the crest of what may be following as a huge wave of new and exciting compounds in the near future.

As a concluding comment pertaining to the overall mechanics of our review for this topic, a search of *taxol or docetaxel or paclitaxel or taxotere* in SciFinder Scholar generated about 50 000 hits.

Our selection of review papers/books and our deliberate focus upon medicinal chemistry and ABDD was culled from nearly 500 key papers such that, in the end, our product represents a mere 1 % of the initial search results. Thus, this article provides only a glimpse of the overall chemical, biological, and clinical story associated with these fascinating compounds. We acknowledge that there are numerous other contributions that we were unable to share, and furthermore, that we may have inadvertently overlooked some that were highly significant within the specific context of our intended discourse. Toward the latter possibility, we apologize in advance to any investigators who may ultimately feel that their work in this arena did not receive the proper attention that it should have been accorded.

Finally, we would like to close our review by reciting an old quote [257], revived recently by Wermuth [258], that was initially stated well before today's *ADMET* phrase ever became popular (e.g., [41,81,101,256]). Clearly ahead of its time, it was intended to assist in alleviating potential PK and toxicity issues for NCE wannabes having novel mechanisms of action by prompting what we have now come to call ABDD. And in addition to its direct reference to drug discovery in general, for the specific case of PAC and all of the words that we have just put together in that regard, this quote also should be thought of as serving to underscore the extremely important role that ABDD activities can play toward elaborating new medicinal chemistry principles and molecular design strategies applicable to better understanding drug actions in general . . . because it will be only from our growing knowledge about such things that the truly new drugs of the even more distant future will be able to be best imagined and ultimately realized.

"The most fruitful basis for the discovery of a new drug is to start with an old drug."

—Sir James Black, winner of the 1988 Nobel Prize in Physiology and Medicine [257]

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LIST OF ABBREVIATIONS

ABDD	analogue-based drug discovery
ADEPT	antibody-directed enzyme prodrug therapy
Asp	aspartic acid
AUC	area under the drug concentration–time curve
BBB	blood–brain barrier
BBN	bombesin
BMS	Bristol-Myers Squibb
BOC	*t*-butoxycarbonyl
CAB	cabazitaxel
CAC	chloroacetyl
CNS	central nervous system
CoMFA	comparative molecular field analysis
DAB	deacetyl-baccatin III
DHA	docosahexenoic acid
DOC	docetaxel
DTT	dithiothreitol
EDA	ethylene diamine
EGFR	epidermal growth factor receptor
EPO	epothilone
EPR	enhanced permeability and retention
EPT	4'-*O*-demethylepip-odophyllotoxin
ER	estrogen receptor
FCS	fetal calf serum
FITC	fluorescein isothiocyanate
FR	folic acid receptor
GDP	guanosine diphosphate
GI_{50}	50 % inhibition of cell growth
GI	gastrointestinal
Glu	glutamic acid
GLUT	glucose transporter protein
GRP	gastrin-releasing peptide
GTP	guanosine triphosphate
HTS	high-throughput screen
IgG	immunoglobulin G
IND	investigational new drug
LA	linoleic acid
LNA	linolenic acid
mAbs	monoclonal antibodies
MAP	microtubule-associated protein
mCRPC	metastatic castration-resistant prostate cancer
MDR	multidrug resistance
MTD	maximum tolerated dose
NCE	new chemical entity
NMR	nuclear magnetic resonance
PABA	*p*-aminobenzyl alcohol
PAC	paclitaxel
PAMAM	poly-(amidoamine)
PEG	polyethylene glycol

Pgp	P-glycoprotein
PSA	prostate specific antigen
PUFA	polyunsaturated fatty acid
RDPR	ribonucleoside diphosphate reductase
RMD	restrained molecular dynamics
RTI	Research Triangle Institute
SAR	structure–activity relationship
$t_{1/2}$	half-life
Topo II	topoisomerase II enzyme
TAP	tumor-activated prodrug
U.S. EPA	United States Environmental Protection Agency

