Reviews

The Role of Natural Product Chemistry in Drug Discovery†

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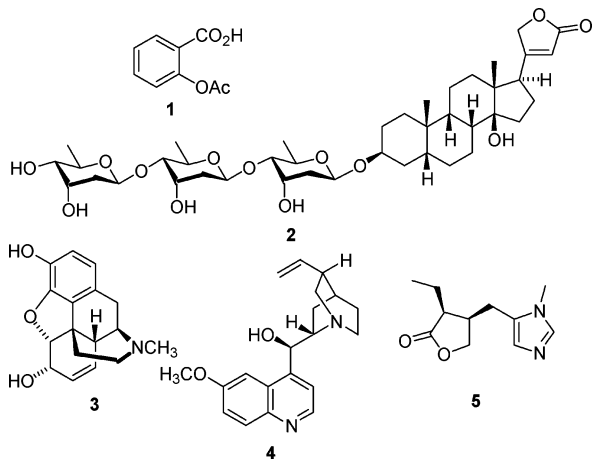
Although traditionally natural products have played an important role in drug discovery, in the past few years most Big Pharma companies have either terminated or considerably scaled down their natural product operations. This is despite a significant number of natural product-derived drugs being ranked in the top 35 worldwide selling ethical drugs in 2000, 2001, and 2002. There were 15 new natural product-derived drugs launched from 2000 to 2003, as well as 15 natural product-derived compounds in Phase III clinical trials or registration at the end of 2003. Recently, there has been a renewed interest in natural product research due to the failure of alternative drug discovery methods to deliver many lead compounds in key therapeutic areas such as immunosuppression, anti-infectives, and metabolic diseases. To continue to be competitive with other drug discovery methods, natural product research needs to continually improve the speed of the screening, isolation, and structure elucidation processes, as well addressing the suitability of screens for natural product extracts and dealing with issues involved with large-scale compound supply.

Introduction

For thousands of years medicine and natural products (NPs) have been closely linked through the use of traditional medicines and natural poisons.[1−5] Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines such as aspirin (**1**), digitoxin (**2**), morphine (**3**), quinine (**4**), and pilocarpine (**5**).[1−5]



The discovery of antibacterial filtrate "penicillin" by Fleming in 1928, re-isolation and clinical studies by Chain, Florey, and co-workers in the early 1940s, and commercialization of synthetic penicillins revolutionized drug discovery research.[6−9] Following the success of penicillin, drug companies and research groups soon assembled large microorganism culture collections in order to discover new antibiotics. The output from the early years of this antibiotic research was prolific and included examples such as streptomycin (**6**), chloramphenicol (**7**), chlortetracycline (**8**), cephalosporin C (**9**), erythromycin (**10**), and vancomycin (**11**).[1,4,8,9] All of these compounds, or derivatives thereof, are still in use as drugs today.

One of the next breakthroughs in drug discovery was the use of mechanism-based screening for bioassay-guided fractionation. Through continual improvement of screening formats, reagent production, robotics, and data management, mechanism-based screening has since become the mainstay of high-throughput screening (HTS). Some of the first compounds identified in the early 1970s using mechanism-based screening methods included the β-lactamase inhibitor clavulanic acid (**12**) from *Streptomyces clavuligerus*[10] and the HMG-CoA reductase inhibitor mevastatin (**13**) (then named ML-236B) from *Penicillium citrinum*.[11] Mevastatin (**13**) (then also named compactin) was also reported as an antifungal agent from *P. brevicompactum*.[12] A mixture of clavulanic acid (**12**) and amoxicillin (**14**) (the combination is called Augmentin) is still being used today as a front line antibiotic, while mevastatin (**13**) and lovastatin (**15**) were the lead compounds for a series of antilipidemic drugs collectively known as the "statins" (Figure 1).[13,14]

Status of Natural Products in Drug Discovery Today

Despite competition from other drug discovery methods, NPs are still providing their fair share of new clinical candidates and drugs. This was demonstrated recently by Newman, Cragg, and Snader, who analyzed the number of NP-derived drugs present in the total drug launches from 1981 to 2002.[15,16] They concluded that NPs were still a significant source of new drugs, especially in the anticancer and antihypertensive therapeutic areas.[15] In another study, Proudfoot reported that 8 out of 29 small molecule drugs launched in 2000 were derived from NPs or hormones and concluded that HTS did not have a significant impact on the derivation of these drugs.[17]

NP-derived drugs are well represented in the top 35 worldwide selling ethical drug sales of 2000, 2001, and 2002

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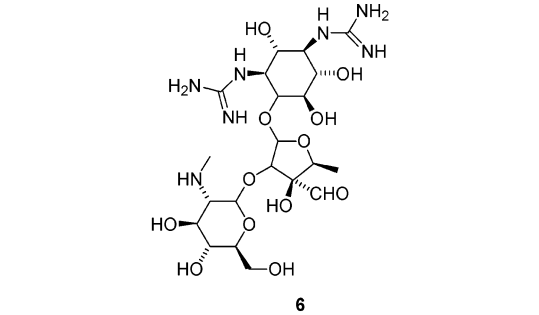

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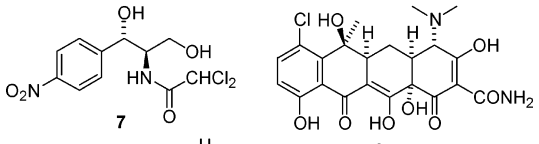

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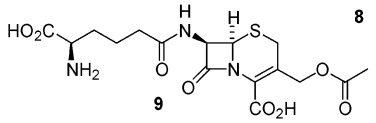

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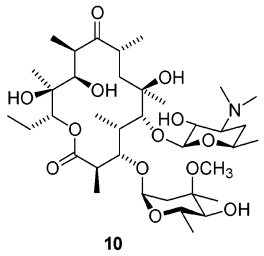

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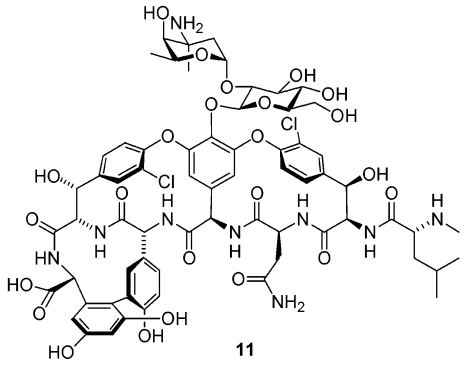

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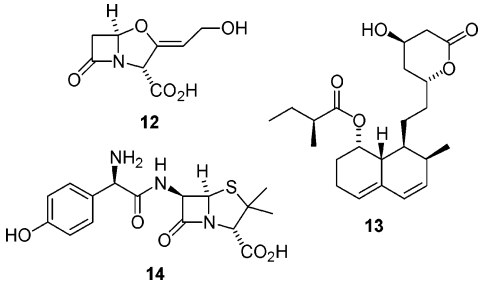


12 **13** **14**

(Table 1 and Figure 2). The percentage of NP-derived drugs was 40% in 2000 and remained approximately constant at 24% in 2001 and 26% in 2002. Therefore, in addition to being a proven and important source of drug leads, NP-derived drugs also contribute significantly to the profitability of many companies.

There has also been a steady introduction of new NP and NP-derived drugs in the United States, Europe, and Japan from 2000 to 2003 (Table 2). A total of 15 were launched (one in 2000, four in 2001, five in 2002, and five in 2003), which included new drug types such as the antimalarial arteether (**16**),[18,19] the echinocandin-derived antifungal caspofungin (**20**),[20,21] the anti-Alzheimer's drug galantamine (galanthamine) (**25**),[22,23,28] and the antibacterial lipopeptide daptomycin (**38**).[25−27,29] Also noteworthy was the launch of the biologic gemtuzumab ozogamicin (**40**) (Mylotarg, Wyeth) in 2000,[32] which is a conjugate of recombinant humanized IgG4 kappa antibody and calicheamicin (**41**),[33] an actinomycetes-derived antibiotic of the ene-diyne class.[34] The drug works by releasing a calicheamicin derivative from the antibody that binds to DNA in the minor groove, resulting in DNA double-strand breakage and cell death.[32] The developers of gemtuzumab ozogamicin (**40**) were awarded the 2003 Discoverers Award from the Pharmaceutical Research and Manufacturers of America (PhRMA).[35] Drugs with links to NPs and those derived from hormones and protein fragments launched since 2000 include bivalirudin (2000) as an anticoagulant (lead: hirudin),[18,19] atosiban (2000) for preterm labor (lead: pituitary hormone oxytocin),[19] ganirelix acetate for female infertility (2000) (lead: luteinizing hormone-releasing hormone [LH-LR]),[18,19] taltirelin (2000) as a CNS

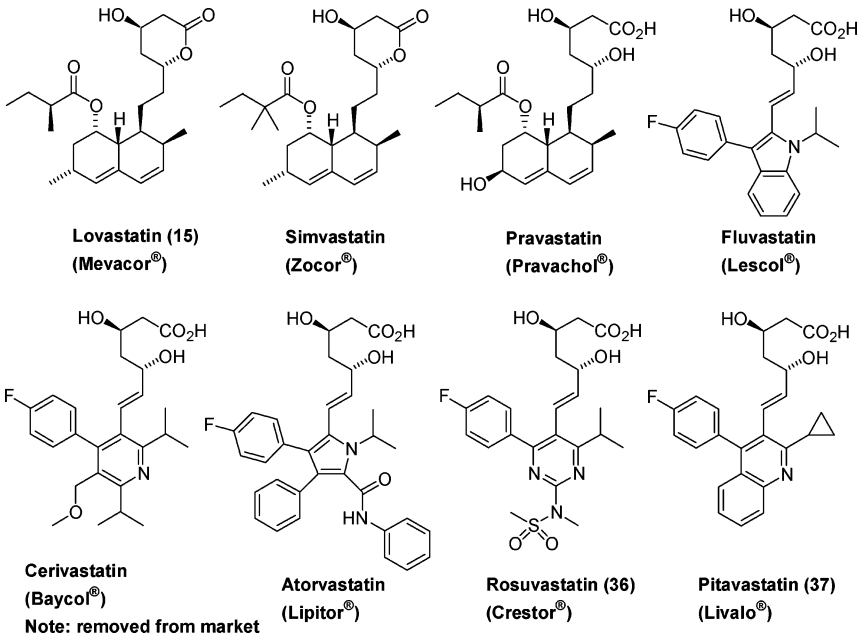


Figure 1. Statin class of antilipidemic drugs derived from the lead compound mevastatin (compactin, ML-236B) (**13**).

Table 1. Top 35 Worldwide Ethical Drug Sales for 2000, 2001, and 2002[a] with Natural Product-Derived Drugs in Blue,[b] Biologically Derived Drugs in Magenta,[c] and Synthetically Derived Drugs in Black[d]

Rank	2000	2001	2002
1	Omeprazole	Atorvastatin	Atorvastatin
2	Atorvastatin	Omeprazole	Simvastatin
3	Simvastatin	Simvastatin	Omeprazole
4	Amlodipine	Lansoprazole	Erythropoietin (J&J)
5	Lansoprazole	Amlodipine	Amlodipine
6	Loratadine	Erythropoietin (J&J)	Lansoprazole
7	Erythropoietin (J&J)	Loratadine	Olanzapine
8	Celecoxib	Celecoxib	Paroxetine
9	Fluoxetine	Olanzapine	Celecoxib
10	Olanzapine	Paroxetine	Sertraline
11	Paroxetine	Sertraline	Interferon α-2b+ribarvarin
12	Sertraline	Metformin/Metformin+Glyburide	Rofecoxib
13	Rofecoxib	Rofecoxib	Salmeterol+Fluticasone propionate
14	Erythropoietin (Amgen)	Erythropoietin (Amgen)	Gabapentin
15	Metformin/Metformin+Glyburide	Pravastatin (BMS)	Pravastatin (BMS)
16	Estrone	Estrone	Erythropoietin (Amgen)
17	Amoxicillin + Clavulanic acid	Amoxicillin + Clavulanic acid	Alendronate Sodium
18	Enalapril	Fluoxetine	Losartan/Losartan+Hydrothiazide
19	Pravastatin (BMS)	Risperidone	Risperidone
20	Insulin	Losartan/Losartan+Hydrothiazide	Venlafaxine
21	Ciprofloxacin	Insulin	Esomeprazole magnesium
22	Losartan/Losartan+Hydrothiazide	Ciprofloxacin	Fexofenadine
23	Pravastatin (Sankyo)	Gabapentin	Clopidogrel bisulfate
24	Risperidone	Alendronate sodium	Insulin
25	Paclitaxel	Leuprolide acetate	Estrone
26	Leuprolide Acetate	Fexofenadine	Loratadine
27	Azithromycin	Venlafaxine	Amoxicillin+Clavulanic acid
28	Interferon α-2b+Ribarvarin	Sidenafil	Sidenafil
29	Sidenafil	Azithromycin	Valsartan
30	Gabapentin	Interferon α-2b+Ribarvarin	Citalopram hydrobromide
31	Fluticasone propionate	Pravastatin (Sankyo)	Leuprolide Acetate
32	Clarithromycin	Filgrastim	Oxycodone HCl
33	Filgrastim	Fluticasone propionate	Azithromycin
34	Cyclosporin	Enoxaparin	Montelukast sodium
35	Lisinopril	Vaccines (Aventis)	Rituximab

[a] Top 35 worldwide ethical drug sales data supplied by Wood Mackenzie, Boston, MA. [b] NP-derived indicates that the drug is either a NP, a semisynthetic derivative of a NP, or a synthetic drug that is modeled on a NP pharmacophore. [c] Biologically derived indicates that the drug is hormone or protein derived. [d] Erythropoietin is sold by both Johnston & Johnston (J&J) and Amgen, while pravastatin is marketed in Japan by Sankyo and the United States by Bristol-Myers Squibb (BMS).

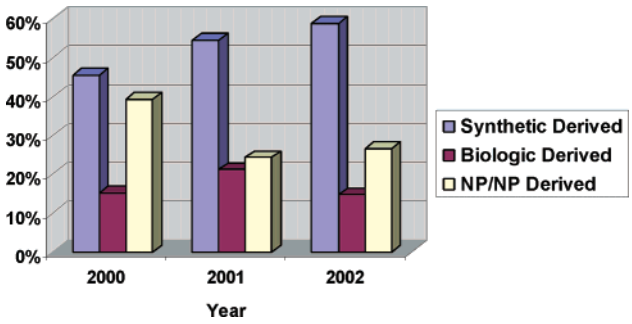


Figure 2. Percentage of NP and NP-derived, biologic-derived, and synthetic-derived drugs in the top 35 worldwide ethical drug sales for 2000, 2001, and 2002 (data derived from Table 1 with "Interferon α-2b+ribarvarin" counted as a biologic, "Salmeterol+Fluticasone propionate" as NP-derived, and erythropoietin and pravastatin counted only once per year).

stimulant (lead: thyrotropin-releasing hormone [TRH]),[17−19] nestiritide (2001) as a treatment for acute decongestive heart failure (lead: recombinant form),[20] acemannan (2001) for wound healing (lead: manno-galacto acetate isolated from *Aloe vera*),[21] fondaparinex sodium (2002) as an antithrombotic (lead: heparin),[21,22,24] abarelix (2003) for advanced prostate cancer (lead: gonadotropin releasing hormone [GnRH]),[25−27] and enfuviritide (2003) for treatment of HIV infection (lead: viral transfusion protein gp120).[25−27,36]

In 1998, Shu published a review on NPs in drug development from an industrial perspective listing most compounds that were then in clinical trials.[37] In that review, NP-derived drugs were well represented in the anticancer, anti-infectives, immunosuppression, and neurological disease therapeutic areas, and some of these compounds have since progressed further into clinical trials or onto the market. There were 15 NP or NP-derived drugs in Phase III clinical trials or registration as of December 31, 2003 (Table 3). Trabectedin (**66**), which was filed in Europe for treatment of soft tissue sarcoma but was rejected in October 2003, is currently being studied as a single agent and in combination in other cancer indications with a scheduled launch in 2006.[38] The launch of oritavancin (**59**), which was scheduled for 2005, also may be delayed, as there have been problems with its manufacture.[39] Anidulafungin (**42**), dalbavancin (**44**), everolimus (**50**), exatecan (**52**), rubitecan (**62**), and ziconotide (**70**) are scheduled for filing and/or launch in 2004, while FTY720 (**54**), ramoplanin (**61**), and tigecycline (**63**) are scheduled for 2005, M6G (**58**) for 2006, and vinflunine (**67**) for 2007. There is no scheduled launch date available for edotecarin (**46**) and ixabepilone (**56**).

Current State of Industrial Natural Product Research

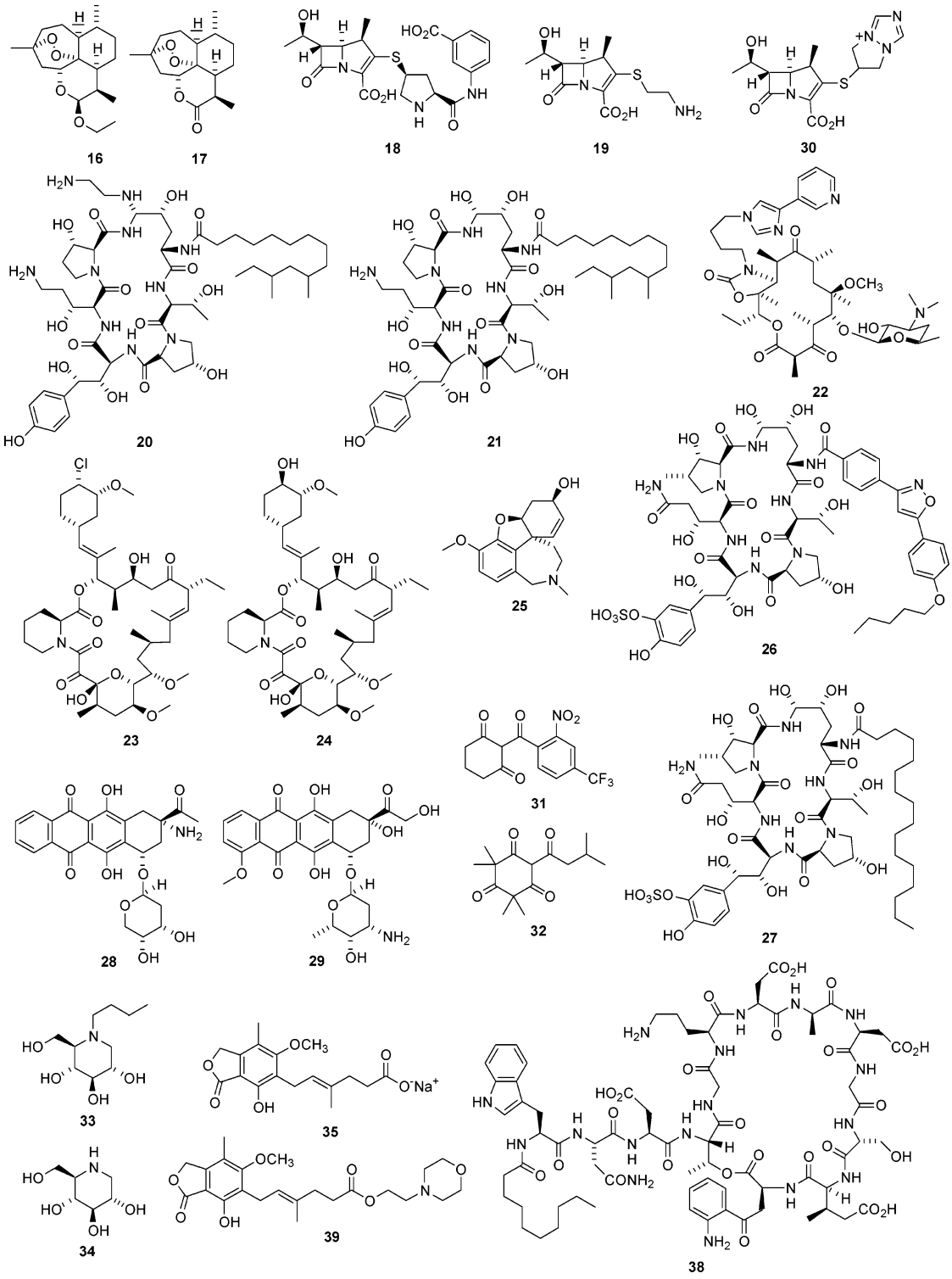
Drug discovery is a complex, interdisciplinary pursuit of chemistry, pharmacology, and clinical sciences, which has benefited humankind immensely over the last 100 years.[40,41] Although drug discovery has been traditionally a difficult and expensive process, the amount of money currently being invested in R&D and clinical development has skyrocketed, while the output of newly launched drugs has fallen.[42−44] This has prompted much discussion as to why this has occurred and how this will effect the future of the industry.[45−47] However, there may be hope on the horizon with a record number of new products entering the Pharmaprojects R&D database in 2002−2003.[48]

Table 2. NP or NP-Derived Drugs[a] Launched in Either the United States, Europe, or Japan in 2000−2003[18−27]

year	name	lead structure	company (originator)	disease	mechanism of action	method of manufacture and original source
2000	arteether (**16**) (Artemotil)	artemisinin (**17**)	Artecef BV (Central Drug Institute)	antimalarial	thought to involve heme detoxification	semisynthetic and template artemisinin originally isolated from the plant *Artemisia annua*
2001	ertapenem (**18**) (Invanz)	thienamycin (**19**)	Merck (AstraZeneca)	antibacterial	bacterial cell wall synthesis inhibitor	synthetic; structure based on thienamycin originally isolated from *Streptomyces cattleya*
2001	caspofungin (**20**) (Cancidas)	pneumocandin B (**21**)	Merck (Merck)	antifungal	1,3-β-D-glucan synthesis inhibitor	semisynthetic and template pneumocandin B originally isolated from the fungus *Glarea lozoyensis*
2001	telithromycin (**22**) (Ketek)	erythromycin (**10**)	Aventis (Aventis)	antibacterial	inhibition of protein synthesis	semisynthetic and template erythromycin originally isolated from *Saccharopolyspora erythraea* (formally *Streptomyces erythreus*)
2001	pimecrolimus (**23**) (Elidel)	ascomycin (**24**)	Novartis (Novartis)	atopic dermatitis	thought to inhibit T cell activation and prevents release of inflammatory cytokines	semisynthetic and template ascomycin originally isolated from *Streptomyces hygroscopicus* var. *ascomyceticus*
2002[b]	galantamine (**25**) (Reminyl)	natural product	Johnson & Johnson (Trad. Med. from Eastern Europe)	Alzheimer's disease	thought to involve inhibition of acetylcholinesterase	synthetic; NP originally isolated from the plant *Galanthus* spp. and later from *Narcissus* spp.
2002	micafungin (**26**) (Funguard)	FR901379 (**27**)	Fujisawa (Fujisawa)	antifungal	1,3-β-D-glucan synthesis inhibitor	semisynthetic and template FR901379 isolated from the fungus *Coleophoma empetri*
2002	amrubicin hydrochloride (**28**) (Calsed)	doxorubicin (**29**)	Sumitomo (Sumitomo)	anticancer	inhibition of topoisomerase II	synthetic and based upon doxorubicin, which was originally derived from *Streptomyces peucetius*
2002	biapenem (**30**) (Omegacin)	thienamycin (**19**)	Meiji Seika (Wyeth)	antibacterial	bacterial cell wall synthesis inhibitor	synthetic; structure based on thienamycin originally isolated from *Streptomyces cattleya*
2002	nitisinone (**31**) (Orfadin)	leptospermone (**32**)	Rare Diseases Therapeutics (AstraZeneca)	antityrosinaemia	inhibition of 4-hydroxyphenylpyruvate dioxygenase	synthetic. structure based on leptospermone originally isolated from the plant *Callistemon citrinus* and related to triketone herbicides
2003	miglustat (**33**) (Zavesca)	1-deoxynojirimycin (**34**)	Actelion/Teva (CellTech)	type 1 Gaucher disease	inhibition of glucosylceramide synthase	synthetic; lead compound 1-deoxynojirimycin isolated from *Streptomyces trehalosaticus* and various plants
2003	mycophenolate sodium (**35**) (Myfortic)	natural product	Novartis[c]	immunosuppression	inhibitor of inosine monophosphate dehydrogenase	synthetic; NP originally isolated from the fungus *Penicillium brevicompactum*
2003	rosuvastatin (**36**) (Crestor)	mevastatin (**13**)	AstraZeneca (Shionogi & Co)	dyslipidemia	inhibition of HMG-CoA reductase	synthetic; structure based on mevastatin originally isolated from *Penicillium citrimun* and *P. brevicompactum*
2003	pitavastatin (**37**) (Livalo)	mevastatin (**13**)	Sankyo/Kowa (Kowa/Nissan Chemical)	dyslipidemia	inhibition of HMG-CoA reductase	synthetic; structure based on mevastatin originally isolated from *Penicillium citrimun* and *P. brevicompactum*
2003	daptomycin (**38**) (Cubicin)	natural product	Cubist (Lilly)	antibacterial	inhibition of protein, DNA and RNA synthesis	natural product that is isolated from *Streptomyces roseosporus*

[a] Other drugs launched with connection to NPs: **Vitamin D:** maxacalcitol (2000) and falecalcitriol (2001). **Steroid:** drospirenone (2000), exemestane (2000), trimegestone (2001), fulvestrant (2002), norelgestromin (2002), and dutasteride (2003). **Tryptamine:** almotriptan (2000), alosetron hydrochloride (2000), ramatroban (2000), eletripan (2001), tagaserod maleate (2001), and frovatriptan (2002). **Prostaglandin:** bimatoprost (2001), travoprost (2001), and treprostinil sodium (2002). [b] Galantamine (galanthamine) (**25**) was launched in Austria in 1996 as Nivalin and then in 2002 as Reminyl in Europe and the United States.[28] [c] Mycophenolic acid (**35**) was the first antibacterial discovered in 1893.[4,30] Its mofetil derivative (**39**) (CellCept, Roche) was launched in 1995 as an immunosuppressant.[31]

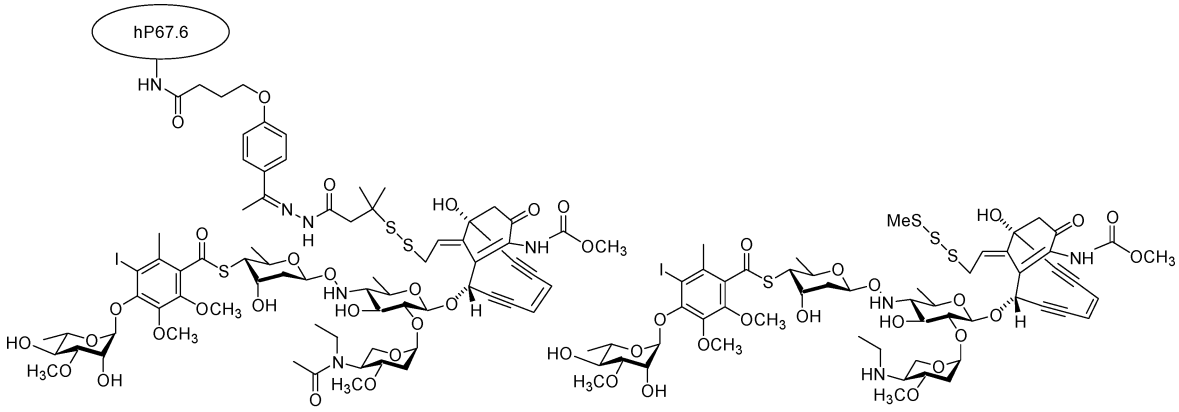
Chart 1



Given that NPs have historically provided many novel drugs leads, one would assume that NPs would still play a pivotal role in the drug discovery strategy of Big Pharma. However, most Big Pharma companies have terminated or significantly scaled down their NP operations in the last 10 years.[49−52] To a certain extent the downsizing or termination of these NP research programs has been offset by biotech companies offering NP-related services such as pure NP libraries and more traditional extract based screening services.[53−58] To better understand why Big Pharma has scaled down its NP research programs, it is prudent to examine the differences between the pharmaceutical industry of today and that of 10−20 years ago (Table 4).

The advent of combinatorial chemistry about 15 years ago created huge excitement in the pharmaceutical industry, and most Big Pharma companies quickly changed their drug discovery strategies to include a significant proportion of combinatorial chemistry.[66,67] The impending structure of the human genome and the promise of a plethora of new targets added to the excitement of the time. The basic premise was that combinatorial chemistry would generate

Chart 2



libraries consisting of millions of compounds, which would be screened by HTS and produce drug leads by sheer weight of numbers. In addition, most synthetic compound libraries have none of the IP issues that are involved with NPs.[52,68,69] The leads would be delivered in quicker time and in greater numbers for all therapeutic areas compared to traditional drug discovery methods, and as a consequence, it was not surprising that NP research was often assigned a lower priority. However, results from early combinatorial libraries were often disappointing, and by the mid-1990s there were serious doubts about the usefulness and value of most large libraries generated to that time.[65,66,70] For example, in a recent article Lipinski was quoted as saying, "The combinatorial libraries in the early years were so flawed that if you took the libraries across Pharma from 1992 to 1997 and stored them in dumpsters you would have improved productivity".[49] However, in the ensuing years, more attention has been placed on the quality and diversity of the combinatorial libraries, and combinatorial chemistry is used now predominantly for lead optimization and the generation of focused compound libraries.[67]

The reason for the lack of lead compounds from synthetic libraries in some therapeutic areas such as anti-infectives, immunosuppression, oncology, and metabolic diseases may be due to the different chemical space occupied by NPs and synthetic compounds.[71−74] This different chemical space makes NPs an attractive alternative to synthetic libraries, especially in therapeutic areas that have a dearth of lead compounds. In an interesting development, some groups and companies have begun to synthesize more complex structures to match the chemical space occupied by NPs, and this has been described as "diversity orientated synthesis" (DOS) by Schreiber.[75,76] NPs have been used also as starting templates in the synthesis of combinatorial libraries.[73,77−81] NP pharmacophores are well represented in lists of "privileged structures", which makes them ideal candidates for building blocks for biologically relevant chemical libraries.[82,83]

Therefore, the above arguments strongly suggest that NPs should be incorporated into a well-balanced drug discovery program. However, NP research must constantly address any problems, either real or perceived, if it is to continue to be relevant to drug discovery and compete with other methods. Some of the challenges currently faced by NP drug discovery research are discussed below.

Challenges Faced by Natural Product Chemistry

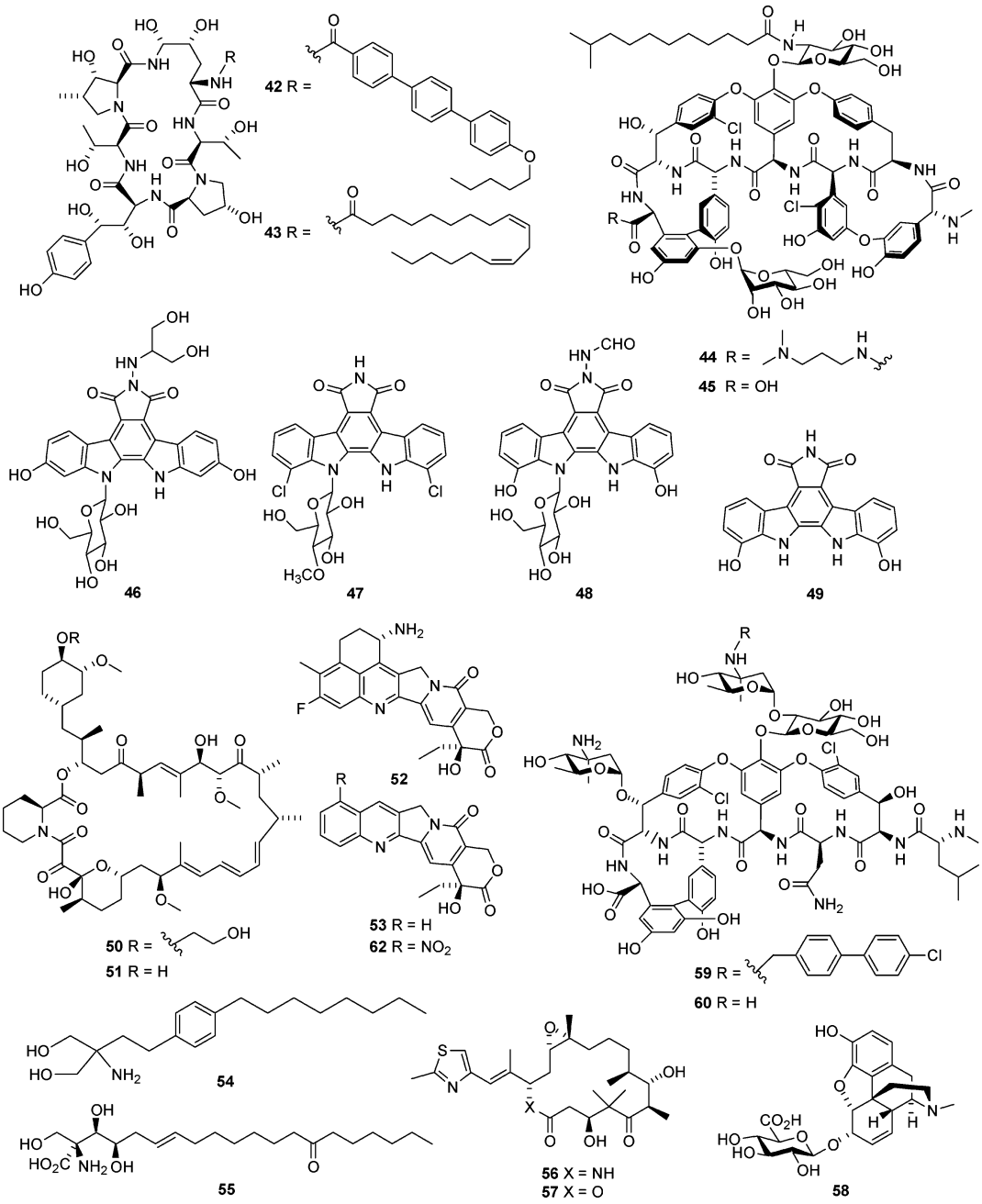
Screening. The advent of routine HTS has been one of the most important changes to the drug discovery process.[84−86] Screening of one hundred thousand samples in a routine assay can now be completed in just over a week using 384-well formatting, a data handling system, and limited robotics. This screening time can be decreased further using higher density formats and advanced robotics.[84,87,88] Therefore, the number of compounds or extracts that can be screened for each drug target is generally not the rate-limiting step. More important considerations are the cost of the screen consumables, time required, and the people resources allocated to each screen. The cost of a screen can escalate quickly if an expensive screen format has to be used or the substrate or reagents are difficult to make, have a short half-life, and/or are expensive to purchase.

While the number of screening points available from HTS has risen dramatically, the percentage generally allocated to NP samples has decreased significantly or is nonexistent. This poses an interesting question: is the smaller number of new lead compounds currently being discovered due to an increase in the difficulty of drug discovery or from a lack of NP screening? If it is indeed from a lack of NP screening, then NPs represent a wealth of drug discovery opportunities for new targets and older targets that have not been screened exhaustively against NPs.

The choice of what biological targets to screen against a NP library is an important decision that is critical to the long-term success of a NP drug discovery program.[89] Each assay needs to be thoroughly examined in order to evaluate the probability of discovering a novel lead compound and the likelihood that a compound derived from this lead will enter a preclinical program. A survey of previously reported synthetic and natural product inhibitors is also essential. Assay parameters that need to be considered include the druggability of the target,[90−92] the suitability for the screening NP extracts, and the existence of a progression pathway from lead compound to the clinic. From all of the above information, a decision must be made on whether to proceed or not with screening.

Timing of Screening Campaign. The decision when to screen NP extracts compared to compound libraries is extremely important for the successful integration of NP hits into a lead discovery program. This is because no matter how quickly the active compounds can be isolated and their structures identified, there will always be a lag time behind the evaluation of pure compounds whose structure and method of synthesis is known at the onset. In fact, screening of NP extracts well before a synthetic library would be preferable, but in practice this rarely, if ever, happens. Alternatively, NP extracts may be used as

Chart 3



a last resort when no lead series have been identified after completion of all other screening. While in principle this seems attractive, the screening of these types of drug targets must not be overdone, as using NPs only for difficult targets unfairly biases its output compared to other techniques. Therefore, screening NP libraries against various types of drug targets in a way complementary to compound libraries offers the most efficient way of discovering a new drug lead.

Compatibility of NP Extracts with HTS. The screening of NP extract libraries is generally more problematic than screening compound libraries.[84−86,93−98] This is because NP extracts contain complex mixtures of mostly uncharacterized compounds, some of which have undesirable properties. For example, compounds present in a NP extract may autofluoresce or have UV absorptions that interfere with the screen readout. Compare this to compound screening where the structure and physical properties of the compounds are already known and can be eliminated on this basis. An added complication is that interfering compounds may be present in the extract in addition to compounds of interest, which may mask the biological effect. Compounds or families of compounds also may be present in an extract, which can interfere with the screen in a nonspecific manner.[99] Examples include plant tannins that can interfere with enzymatic assays and fatty acids present in high concentrations[99,100] that can be nonspecific binders due to their detergent-like properties.[99,101] Interestingly, recent studies have proposed a model for identification and prediction of promiscuous aggregating inhibitors that may explain the high hit rate of certain NPs.[102−105] The discrimination of real hits from false positives sometimes can be achieved by judicious use of detergent concentrations in the assay and by varying the enzyme concentration.[103,105]

The ideal assay for NP extract screening would not be affected by such interfering compounds, have an adequate signal-to-noise ratio, and have excellent screen reproduc-

Table 3. Natural Product or Natural Product-Derived Drugs in Phase III Clinical Trials or Registration[a]

compound name	therapeutic area	method of manufacture, lead compound, and producing organism	company
anidulafungin (**42**) (LY-303366)	antifungal	semisynthetic; lead compound and template echinocandin B (**43**) originally isolated from *Aspergillusrugulovalvus* (formerly *Aspergillus rugulosus*)	Vicuron Pharmaceuticals
dalbavancin (**44**) (BI-397)	antibacterial	semisynthetic; lead compound and template A40926 (**45**) antibiotic complex originally isolated from *Nonomuraea* sp. ATCC 39727	Vicuron Pharmaceuticals
edotecarin (**46**) (J-107088)	anticancer	synthetic; original lead compound rebeccamycin (**47**) isolated from *Saccharothrix aerocolonigenes*; related to NB-506 (**48**), which is a semisynthetic derivative of the natural product BE-13793C (**49**)	Pfizer and Banyu
everolimus (**50**) (SDZ RAD, Certican)	immuno-suppression	semisynthetic; lead compound sirolimus (rapamycin) (**51**) originally isolated from *Streptomyces hygroscopicus*	Novartis
exatecan (**52**) (DX-8951f)	anticancer	synthetic; lead compound camptothecin (**53**) originally isolated from the plant *Camptotheca acuminata*	Daiichi Pharmaceutical
FTY720 (**54**)	immuno-suppression	synthetic; lead compound myriocin (**55**) originally isolated from the fungi *Mycelia sterilia* and *Myriococcum albomyces* and subsequently identified as an immunosuppressant from the fungus *Isaria sinclairii*	Novartis
ixabepilone (**56**) (BMS 247550)	anticancer	semisynthetic; lead compound and template epothilone B (**57**) originally isolated from the myxobacterium *Sorangium cellulosum*	Bristol-Myers Squibb
M6G (**58**) (morphine-6-glucuronide)	pain	semisynthetic; lead compound and template morphine (**3**) derived from Opium poppy, *Papaver somniferum*	CeNeS
oritavancin (**59**) (LY-333328)	antibiotic	semisynthetic; lead compound and template chloroeremomycin (LY264826) (**60**) originally obtained from *Nocardia orietalis*	InterMune
ramoplanin (**61**)	antibacterial	natural product; ramoplanin (**61**) complex isolated from *Actinoplanes* sp. ATCC 33076	Oscient Pharmaceuticals
rubitecan (**62**) (Orathecin)	anticancer	semisynthetic; lead compound and template camptothecin (**53**) originally isolated from the plant *Camptotheca acuminata*	SuperGen
tigecycline (**63**) (Tygacil)	antibiotic	semisynthetic; lead compound tetracycline (**64**) originally isolated from *Streptomyces aureofaciens*; produced by semisynthesis from demeclocycline via minocycline (**65**) (Minocin/Arestin)	Wyeth
trabectedin (**66**) (ET-743, Yondelis)	anticancer	semisynthetic; lead compound trabectedin (**66**) originally isolated from the ascidian *Ecteinascidia turbinata*; produced by semisynthesis from safracin B derived from the bacterium *Pseudomonas fluorescens*	PharmaMar/ Johnson & Johnson
vinflunine (**67**) (Javlor)	anticancer	semisynthetic; lead compound vinblastine (**68**) originally isolated from the plant *Catharanthus roseus*; produced by semisynthesis from vinorelbine (Navelbine) (**69**), which is in turn derived from vinblastine (**68**)	Pierre Fabre
ziconotide (**70**) (Prialt, SNX-111)	chronic pain	synthetic; lead compound ω-conotoxin MVIIA (**70**) originally isolated from the venom of the cone shell, *Conus magus*; ziconotide is the synthetic version of **70**	Elan

[a] Current to December 31, 2003.

ibility. Although this is difficult and rarely achieved, careful design of the initial primary assay and subsequent secondary assays can be the key to a rapid and successful NP extract screening campaign. First of all, the screening format of the primary screen needs to be determined. Homogeneous assays are preferred in HTS, and common screening formats include fluorescence resonance energy transfer (FRET), fluorescence polarization (FP), time-resolved fluorescence (TRF), flash plate, scintillating proximity assay (SPA), and whole cell assays.[106] Nonhomogeneous assays can also be used but involve an extra step, which adds extra time to the process. However, there will always be a number of extracts that contain interfering and/or nuisance compounds no matter how robust a primary screen is or how carefully the extracts are prepared.[99] Therefore, the key is to design a complementary orthogonal assay or assays to remove as many false positive hits as possible. Dereplication (see below) can then be used to remove nuisance compounds or group like-extracts.

Some other new screening methods[87] include the novel microarray compound screening (microARCS) technology, which utilizes agarose matrixes to introduce a majority of the reagents throughout the assay,[107] and on-line biochemical detection coupled to mass spectrometry, which already has been used for the screening of natural products extracts.[108]

An example from our laboratory of the development of an efficient HTS FRET assay, which was used as the

Chart 4

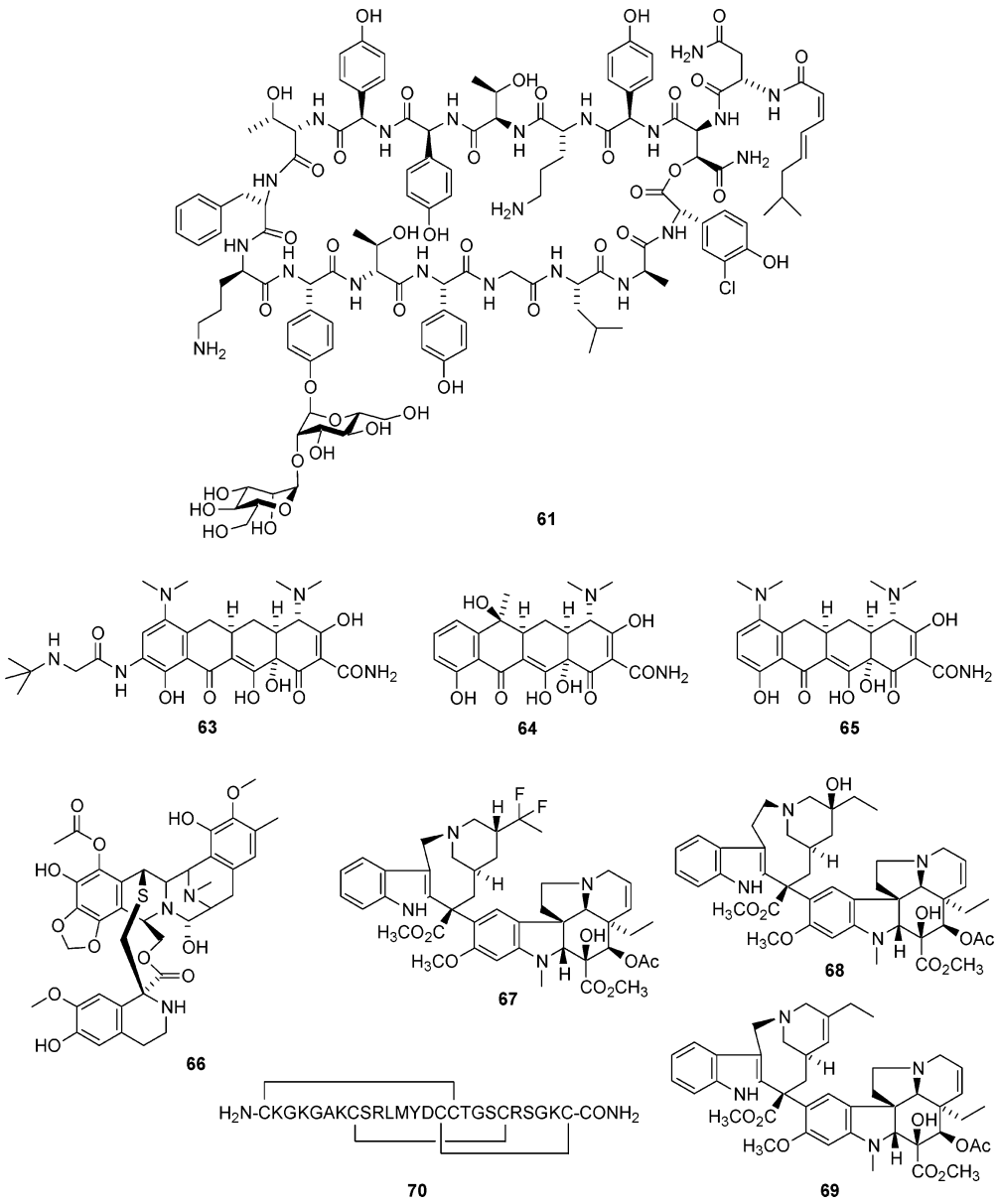


Table 4. Past and Present Scenarios of Drug Discovery Processes in Big Pharma Companies

past scenario	present scenario
limited numbers of synthetic compounds available	larger numbers of synthetic compounds available
natural products offered opportunity to increase chemical diversity	combinatorial chemistry can offer an alternative means to increase chemical diversity
natural products were one of the few methods available	introduction of methods such as •rational drug design[59,60] •SAR by NMR[61−64] •high-throughput X-ray crystallography[63−65] •new and improved synthetic methodologies available
limited biological targets available	large number of biological targets available from proteomics
screen lifetime of many months to several years	average screen lifetime usually less than 1 month
ample capacity for testing natural product extracts	increased pressure on screening slots due to increase in the number of synthetic compounds available for screening
sufficient time for natural product characterization	restricted time for natural product characterization

primary assay, and an FP orthogonal assay was described by Flotow and co-workers for the antimalarial target plasmepsin II.[109] The FRET and FP assays were used to eliminate most of the false-positive extracts, and the remaining extracts that underwent dereplication were devoid of color inference. Two of the active extracts identified were derived from the stem bark and leaves of the plant *Albizia adinocephala* (J.D.Sm.) Britt. & Rose ex Record (Leguminosae) collected from Panama. Bioassay-guided fractionation of the stem bark extract led to the isolation of two new macrocyclic spermine alkaloids, budmunchiamines L4 and L5, as the active components of the plant.[110]

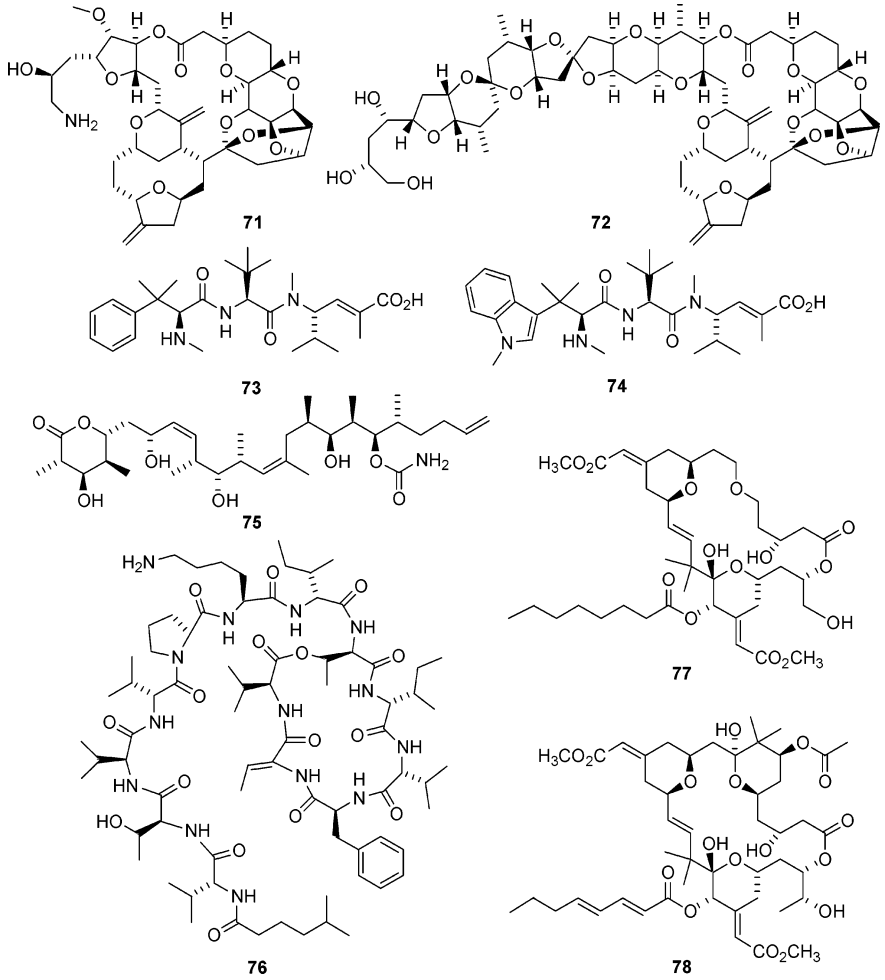
Screening of Crude Extracts, Prefractionated Extracts, or Pure NPs. Traditionally, crude extracts have

Table 5. Natural Product-Derived Compounds in Oncology Clinical Trials Produced by Total Synthesis

compound	lead compound	clinical trial (company)	comment
E7389 (**71**)[139]	halichondrin B (**72**)	Phase I (Eisai)	fully synthetic eastern hemisphere of halichondrin B
HTI-286 (**73**)[140]	hemiasterlin (**74**)	Phase I (Wyeth)	hemiasterlin not available in sufficient quantities for clinical trials
discodermolide (**75**)[141]	discodermolide (**75**)	Phase I (Novartis)	gram scale syntheses developed
ZK-EPO[a,142]	epothilone B (**57**)	Phase I (Schering AG)	first totally synthetic epothilone in clinical trials
kahalalide F (**76**)[143]	kahalalide F (**76**)	Phase II (PharmaMar)	compound for Phase II clinical trials made by total synthesis
bryolog (**77**)[b,144]	bryostatin 1 (**78**)	preclinical[c] (GPC Biotech)	simpler analogue of bryostatin

[a] Structure for ZK-EPO not available. [b] Representative structure of bryolog family. [c] In March 2004 GPC Biotech halted clinical studies of both the bryologs and bryostatin 1 (**78**).

Chart 5



been used for screening, but the extra screening capacity available from HTS has enabled the possibility of economically screening prefractionated extracts. The prefractionation process can produce fractions that can range from relatively crude fractions to mixtures of only a few compounds. Methods used to generate these libraries include solid absorbents [column chromatography/high-performance liquid chromatography (HPLC)][56−58,111−114] and liquid−liquid methods [countercurrent chromatography (CCC) and centrifugal partition chromatography (CPC)].[115−117] There are many pros and cons of screening extracts versus fractions. For example, screening prefractionated extracts will allow quicker identification and dereplication of extracts, allow profiling patterns between extracts to be examined, allow compounds whose activity is masked in a crude extract to be detected, and most importantly, identify active components present in amounts too small to detect by extract screening. However, by screening at higher concentrations, interfering/nuisance compounds will also be more frequently identified. There is also a significant cost involved in preparation and screening of the extra fractions generated by prefractionation. Finally, as the total number of prefractionated extracts screened is inversely proportional to the biodiversity screened, a decision must be made whether the loss of biological diversity is adequately compensated by prefractionation. The answer to this question will help determine the optimal number of fractions that should be generated for each extract.

The advent of sophisticated separation and analytical instruments has enabled some companies to assemble large libraries of pure NPs, which can then be screened in a manner analogous to pure compound libraries.[56−58] The advantage of these libraries is that no further purification is generally required, which enables active compounds to

be evaluated on an equivalent basis to synthetic compound libraries. The disadvantages are that it is a time-consuming process and minor active components may be missed using an isolation strategy based solely on peak collection.

Dereplication. The term "dereplication" is the process of identifying known compounds that are responsible for the activity of an extract before bioassay-guided isolation has started.[99,118−121] The dereplication process, which has been an ongoing concern in NP chemistry since the beginning of antibiotic research, is used to eliminate, group, and/or prioritize extracts for further study and can save considerable research time. The dereplication procedure also can be extended to group like-extracts that contain the same or similar unidentified compounds that are responsible for the biological activity. This grouping of extracts with like dereplication profiles significantly reduces the possibility of different chemists independently isolating and identifying the same active component. The most generic procedure used today involves separation of an extract using reversed-phase HPLC and splitting of the eluent postcolumn into a mass spectrometer (usually MS/MS to match fragmentation ions) and a fraction collector using microtiter plates to test tubes depending on the scale. The fractions are screened and the retention time, UV spectra, MS data, and activity of fractions are analyzed for common interfering compounds and known inhibitors using commercial and in-house databases. This method has been so successful that it has also been used in combinatorial chemistry to analyze reaction mixtures.[122]

Other methods for dereplication that have been used include separation by solid-phase extraction cartridges[121] and CCC.[115] Alternative methods for analysis include affinity capillary electrophoresis,[123] LC-NMR,[124−126] and on-line biochemical assay.[108]

Isolation and Structure Elucidation. The bioassay-guided fractionation procedure used to identify bioactive natural products is often perceived as rate limiting and resource intensive. However, the rapid improvement of instrumentation and robotics used to revolutionize other aspects of drug discovery can also be used to improve the speed of the isolation and structure elucidation of NPs. After dereplication, extracts then undergo bioassay-guided fractionation to ultimately provide the active compound or compounds. An increase in the speed of bioassay-guided fractionation has been facilitated by a marked improvement in HPLC automation, MS, and column technology, as well a rapid turnaround of screening results provided by HTS. The advent of new probe technology[127] and higher magnetic fields has led to a significant shortening in acquisition time for NMR data, and the structure elucidation of NPs can be achieved routinely on amounts less than 1 mg.[128]

Progress has also been made in automated structure-solving algorithms,[129−131] but presently none can rival the structure elucidation skills of an experienced NP chemist. However, these programs can be a valuable tool to search for alternative structures that fit the same NMR data.

Compound Development. Perhaps the biggest obstacle to NP chemistry is the continual resupply of large amounts of NP required for further biological evaluation. The identification of a sustainable source of the NP needs to be addressed if a semisynthesis or total synthesis is not available. This is not so much trouble if there is a microbial source of the compound, which explains why most companies prefer the screening of microorganism extracts. A systematic approach called OSMAC (one strain−many compounds) to increasing the yield and diversity of compounds produced by microorganisms was recently discussed by Zeeck and co-workers,[132] while advances are being achieved rapidly in the area of microbial combinatorial biosynthesis.[133] A recent review discussing the diversification of microbial NPs for drug discovery details other recent advances.[134] There has also been progress with organisms usually considered to be problematic. For example, the resupply of compounds from plants can be enhanced for minimal environmental damage by tissue culture[135] and genomic methods,[136] while study has continued into the aquaculture of marine invertebrates[137] and the identification, cloning, and expression of symbiotic microorganism genes.[138]

There is often also a worry that the complex nature of some NP chemical structures, which are critical for the biological activity of the compound, may impede the lead optimization process. However, over the past few years there has been significant effort devoted to the semisynthesis and synthesis of complex NPs. The success of this strategy is evident by the fact that 14 of the 15 NP-derived drugs launched from 2000 to 2003 (Table 2) are manufactured by either semisynthesis (5) or synthesis (9). Of these launched drugs, the vinblastine (**68**), vancomycin (**11**), and echinocandin [pneumocandin B (**21**) and FR901379 (**27**)] templates would be extremely difficult to synthesize in an economical manner. Similarly, of the 15 NP-derived compounds in clinical candidates at the end of 2003 (Table 3), 10 are produced by semisynthesis and four by total synthesis. This synthetic effort has been particularly evident for NP-derived anticancer drugs where total synthesis has been critical in lead optimization and for obtaining enough material for clinical trials (Table 5).

Conclusion

A common misperception has been that NP research has not kept pace with other drug discovery techniques and, as a consequence, become uncompetitive for lead discovery. However, improvements in instrumentation, robotics, and bioassay technology have increased the speed of bioassay-guided isolation and structure elucidation of NPs considerably, and these improvements have allowed NP research to be more competitive with synthetic compound screening. Another misconception has been that NP research has failed to deliver many new compounds that have undergone clinical evaluation over the last few years. However, in reality, 15 NP-derived drugs have been launched in the key markets of the United States, Europe, and Japan over the last three years, and an additional 15 NP-derived compounds were in Phase III clinical trials at the end of 2003. These NP-derived drugs also contribute considerably to the profitability of many Pharma and biotech companies. These factors, as well as an inadequate number of lead compounds in many therapeutic areas and the unique chemical space occupied by NPs, have led to a renewed interest in NP research. However, this renewed interest can be sustained only if NP research can continue to be competitive with other drug discovery techniques. Key factors to remaining competitive include continual improvements in the speed of dereplication, isolation, structure elucidation, and compound supply processes and prudent selection of drug targets for the screening of NP libraries.

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