IN THE HIGH COURT OF AUSTRALIA
SYDNEY REGISTRY

No. S28 of 2015

BETWEEN:

YVONNE D'ARCY
Appellant

and

MYRIAD GENETICS INC
First Respondent

GENETIC TECHNOLOGIES LIMITED ABN 17 009 212 328
Second Respondent

AFFIDAVIT

I, Sherry M. Knowles, of 400 Perimeter Center Terrace NE, Atlanta, Georgia, 30346, United States of America, attorney, state under oath as follows:

1. I am an intellectual property attorney with over 25 years of experience in global corporate and private practice, with a focus in pharmaceuticals and biotechnology. I am currently the Principal of Knowles Intellectual Property Strategies, LLC, a legal and consulting firm focused on providing global guidance on complex IP matters, including opinions and strategy, licensing, litigation, patent prosecution, obtaining and protecting the full value of innovation, investor support and monetization of assets.

Date of document:	11 March 2015
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2. From 2006-2010, I was the Senior Vice President and Chief Patent Counsel at GlaxoSmithKline, where I served as the worldwide head of patents for all litigation and transactional matters, and managed a global department of over 200 people in 12 offices. In this position, I was a member of the Product Management Board, the Technology Investment Board, the Scientific Advisory Board, the Legal Management Team (consisting of all of the direct reports to the General Counsel), and I chaired the Global Patents Management Team.

3. In 2008, Managing IP Magazine named me as one of the top 10 most influential people in Intellectual Property. In 2010, the New Jersey Intellectual Property Lawyers Association awarded GSK, with me as the representative, the Jefferson Medal for exceptional contribution to Intellectual Property. In 2010, Managing IP Magazine named the GSK Global Patent Team the "In-House IP Team of the Year" for 2009 for the constructive approach to IP in the developing world, the engagement with public policy in Europe and the successful resolution of the USPTO rules matter in the US.

4. In November 2011, Intellectual Asset Management Magazine listed me among the top fifty key individuals, companies and institutions that have shaped the IP marketplace in the last eight years. I am also listed in the IAM 250 "World's Leading IP Strategists," published by IAM Magazine in 2011 and the IAM 300 "World's Leading IP Strategists," published by IAM Magazine in 2012, 2013, 2014 and 2015. I am included in the list of Top 250 Women in IP by Managing IP Magazine for 2014.

5. I was Chair of the IP Subcommittee of PhRMA in 2008, and Chair Emeritus of the PhRMA IP Subcommittees in 2009 and 2010. From 2006-2010, I was a member of InterPat, which is the association of Chief Patent Counsels of the major pharmaceutical companies, and from 2008-2010 was a member of the Executive Committee of InterPat. I was the Chair of the work stream on data exclusivity for InterPat from 2006-2010.

6. Prior to working with GlaxoSmithKline, I spent almost 20 years in private law firm practice. I was a partner in and founder of the Biotechnology and Pharmaceutical Intellectual Property Practice at King & Spalding LLP, where I represented companies, foundations and universities in connection with patent prosecution, litigation, contracts, licensing, financing and other corporate intellectual property issues relating to pharmaceutical, biotechnology and chemical inventions.

7. I received my B.S. with distinction in chemistry from Duke University and received my M.S. in organic chemistry from Clemson University. Prior to attending law school, I spent several years at SmithKline Beecham (now GlaxoSmithKline) as a pharmaceutical synthetic chemist. I received my J.D., *magna cum laude*, from the University of Georgia where I was a Benjamin Phillips Scholar and was elected to the Order of the Coif.

8. I am an attorney qualified in the United States of America and admitted to practice before the U.S. Patent and Trademark Office, the U.S. Court of Appeals for the Federal Circuit and the state of Georgia.

9. I have been asked by the Institute of Patent and Trade Mark Attorneys of Australia to provide an affidavit describing my knowledge and experience regarding the importance of patent protection to the development of biologics and natural products and, in particular, patents which claim isolated molecules from natural products, *per se*.

Biological and natural products

10. The Natural Products Branch of the Developmental Therapeutics Program of the U.S. National Cancer Institute, a part of the US National Institutes of Health (**NIH**), has carried out a thirty year study of natural products as a source of new drugs. They have published reviews in 1997, 2003, 2007 and 2012. The data collected cover drugs developed in the period from January 1st 1981 to December 31st 2010 for all diseases world-wide, and from 1950 to December 2010 for all approved antitumor drugs world-wide. Now shown to me and

marked **Exhibit SMK-1** is a true copy of the 2012 publication by D. Newman and G. Craig, *J. Nat. Prod.* 2012 March 23; 5(3): 311-335.

11. The NIH article summarizes that from the 1940s through to the 2012 study, 48.6% of all anti-cancer agents have either been natural products or directly derived from them. Further, "the influence of natural product structures is quite marked with ... the anti-infective area being dependent on natural products and their structures." *Id.* p. 311.

12. During the years 1981-2010, the review identified 1355 new approved drugs. The article categorized approved drugs as biological ("B"), natural product ("N"), natural product (botanical) ("NB"), derived from a natural product (usually a semi-synthetic modification) ("ND"), totally synthetic ("S"), made by total synthesis but of a natural product ("S*"), a natural product mimic ("NM") or a vaccine ("V"). Among their specific observations are that:

    (a) During the review period, there were 15% B, 4% N, 22% ND, 29% S, 11%S/NM, 4% S*, 11% S*/NM, and 6% V.

    (b) The natural products field was still producing or was involved in about 50% of all small molecules in the years 2000-2010 (36.5% mean and 8.6% sd).

    (c) In 2010, half of the 20 approved small molecule NCEs fell into the "N" category including the majority of anti-tumor agents.

    (d) Overall, in the antibacterial area, "N" and "ND" compounds account for just under 75% of the approved agents.

    (e) For anti-cancer drugs, of 99 small molecules, 79 were either natural products or based on a natural product.

13. The Tufts Center for the Study of Drug Development on November 18, 2014, issued the results of their recent study which concluded that developing a new prescription medicine now takes longer than ten years at an estimated cost of

$2.5 billion dollars. This figure includes an average out-of-pocket cost of $1.4 billion dollars and a time cost (expected returns that investors forego while a drug is in development) of $1.1 billion dollars. Now shown to me and marked **Exhibit SMK-2** is a true copy of the November 2014 press release.

14. There are generally three kinds of patent claims that might cover an isolated natural product: product *per se* (also referred to as composition of matter), method of use and method of manufacture. Patent claims to the novel isolated natural product itself are typically required to obtain comfort by a company that market protection is strong enough to assure reimbursement and an adequate return. Companies usually base their long-range forecasts on projected patent protection from such product claims (as opposed to method of use or method of manufacture claims).

15. It is my experience based on 25 years in the field of pharmaceuticals and biotechnology intellectual property, that given the long time and high cost commitment of developing drugs, pharmaceutical companies would not proceed without assurance of sufficient market protection to recover the investment, to make a profit and to be compensated for the very high risk of failure.

16. It is also my experience based on 25 years in the field of pharmaceuticals and biotechnology IP, that corporations closely monitor the law and potential changes in the law, which can affect corporate behavior. Corporations prefer to invest their capital in projects that enjoy a well-settled expectation of long term legal stability and certainty.

17. As part of my practice, I have represented a number of venture capital and investment banking firms that have considered and continue to consider whether to invest in an emerging (i.e., pre-revenue) or growing small biotech or pharmaceutical company that has a drug in development. One of the main considerations during due diligence investigations is whether the bankers can be convinced that the patent position on the drug in development is solid and

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will protect the market and the investment. Where a product is not patentable or the law on the patentability of the product is weak or predicted to change, the investors are usually not interested in proceeding with investment.

18. As part of my practice, I have in addition represented a large number of emerging companies developing a range of pharmaceutical and biotech products, including isolated natural products. These companies make the decision whether to develop an identified drug, in significant part on the strength of the patent positions on the drugs they have identified as active. These companies typically select the drugs to advance based on whether they can obtain patent protection for a composition of matter, that is, the drug itself.

19. During my 25 years representing pharmaceutical and biotechnology clients, I am personally aware of numerous potential products which were not developed because the companies were not satisfied that sufficient patent protection would be available. One typical scenario is where it has been discovered that there is a new use for an old drug, and therefore the investment could only be protected through method of use or manufacture claims and not product claims per se. This scenario is analogous to the situation that would occur if an isolated natural product could not be patented as a product per se, and where the company would have to rely on claims to methods of use and manufacture. I am also aware of companies that stopped considering the development of a product after a change in the law that adversely affected the ability to maintain patent protection.

20. Of the drug categories in the NIH study, only the "S" category is clearly outside of the scope of being either a natural product or based on a natural product. If the law evolved that isolated natural products and their derivatives are not patentable, and projecting this back in time, this would leave 968 approved drugs at risk of no patent protection, and thus using the assumption that corporations act rationally and would not develop drugs without market protection, at risk of not ever having been developed at all. If the number is confined to biologicals, natural products, derivatives of natural products and

vaccines (often made of pieces of natural products), 47% of drugs would be at risk, or 636 drugs over a ten year period.

21. During the period from 1981 to 2010 for which the NIH collected its data, patent protection was available in the United States and many other jurisdictions around the world, including in Australia, in respect of inventions that would fall within the definition of "biological" or "natural product" used in the NIH review. I am also aware that patents were issued in respect of many of the natural product-based drugs set out in Table 1 below, *per se* (that is, the patents comprised or included claims to the drug itself compared with claims to methods of formulation of the drug or methods of treatment with the drug). For example, Epogen (erythropoietin or EPO) is the subject of numerous US patents and I understand is also the subject of Australian Patent No 660650. AU 600650 includes claims to isolated erythropoietin (EPO) and to nucleic acid sequences encoding human EPO. It was the work done by scientists at Amgen which lead to the isolation of the gene encoding human EPO. This development enabled for the first time the production of commercial quantities of EPO which resulted in the dramatic improvement in the welfare of patients undergoing dialysis and of patients receiving chemotherapy.

22. I have been working with Matthew J. Higgins, Ph.D., an Assistant Professor of Strategic Management at the Georgia Institute of Technology, and Faculty Research Fellow of the National Bureau of Economic Research, through the IMS Health and Pharmaprojects program, to collect data on the number of dosages of top-selling natural product therapeutics that were sold in the United States for a ten year period from 2001 to 2011 for a range of drugs. A sample summary of the ten year sales units for just several of these natural product-based drugs is set out in Table 1 below.

Table 1

Dosage Numbers sold in the U.S. for Selected Natural Product Drugs

Natural product	**Used to treat**	**Sales units**
Clavulanic acid	Bacterial infections	5,338,207,765
Penicillin	Bacterial infections	3,483,851,173
Tetracycline	Bacterial infections	1,922,758,255
Taxol	Cancer	1,554,822,780
Epogen	Anemia	384,546,232
Adriamycin	Cancer	10,433,433
Insulin	Diabetes	8,035,843
Vincristine	Cancer	4,994,779
Vinblastine	Cancer	1,230,034
Streptomycin	Bacterial infections	447,367
Total: 12,709,327,661 dosages		

23. Based on the data of just these ten selected top-selling natural product therapeutics, patients in the United States alone have benefited by taking almost 13 billion doses of these drugs that arguably would not have been patentable under a patent law holding that isolated natural products are not patentable, and thus in the main not commercialized or available.

The use of Natural Products in the treatment of Breast Cancer

24. According to the Susan G. Komen for the Cure Foundation, 2.9 million women alive now in the United States alone have experienced breast cancer. Globally, a woman is diagnosed with breast cancer every 19 seconds and a woman dies of breast cancer every 74 seconds.

25. The Susan G. Komen for the Cure Foundation has published that there are now eight common front line combination treatments for early and locally advanced breast cancer. Adriamycin, a fermentation natural product of bacteria is in five of the eight front line therapies, as shown in Table 2 below. Without the commercialization of Adriamycin with the expectation of patent protection, five out of the eight front line therapies for breast cancer would not exist, which would have dramatically increased the death rate from this disease.

Table 2

The Eight Front Line Treatments for Breast Cancer

ACTH	Adriamycin Cyclophosphamide Taxol Herceptin
CAF	Cyclophosphamide Adriamycin 5-Fluorouracil
AC	Adriamycin Cyclophosphamide
TAC	Taxotere Adriamycin Cyclophosphamide
AC---T	Adriamycin/Cyclophosphamide Followed by Taxol
AC---D	Adriamycin/Cyclophosphamide Followed by Docetaxel (Taxotere)
TC	Cyclophosphamide/Taxotere
TCH	Docetaxel, carboplatin and Herceptin

26. Now shown to me and marked **Exhibit SMK-3** is a true copy of U.S. Patent No. 3,590,028 claiming Adriamycin.

Recent developments in the United States

27. The Supreme Court of the United States addressed the patentability of isolated genes in *Association for Molecular Pathology v Myriad Genetics, Inc.*, 569 U.S. __ (2013) (Myriad). The decision has been deeply criticized by the majority of the U.S. Patent Bar as inconsistent with statutory law, bad policy and creating the consequence of adversely impacting the development of new isolated natural product-based drugs.

SMK

28. Following the decision in *Myriad*, the U.S. Patent and Trademark Office issued the "Procedure for Subject Matter Eligibility Analysis of Claims Reciting or Involving Laws of Nature/Natural Principals, Natural Phenomena and/or Natural Products" (March 14, 2014) which was also roundly criticized by the majority of the U.S. Patent Bar as expanding the already detrimental *Myriad* analysis and applying the expanded analysis to products that have not yet been litigated, therefore *de facto* expanding the *Myriad* isolated gene ruling to a host of other natural products, including chemicals derived from natural sources, antibiotics, fats, oils, petroleum derivatives, resins, toxins, foods, metals and metallic compounds, nucleic acids, organisms, proteins, peptides and other substances derived from nature.

29. The scope of the March 2014 U.S. PTO *Myriad* Guidance illustrates how difficult, or impossible, it is to cabin in a judicial ruling that isolated genes are not patentable and to prevent an extension of such law to create a loss of patent eligibility that is applied to all isolated natural products.

30. In December 2014, after substantial negative feedback from the U.S. Patent Bar, the U.S. Patent and Trademark Office withdrew the March 2014 Guidance and issued new Guidance in place thereof ("Interim Guidance on Patent Subject Matter Eligibility", December 16, 2014, referred to below as "revised *Guidance*"). The revised *Guidance*, however, did not decrease the wide scope of natural products caught in the net; it simply added a few illustrations of how a product isolated from nature might not be considered a natural product. The revised Guidance said that if a product isolated from nature is markedly different from the product in nature through a man-made transformation (not an inherent change due to isolation), then it may be considered outside of the definition of a natural product. The very small number of products this carve-out might apply to, if any, is demonstrated by the fact that the U.S. PTO continued to hold that isolated taxol (found in the bark of the Pacific yew tree), which is useful to treat cancer is not patentable *per se,* even though a patient

might have to eat an entire forest to get a therapeutic effect, and in doing so, would no doubt die in the process instead of being cured.

31. Based on my understanding of the revised *Guidance*, it appears that only those drugs which the NIH classifies as synthetic are likely outside of the terms of the revised *Guidance*. Applying this to the NIH data set for all drugs developed from 1981 to 2010, at least 47 per cent of those drugs would be given close scrutiny and could be at risk of not being entitled to patent protection per se. The drugs particularly at risk would be (i) bacterial fermentation products that are often the basis for antibiotics, which are now in critical demand due to the emergence of drug-resistant bacteria, and which may also have anti-cancer properties (ii) human antibodies, that are used to treat a host of disorders, including cancer, and (iii) vaccines which are made of one or a mixture of naturally occurring proteins or protein fragments.

32. It has now been about one year since the first guidance was issued and several months since the revised *Guidance* has been issued. I am aware that it is having a significant negative effect on the prosecution of patent application claims to isolated natural products in the U.S.

33. Hans Sauer, the Associate General Counsel for Intellectual Property of the Biotechnology Industry Organization ("BIO") stated publicly at a forum on the Guidelines held at the U.S. PTO on January 21, 2015 that:

> "BIO's members continue to be concerned with patentability in the United States. Few areas of substantive patent law have received as much discussion within BIO's community. BIO's members view the development of extra-statutory law in this area as a significant departure from internationally accepted norms of patentability with negative implications for innovative, industrial, agricultural and pharmaceutical products and processes. Inventive preparations based on naturally occurring substances have historically been of great importance in biotechnology and innovation in this area has been spurred by, at least in part, by the availability of patent protection. This is true for every sector of biotechnology; examples include vaccine antigens, crop protection products, plant biotechnology, industrial enzymes,

immunosuppressants, anti-cancer substances and antibiotic drugs."

Effect of denial of patent protection for biologics and natural products

34. As discussed above, based on my professional experience and my interactions with other people who are involved in the business of developing and commercializing new drugs, the availability of patent protection for a new drug *per se* is an important factor in a company's decision whether or not to invest in the development and commercialization of the new drug.

35. Based on my understanding of patent law and science, and my professional experience, it is my view that the U.S. Supreme Court decision in the *Myriad* case that isolated gene products are not patentable as natural products, is wrong on its face, inconsistent with years of precedent to the contrary, and opens a Pandora's Box of seriously negative downstream effects as seen in the U.S. PTO's revised *Guidance* expanding its scope, and rejections of pending patent applications on subject matter caught in the web. The highest public interest is human health. The U.S. *Myriad* decision and its' expansive interpretation and applications are likely to have, and is having, a detrimental effect on the development of new drugs based on biologics and natural products and medical treatment of humans with such drugs, such as those drugs of the same type as described in the NIH review and the dosage sales data provided above.

SWORN by the deponent
at Atlanta, Georgia on 11 March 2015.

Before me:

Signature of deponent

Kristi L. Rebel, Notary Public

Sherry M. Knowles

KRISTI REBEL
NOTARY PUBLIC
My Commission Expires 04-29-2016
DEKALB COUNTY, GEORGIA

IN THE HIGH COURT OF AUSTRALIA
SYDNEY REGISTRY No. S28 of 2015

Affidavit of Sherry M. Knowles sworn on 11 March 2015

INDEX OF EXHIBITS

EXHIBIT	DESCRIPTION	PARAGRAPH	PAGE
SMK-1	'Natural Products as Sources of New Drugs over the 30 Years from 1981 to 2010'	10	17
SMK-2	Press release from The Tufts Center for the Study of Drug Development dated November 18, 2014	13	72
SMK-3	U.S. Patent No. 3,590,028 claiming Adriamycin	26	77

10

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BETWEEN:

YVONNE D'ARCY
Appellant

and

MYRIAD GENETICS INC
First Respondent

GENETIC TECHNOLOGIES LIMITED ABN 17 009 212 328
Second Respondent

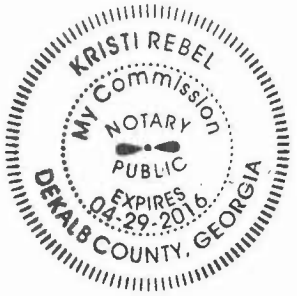
EXHIBIT SMK-1

This is the exhibit marked **Exhibit SMK-1** produced and shown to Sherry M. Knowles at the time of swearing her affidavit this 11 March 2015.

'Natural Products as Sources of New Drugs over the 30 Years from 1981 to 2010'

Before me

Kristi L. Rebel, Notary Public



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Natural Products as Sources of New Drugs over the 30 Years from 1981 to 2010†

David J. Newman* and **Gordon M. Cragg**
Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute-Frederick, P. O. Box B, Frederick, MD, 21702

Abstract

This review is an updated and expanded version of the three prior reviews that were published in this journal in 1997, 2003 and 2007. In the case of all approved therapeutic agents, the time frame has been extended to cover the 30 years from January 1st 1981 to December 31st 2010 for all diseases world-wide, and from 1950 (earliest so far identified) to December 2010 for all approved antitumor drugs world-wide. We have continued to utilize our secondary subdivision of a "natural product mimic" or "NM" to join the original primary divisions, and have added a new designation "natural product botanical" or "NB" to cover those botanical "defined mixtures" that have now been recognized as drug entities by the FDA and similar organizations. From the data presented, the utility of natural products as sources of novel structures, but not necessarily the final drug entity, is still alive and well. Thus, in the area of cancer, over the time frame from around the 1940s to date, of the 175 small molecules, 131 or 74.8% are other than "S" (synthetic), with 85 or 48.6% actually being either natural products or directly derived there from. In other areas, the influence of natural product structures is quite marked with, as expected from prior information, the anti-infective area being dependent on natural products and their structures. Although combinatorial chemistry techniques have succeeded as methods of optimizing structures, and have been used very successfully in the optimization of many recently approved agents, we are only able to identify only one de novo combinatorial compound approved as a drug in this 30-year time frame. We wish to draw the attention of readers to the rapidly evolving recognition that a significant number of natural product drugs/leads are actually produced by microbes and/or microbial interactions with the "host from whence it was isolated", and therefore we consider that this area of natural product research should be expanded significantly.

Introduction

It is fourteen years since the publication of our first,[1] eight years since the second,[2] and four years[3] since our last full analysis of the sources of new and approved drugs for the treatment of human diseases, although there have been intermediate reports in specific areas such as cancer,[4, 5] and anti-infectives,[6] together with a more general discussion on natural products as leads to potential drugs.[7] All of these articles demonstrated that natural product and/or

†Dedicated to Dr. Gordon M. Cragg, Chief of the NCI's Natural Products Branch from 1989 to 2004, for his pioneering work on bioactive natural products and on a more personal note, for his advice, support and friendship to me (DJN) over the last twenty-plus years. May his advice and help continue for a long time into the future.

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Supplementary Information **Available**. An Excel 2003 workbook with the full data sets is available free-of-charge via the Internet at http://pubs.acs.org

The opinions discussed in this review are those of the authors and are not necessarily those of the U.S. Government

natural product structures continued to play a highly significant role in the drug discovery and development process.

That Nature in one guise or another has continued to influence design of small molecules is shown by inspection of the information given below, where with the advantage of now 30 years of data, the system has been able to be refined. We have eliminated some duplicated entries that crept into the original datasets and have revised a few source designations as newer information has been obtained from diverse sources. In particular, as behooves authors from the National Cancer Institute (NCI), in the specific case of cancer treatments, we have continued to consult the records of the FDA, and added comments from investigators who have informed us of compounds that may have been approved in other countries and that were not captured in our earlier searches. As was done previously, the cancer data will be presented as a stand-alone section from the beginning of formal chemotherapy in the very late 1930s or early 1940s to the present, but information from the last 30 years will be included in the datasets used in the overall discussion.

A trend mentioned in our 2003 review[2] in that though the development of high-throughput screens based on molecular targets had led to a demand for the generation of large libraries of compounds, the shift away from large combinatorial libraries that was becoming obvious at that time has continued, with the emphasis now being on small focused (100-~3000 plus) collections that contain much of the "structural aspects" of natural products. Various names have been given to this process, including "diversity oriented syntheses",[8-12] but we prefer to simply refer to "more natural product-like", in terms of their combinations of heteroatoms and significant numbers of chiral centers within a single molecule,[13] or even"natural product mimics" if they happen to be direct competitive inhibitors of the natural substrate. It should also be pointed out that Lipinski's fifth rule effectively states that the first four rules do not apply to natural products nor to any molecule that is recognized by an active transport system when considering "druggable chemical entities".[14-16] Recent commentaries on the "industrial perspective in regard to drug sources[17] and high throughput screening[18] have been published by the GSK group and can be accessed by interested readers.

Although combinatorial chemistry in one or more of its manifestations has now been used as a discovery source for approximately 70% of the time covered by this review, to date, we still can only find one de novo new chemical entity (NCE) reported in the public domain as resulting from this method of chemical discovery and approved for drug use anywhere. This is the antitumor compound known as sorafenib (Nexavar®, **1**) from Bayer, approved by the FDA in 2005 for treatment of renal cell carcinoma, and then in 2007, another approval was given for treatment of hepatocellular carcinoma. It was known during development as BAY-43-9006 and is a multi-kinase inhibitor, targeting several serine/threonine and receptor tyrosine kinases (RAF kinase, VEGFR-2, VEGFR-3, PDGFR-beta, KIT and FLT-3). It has been approved in Switzerland, the European Union and the People's Republic of China, with additional filings in other countries. Currently, it is still in multiple clinical trials in both combination and single agent therapies, a common practice once a drug is approved for an initial class of cancer treatment.

As mentioned by the present authors and others in prior reviews on this topic, the developmental capability of combinatorial chemistry as a means for structural optimization, once an active skeleton has been identified, is without par. An expected surge in productivity however, has not materialized. Thus, the number of new active substances (NASs) from our dataset, also known as New Chemical Entities (NCEs), which we consider to encompass all molecules, including biologics and vaccines, hit a 24-year low of 25 in 2004 (although 28% of these were assigned to the ND category), leading to a rebound to 54 in 2005, with 24% being N or ND and 37% being biologics (B) or vaccines (V), as we

discuss subsequently. The trend to small numbers of approvals continues to this day as can be seen by inspection of Figures 2 and 4 (see Discussion section below).

Fortunately, however, research being conducted by groups such as Danishefsky's, Ganesan's, Nicolaou's, Porco's, Quinn's, Schreiber's, Shair's, Tan's, Waldmann's, and Wipf's, together with those of other synthetic chemists, is continuing the modification of active natural product skeletons as leads to novel agents. This was recently exemplified by the groups of Quinn[19] and Danishefsky[20] or the utilization of the "lessons learned" from studying such agents as reported by the groups of Tan[21, 22] and Kombarov[23] to just some of the some recent publications. Thus, in due course, the numbers of materials developed by linking Mother Nature to combinatorial synthetic techniques should increase. These aspects, plus the potential contributions from the utilization of genetic analyses of microbes will be discussed at the end of this review.

Against this backdrop, we now present an updated analysis of the role of natural products in the drug discovery and development process, dating from 01/1981 through 12/2010. As in our earlier analyses,[1-3] we have consulted the *Annual Reports of Medicinal Chemistry* in this case from 1984-2010,[24-50] and have produced a more comprehensive coverage of the 1981-2010 time frame through addition of data from the publication, *Drug News and Perspective*,[51-71] and searches of the Prous (now Thomson-Reuter's *Integrity*™) database, as well as by including information from individual investigators. As in the last review, biologics data prior to 2005 were updated using information culled from disparate sources that culminated in a 2005 review on biopharmaceutical drugs.[72] We have also attempted to capture vaccine data in the last few years, but this area of the database is not as complete as we would hope.

We have also included relevant references in a condensed form in Tables 2-5 and 8-10. If we were to provide the full citations, the numbers of references cited in the present review would become overwhelming. In these tables, "ARMC ##" refers to the volume of *Annual Reports in Medicinal Chemistry* together with the page on which the structure(s) and commentary can be found. Similarly, "DNP ##" refers to the volume of *Drug News and Perspective* and the corresponding page(s), though this journal has now ceased publication as of the 2010 volume, and an "I######" is the accession number in the Prous (now Thomson-Reuters, *Integrity*™) database. Finally, we have used "Boyd" to refer to a review article[73] on clinical antitumor agents and "M'dale" to refer to *Martindale*[74] with the relevant page noted.

It should be noted that the "Year" header in all tables is equivalent to the "Year of Introduction" of the drug. In a number of cases over the years, there are discrepancies between sources as to the actual year due to differences in definitions. Some reports will use the year of approval (registration by non-USA/FDA organizations) while others will use the first recorded sales. We have generally taken the earliest year in the absence of further information.

Results

As in previous reviews, we have only covered New Chemical Entities (NCEs) in the present analysis. As mentioned in the earlier reviews, if one reads the FDA and PhRMA web sites, the numbers of NDA approvals are in the high ten to low hundred numbers for the last few years. If, however, combinations of older drugs and old drugs with new indications, and/or improved delivery systems are removed, then the number of true NCEs has ranged between the 20s to just over 50 per year since 1989. If one now removes biologicals and vaccines thus noting only "small molecules", then the figures show that over the same time frame, the

numbers have ranged from close to 40 for most of the 1989 to 2000 time frame, dropping to 20 or less from 2001 to 2010 with the exception of 2002 and 2004 when the figures climbed above 30 (cf., Figures 2 and 4 below).

For the first time, now with 30 years of data to analyze, it was decided to add two other graphs to the listings, of which one might be of significant interest to the natural products community. In Figure 5 the percentage of approved NCEs have been plotted per year from 1981 to 2010 where the designation is basically an "N" or a subdivision ("NB" or "ND") with the total numbers of small molecules approved by year as a point chart in Figure 6. Thus, we have deliberately not included any designations that could be considered as "inspired by a natural product structure", although from the data provided either in the tables or from the supporting information, any reader who so desires, may calculate their own particular variation(s) on Figure 5.

As in our earlier reviews,[1-3] the data have been analyzed in terms of numbers and classified according to their origin using the previous major categories and their subdivisions.

Major Categories of Sources

The major categories used are as follows:

"B" Biological; usually a large (>45 residues) peptide or protein either isolated from an organism/cell line or produced by biotechnological means in a surrogate host.

"N" Natural product.

"NB" Natural product "Botanical" (in general these have been recently approved).

"ND" Derived from a natural product and is usually a semi-synthetic modification.

"S" Totally synthetic drug, often found by random screening/modification of an existing agent.

"S*" Made by total synthesis, but the pharmacophore is/was from a natural product.

"V" Vaccine.

Sub-category

"NM" Natural Product Mimic (see rationale and examples below) (For amplification as to the rationales used for categorizing using the above subdivisions, the reader should consult the earlier reviews.[1-3])

In the field of anticancer therapy, the advent in 2001 of Gleevec®, a protein tyrosine kinase inhibitor, was justly heralded as a breakthrough in the treatment of leukemia. This compound was classified as an "/NM" on the basis of its competitive displacement of the natural substrate, ATP, in which the intracellular concentrations can approach 5 mM. We have continued to classify PTK and other kinase inhibitors that are approved as drugs under the "/NM" category for exactly the same reasons as elaborated in the 2003 review,[2] and have continued to extend it to cover other direct inhibitors/antagonists of the natural substrate/receptor interaction whether obtained by direct experiment or by in silico studies followed by direct assay in the relevant system.

Similarly, a number of new peptidic drug entities, although formally synthetic in nature, are simply produced by synthetic methods rather than by the use of fermentation or extraction. In some cases, an end group might have been changed for ease of recovery. In addition, a number of compounds produced totally by synthesis, are in fact isosteres of the peptidic substrate and are thus "natural product mimics" in the truest sense of the term. For further

information on this area, interested readers should consult the excellent earlier review by Hruby,[75] his 2009 "Perspective" review,[76] and very recent work in the same area by Audie and Boyd [77] and VanHee et al.[78] in order to fully appreciate the potential of such (bio)chemistry.

As an example of what can be found by studies around relatively simple peptidomimics of the angiotensin II structure, the paper of Wan et al.[79] demonstrating the modification of the known but non-selective AT_1/AT_2 agonist, L-162313 (**2**, itself related to the sartans), into the highly selective AT_2 agonist **3** (a peptidomimetic structure), led to the identification of short pseudopeptides exemplified by **4**, which is equipotent (binding affinity = 500 pM) with angiotensin II and has a better than 20,000-fold selectivity versus AT_1, whereas angiotensin II has only a five-fold binding selectivity in the same assay,[80] as reported in our 2007 review. The chemistry leading to these compounds was reported in 2007 in greater detail by Georgsson et al.[81] with a thorough discussion of the role of AT_2 receptors in a multiplicity of disease states being published in 2008.[82] To date, we have not found any clinical trials reported on these materials.

In the area of modifications of natural products by combinatorial methods to produce entirely different compounds that may bear little if any resemblance to the original, but are legitimately assignable to the "/NM" category, citations are given in previous reviews.[8, 83-90] In addition, one should consult the reports from Waldmann's group[91,92] and those by Ganesan,[93,94] Shang and Tan,[95] Bauer et al.[21] Constantino and Barlocco,[96] Bade et al.[97] and Violette et al.[98] demonstrating the use of privileged structures as a source of molecular skeletons around which one may build libraries. Another paper of interest in this regard is the editorial by Macarron from GSK,[15] as this may be the first time where data from industry on the results of HTS screens of combichem libraries versus potential targets was reported with a discussion of lead discovery rates. In this paper, Macarron re-emphasizes the fifth Lipinski rule, which is often ignored; "natural products do not obey the other four".

Overview of Results

The data we have analyzed in a variety of ways are presented as a series of bar graphs and pie charts and two major tables in order to establish the overall picture, and then are further subdivided into some major therapeutic areas using a tabular format. The time frame covered is the 30 years from 01/01/1981 - 12/31/2010:

•New Approved Drugs:	With all source categories (Figure 1)
•New Approved Drugs:	By source/year (Figure 2)
•Sources of all NCEs:	Where four or more drugs were approved per medical indication (Table 1), with listings of diseases with < 3 approved drugs
•Sources of Small-Molecule NCEs:	All subdivisions (Figure 3)
•Sources of Small-Molecule NCEs:	By source/year (Figure 4)
•Percent N/NB/ND:	By year (Figure 5)
•Total Small Molecules:	By year (Figure 6)
•Antibacterial Drugs:	Generic and trade names, year, reference and source (Table 2)
•Antifungal Drugs	Generic and trade names, year, reference and source (Table 3)
•Antiviral Drugs	Generic and trade names, year, reference and source (Table 4)
•Antiparasitic Drugs	Generic and trade names, year, reference and source (Table 5)
•Antiinfective Drugs	All molecules, source and numbers (Table 6)
•Antiinfective Drugs	Small molecules, source and numbers (Table 7)
•Anticancer Drugs	Generic and trade names, year, reference and source (Table 8; Figure 7)

•All Anticancer Drugs (very late 1930s-12/2010)	Generic and trade names, year, reference and source Table 9; Figures 8, 9)
•Antidiabetic Drugs	Generic and trade names, year, reference and source (Table 10)

The extensive datasets shown in the figures and tables referred to above highlight the continuing role that natural products and structures derived from or related to natural products from all sources have played, and continue to play, in the development of the current therapeutic armamentarium of the physician. Inspection of the data shows the continued important role for natural products in spite of the current greatly reduced level of natural products-based drug discovery programs in major pharmaceutical houses.

Inspection of the rate of NCE approvals as shown in Figures 2, and 4 - 6 demonstrates that even in 2010, the natural products field is still producing or is involved in ca. 50% of all small molecules in the years 2000 – 2010. This is readily demonstrated in Figures 5 and 6 where the percentage of just the "N" linked materials is shown, with figures ranging from a low of 20.8% in 2009, to a high of 50% in 2010, with the mean and standard deviation for those 11 years being 36.5 ± 8.6, without including any of the natural product inspired classifications (S*, S*/NM and S/NM). What is quite fascinating is that in 2010, fully half of the 20 approved small molecule NCEs fell into the "N" categories, including the majority of the antitumor agents (cf., Tables 2 – 4; 8).

As was shown in the 2007 review, a significant number of all NCEs still fall into the categories of biological ("B") or vaccines ("V"), with 282 of 1355 or (20.8%) over the full 30-year period, and it is to be admitted that not all of the vaccines approved in these 30 years have been identified, although in the last 10 or 11 years probably a great majority have been captured. Thus, the proportion of approved vaccines may well be higher over the longer time frame. Inspection of Figure 2 shows the significant proportion that these two categories hold in the number of approved drugs from 2000, where, in some years, these categories accounted for ca. 50% of all approvals. If the three "N" categories are included then the proportions of nonsynthetics are even higher for these years. This is so in spite of many years of work by the pharmaceutical industry devoted to high-throughput screening of predominately combinatorial chemistry products, and this time period should have provided a sufficient time span for combinatorial chemistry work from the late 1980s onwards to have produced a number of approved NCEs.

Overall, of the 1355 NCEs covering all diseases/countries/sources in the years 01/1981-12/2010, and using the "NM" classifications introduced in our 2003 review,[2] 29% were synthetic in origin, thus demonstrating the influence of "other than formal synthetics" on drug discovery and approval (Figure 1). In the 2007 review, the corresponding figure was 30%.[3]

Inspection of Table 1 demonstrates that overall, the major disease areas that have been investigated (in terms of numbers of drugs approved) in the pharmaceutical industry continue to be infectious diseases (microbial, parasitic and viral), cancer, hypertension, and inflammation, all with over 50 approved drug therapies. It should be noted however, that numbers of approved drugs/disease do not correlate with the "value" as measured by sales. For example, the best selling drug of all is atorvastatin (Lipitor®), a hypocholesterolemic descended directly from a microbial natural product, which sold over $(U.S.) 11 billion in 2004, and, if one includes sales by Pfizer and Astellas Pharma over the 2004 to 2010 time frames, sales have hovered between $(U.S.) 12-14 billion depending upon the year. The first US patent for this drug expired in March 2010 and Ranbaxy, the Indian generics company launched the generic version in the U.S.A. in December 2011, following FDA approval on the last day of the Pfizer patent, November 30th, 2011.

The major category by far is that of antiinfectives including antiviral vaccines, with 270 (23.9%) of the total (1130 for indications ≥4) falling into this one major human disease area. On further analysis (Tables 6 and 7), the influence of biologicals and vaccines in this disease complex is such that only 22.6% are synthetic in origin (Table 6). If one only considers small molecules (reducing the total by 77 to 193; Table 7), then the synthetic figure goes up to 31.6%, marginally greater than in our previous report.[3] As reported previously,[1-3] these synthetic drugs tend to be of two basic chemotypes, the azole-based antifungals and the quinolone-based antibacterials,

Six small-molecule drugs were approved in the antibacterial area from 01/2006 to 12/2010. Three were classified as ND, with the first retapamulin (**5**) being a semisynthetic modification of the well known pleuromutilin structure by GSK in 2007, the second being ceftobiprole medocaril, a cephalosporin prodrug (**6**) from the Roche spin-off company Basilea in 2008 in Switzerland and Canada. The compound was later withdrawn as of September 2010 by Basilea/Janssen-Cilag (J&J) and it is currently back in Phase III trials, with Johnson and Johnson having terminated their license. The third agent was the modified vancomycin, telavancin (**7**) by Astellas Pharma in conjunction with Theravance in 2009. The three synthetic antibacterials in this time frame were the fluoroquinolones, garenoxacin (**8**) from Astellas Pharma in 2007, sitafloxacin from Daiichi (**9**) in 2008, and besifloxacin (**10**) from Bausch and Lomb in 2009. Overall, in the antibacterial area, as shown in Table 7, small molecules account for 104 agents, with "N" and "ND" compounds accounting for just under 75% of the approved agents.

In the antifungal area, only one drug was approved in the 2006 to 2010 time frame. This was the echinocandin derivative, anidulafungin (ND; **11**) approved for use in the USA in early 2006 and was covered in the 2007 review but without a structure. As is the case with a significant number of compounds, the final company was not the originator. This molecule was first synthesized by Lilly under the code number LY-303366, then licensed to Versicor in 1999; Versicor became Vicuron in 2003 and Pfizer purchased Vicuron in 2005.

In contrast to the antibacterial case, in the antifungal area, as shown in Table 7, small molecules account for 28 agents, but in the 30 years of coverage, only three agents fall into the "ND" category, accounting for just over 10% of the approved drugs. This can be seen in the treatment regimens that still use agents such as amphotericin and griseofulvin, which are both listed in the Integrity™ database as being launched in 1958.

In the antiviral area, a very significant number of the agents are vaccines, as mentioned earlier, predominately directed against various serotypes of influenza, as would be expected from the avian flu outbreaks. In the time frame 2006 to 2010, and looking at small molecules, seven drugs were approved for a variety of viral diseases. In contrast to the previous reviews,[1-3] the number of anti-HIV drugs decreased with only three being reported in the four years since the previous report. These were darunavir (S/NM, **12**) in 2006 from Tibotec/Janssen, an HIV protease inhibitor, the first HIV attachment inhibitor, maraviroc (S, **13**), in 2007, from the joint venture between Pfizer and GSK on anti-HIV therapies, and, in the same year the first integrase inhibitor, raltegravir (S, **14**) by Merck. Of definite import during the last five years, however, is the approval of two new drugs for the treatment of hepatitis B in 2006, The first, telbivudine, a simple thymine analogue that is a DNA-polymerase inhibitor with a 2-deoxyribose derivative as the sugar moiety (S*, **15**), was licensed in from Idenix by Novartis. The second, clevudine (S*, **16**), with the same mechanism of action, is also a thymine derivative, but, in this case, the sugar moiety is further substituted by a fluorine atom on the sugar compared to telbivudine. This compound was originally identified at Yale University and the University of Georgia, then was licensed

by the Korean company Bukwang, who then sub-licensed it to Eisai for further development.

The last two compounds, both of which were approved in 2010, are small-molecule inhibitors of the influenza virus.[99] The first, peramivir (S/NM, **17**) can be considered as a successful in silico derivative as it was modeled into the sialidase crystal structure by BioCryst (Birmingham, AL) who subsequently licensed it to Green Cross and then Shionogi in Japan for treatment of influenza A and B. The second molecule, laninamivir (ND, **18**), is basically similar in structure to both zanamivir (1999, ND, **19**) and oseltamivir (1999, ND, **20**), both modeled on *N*-acetyl-neuraminic acid (**21**, the substrate of the sialidases), and for which synthetic routes can come from either quinic acid (**22**) or shikimic acid (**23**),[100] with the latter compound being produced from the star anise plant, *Illicium anisatum*,[101] or via fermentation of genetically modified *E. coli* strains.[102, 103]

In contrast to the antibacterial and antifungal areas, in the antiviral case, as shown in Table 7, small molecules account for 48 drugs, with only four (or 8%) in the 30 years of coverage falling into the "ND" category. However, consistently we have placed modified nucleosides and peptidomimetics, etc., as falling into the "S*" or "S*/NM" categories. If these are added to the four drugs listed above, then the other than synthetic molecules account for 37 or 57% overall.

As reported in our earlier analyses,[1-3] there are still significant therapeutic classes where the available drugs are totally synthetic at the present time. These include antihistamines, diuretics, and hypnotics for indications with four or more approved drugs (cf., Table 1), and, as found previously, there are still a substantial number of indications in which there are three or less approved drugs that are also totally synthetic. As mentioned in our earlier reviews,[2,3] due to the introduction of the "NM" subcategory, indications such as antidepressants, bronchodilators and cardiotonics now have substantial numbers that, although formally "S" or "S*", fall into the "S/NM" or "S*/NM) subcategories, as the information in the literature points to their interactions at active sites as competitive inhibitors.

With anticancer drugs (Table 8), where in the time frame covered (01/1981-12/2010) there were 128 NCEs in toto, with the number of non-biologicals aka small molecules being 99 (77%), a slightly lower percentage compared to the last review's value of 81%.[3] Using the total of 99 as being equal to 100%, the breakdown was as follows, with the values from the last review inserted for comparison: N (11, 11.1% {9, 11.1%}), NB (1, 1% {none}), ND (32, 32.3% {25; 30.9%}), S (20, 20.2% {18, 22.2%}), S/NM (16, 16.2% {12, 14.8%}), S* (11, 11.1% {11, 13.6%}) and S*/NM (8, 8.1% {6, 7.4%}). Thus, using our criteria, only 20.2% of the total number of small-molecule anticancer drugs was classifiable into the S (synthetic) category. Expressed as a proportion of the non-biologicals/vaccines, then 79 of 99 (79.8%) were either natural products per se or were based thereon, or mimicked natural products in one form or another.

In this current review, we have continued as in our previous contribution (2007)[3] to reassess the influence of natural products and their mimics as leads to anticancer drugs from the beginnings of antitumor chemotherapy in the very late 1930s to early 1940s. By using data from the FDA listings of antitumor drugs, coupled to our previous data sources and with help from Japanese colleagues, we have been able to specify the years in which all but 18 of the 206 drugs listed in Table 9 were approved. We then identified these other 18 agents by inspection of three time-relevant textbooks on antitumor treatment,[73, 104, 105] and these were added to the overall listings using the lead authors' names as the source citation.

Inspection of Figure 9 and Table 9 shows that, over the whole category of anticancer drugs approved world-wide, the 206 approved agents can be categorized as follows: B (26; 13%), N (27; 13%), NB (1; 0.5%), ND (57; 28%), S (44; 21%), S/NM (18; 9%), S* (20; 10%), S*/NM (8; 4%) and V (5; 2%). If one then removes the high molecular weight materials (biologicals and vaccines), reducing the overall number to 175 (100%), the number of naturally inspired agents (i.e., N, ND, S/NM, S*, S*/NM) is 131 (74.9%). Etoposide phosphate and various nanopaticle formulations of Taxol® have been included for the sake of completeness.

There are at least two points of definitive interest to natural products scientists in these figures over the last few years, in particular in the last four (2006-2010), when the sources of approved antitumor drugs are considered. Thus, the first antitumor agent that is a "botanical" (or NB), polyphenon E, was approved by the FDA in 2007 for treatment of genital warts linked to human papilloma viruses (HPV),[106] though one can argue from a chemical aspect that Curaderm®, which is a mixture of solamargines and was approved in 1989, was the first of these. We have now listed it as an "NB" rather than an "N" in Table 8. Polyphenon E is currently in a number of trials against various cancers as both a preventative and as a direct agent against chronic lymphocytic leukemia, bladder and lung cancers at the Phase II level, and in breast cancer at Phase I level, with a number of trials being sponsored by NCI.

What is perhaps of equal or perhaps higher significance, is that if one looks at the seven antitumor agents approved in 2010, roughly 20 years after the move away from natural product-based discovery programs by big pharmaceutical companies, then one, romidepsin (**24**) an histone deacetylase inhibitor (HDAC) is a microbial natural product[107-110] without any modification, and, although it has been synthesized, this compound is still produced by fermentation. Of the remaining six, four are derived from natural products, with three, vinflunine (**25**), cabazitaxel (**26**) and the totally synthetic halichondrin B-derived eribulin (**27**), being tubulin-interactive agents, but all binding to different sites on tubulin. Although the vinca and taxane sites are reasonably well described, eribulin appears to bind to site(s) that are different from these.[111, 112] The remaining one in this category, mifamurtide (**28**), is a derivatized muramyl dipeptide approved for the treatment of osteosarcoma.[113] The remaining small molecule, miriplatin hydrate (**29**) is totally synthetic, and is a new member of a very old class, the platinates, although its structure is dissimilar to others in the class in having what might be described as myristyl ester linkages to the platinum atom, giving it significant lipid solubility.[114]

In our earlier papers, the number of non-synthetic antitumor agents approximated 60% for other than biological/vaccines, without using the "NM" subcategory. The corresponding figure obtained by removing the NM subcategory in this analysis is 60%. Thus, the proportion has remained similar in spite of some reassignments of sources and the continued use of combinatorial chemistry as a source of test substances.

In the case of the antidiabetic drugs, both for diabetes I and II, the numbers since our last review have increased by five from 32 to 37 (Table 10), with one of the five falling into the "ND" category (cf., discussion on liragultide below). However, one biologic for which much was expected, being the first inhaled product, Exubera®, was approved in 2005 by the FDA and then withdrawn in 2008. We have, however, still included it in the tabulation. Four of the other five fall into the S/NM category, but the remaining one, liraglutide,[115] is a very interesting derivative of the glucagon-like peptide-1 (GLP-1) and can best be described as [*N*ε-[(*N*α-hexadecanoyl)-γ-L-Glu]-L-Lys26,L-Arg34]-GLP-1(7-37), where two amino acids have been changed in the 7 to 37 portion of the sequence, followed by addition of lipid "tails". Further information on the utility of GLP-1 agonists can be found in the very recent review by Marre and Penformis.[116]

Discussion

As alluded to in our last two reviews,[2,3] the decline or leveling of the output of the R&D programs of the pharmaceutical companies has continued, with the number of drugs of all types dropping in 2006 to 40 NCEs launched, of which 19 (48%) were classified in the "other than small molecules" or B/V categories. The corresponding figures for the next four years (2007-2010) are as follows. In 2007 there were 44 NCEs launched with 18 (41%) classified as B/V. In 2008, 38 NCEs were launched with 14 (37%) classified as B/V. In 2009, 42 NCEs were launched with 18 (43%) classified as B/V. Then in the last year of this analysis, 2010, there were 33 NCEs launched with 13 (39%) classified as B/V. Thus, one can see that an average of 42% of all NCEs in this five year time frame were biologicals or vaccines, and as mentioned earlier, the numbers of vaccines during this time period may have been underestimated.

As mentioned in the discussion of the antitumor agents and the dramatic influence of natural product structures in the approvals in 2010, we would be remiss if comment was not made on one other very important compound also approved that year. The compound in question is fingolimod (**30**, Gilenya®), the first orally active compound for once-a-day treatment of patients with relapsing forms of multiple sclerosis. The details of the derivation of this compound from an old fungal metabolite known as myriocin (**31**) and the many years of modifications required to produce the drug, have been told in detail in two recent reviews.[117, 118] What is also of significance is the recent report that fingolimod (**30**) also might have activity as a radio-sensitizing agent in treatment of prostate cancer.[119]

Although combinatorial chemistry continues to play a major role in the drug development process, as mentioned earlier, it is noteworthy that the trend toward the synthesis of complex natural product-like libraries has continued. Even including these newer methodologies, we still cannot find another de novo combinatorial compound approved anywhere in the world, although reliable data are not on hand on approvals in Russia and the People's Republic of China at this time. We think that it is appropriate to re-echo the comments by Danishefsky that was used in the 2007 review: "In summary, we have presented several happy experiences in the course of our program directed toward bringing to bear nature's treasures of small molecule natural products on the momentous challenge of human neurodegenerative diseases. While biological results are now being accumulated for systematic disclosure, it is already clear that there is considerable potential in compounds obtained through plowing in the landscape of natural products. Particularly impressive are those compounds that are obtained through diverted total synthesis, i.e., through methodology, which was redirected from the original (and realized) goal of total synthesis, to encompass otherwise unavailable congeners. We are confident that the program will lead, minimally, to compounds that are deserving of serious preclinical follow-up. At the broader level, we note that this program will confirm once again (if further confirmation is, indeed, necessary) the extraordinary advantages of small molecule natural products as sources of agents, which interject themselves in a helpful way in various physiological processes.

We close with the hope and expectation that enterprising and hearty organic chemists will not pass up the unique head start that natural products provide in the quest for new agents and new directions in medicinal discovery. We would chance to predict that even as the currently fashionable "telephone directory" mode of research is subjected to much overdue scrutiny and performance-based assessment, organic chemists in concert with biologists and even clinicians will be enjoying as well as exploiting the rich troves provided by nature's small molecules".[120]

A rapid analysis of the entities approved from 2006 to 2010 indicated that there were significant numbers of antitumor, antibacterial, and antifungal agents approved as mentioned above, with the unexpected showing, as exemplified in Figures 5 and 6, that in 2010, of the 20 small molecules approved, the second lowest number in the 30 years of analysis covered in this review, fully half were natural products or directly derived there from, with the majority of these being in the antitumor area, ten years after the approval of the first protein tyrosine kinase inhibitor, Gleevec®, in 2001. Included in the 2010 antitumor approvals was eribulin (**27**), to our knowledge the most complex drug yet approved made totally by synthesis.

It is highly probable that in the near future, totally synthetic variations on complex natural products will be part of the arsenal of physicians. One has only to look at the extremely elegant syntheses of complex natural products reported recently by Baran and his co-workers to visualize the potential of coupling very active and interesting natural products with the skills of synthetic chemists in academia and industry.[121-124] Also of great significance are the modeling of reactions based on Nature such as those described recently by Furst and Stephenson.[125] Further examples of where selective modification via synthesis of very active peptidic-based molecules can also be seen from the recent paper by Luesch's group on improvements of the in vivo antitumor activity of the apratoxins, molecules produced by cyanobacteria.[126]

It is often not appreciated that the major hurdle in bringing a totally synthetic complex molecule to market, is not the basic synthesis but the immense problems faced by process chemists in translating research laboratory discoveries to commercial items.[127,128] In the case of eribulin, the process chemistry group utilized selective crystallization steps rather than chromatography in order to provide the intermediates and the final product itself.

In this review, as we stated in 2003 and 2007,[2,3] we have yet again demonstrated that natural products play a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases. As we mentioned in earlier articles, some of our colleagues argued (though not in press, only in personal conversations at various forums) that the introduction of categories such as S/NM and S*/NM is an overstatement of the role played by natural products in the drug discovery process. On the contrary, we would still argue that these further serve to illustrate the inspiration provided by Nature to receptive organic chemists in devising ingenious syntheses of structural mimics to compete with Mother Nature's longstanding substrates. Even if we discount these categories, the continuing and overwhelming contribution of natural products to the expansion of the chemotherapeutic armamentarium is clearly evident as demonstrated in Figures 5 and 6, and as we stated in our earlier papers, much of Nature's "treasure trove of small molecules" remains to be explored, particularly from the marine and microbial environments.

From the perspective of microbes and their role(s) as sources of novel bioactive entities, it is now becoming quite evident that there are molecules for which the production depends upon the interaction among organisms from similar and also at times, widely different taxa.[129] Recent examples include activation of silent gene clusters in fungi,[130] or the activations of natural product biosyntheses in *Streptomyces* by mycolic acid-containing bacteria,[131] and the production of marine natural products via interactions between sponges and their associated microbes.[132]

Over the last few years, some data have been published indicating, but not as yet fully proving, that a number of fungi isolated from a significant number of different terrestrial plants may contain the full biosynthetic cluster for Taxol® production.[133] The one piece missing in the biosynthetic process, the presence of the gene for taxadiene synthetase was

identified but the production of the metabolite was not fully confirmed in the view of some.[134,135] The possibilities relating to the production of this agent via fungi have been discussed recently by Flores-Bustamente et al.[136] and recently further evidence of production from a *Taxus globosa* source was reported.[137]

A point emphasized in the review by Flores-Bustamente et al,[136] is effectively the same as those made following the reports a few years ago of multiple unexpected (silent) gene clusters in *Aspergillus nidulans* by Bok et al.[138] That work demonstrated that one has to be able to find the "genetic on-switch" to be able to obtain expression of such clusters outside of the host, as exemplified by further work from the Wisconsin group.[139] Similarly, as recently demonstrated by the group from the Leibnitz Institute in Jena following full genomic analyses of interactions between *Aspergillus nidulans* and *Streptomyces rapamycinicus*, the majority of biosynthetic clusters are "silent" under normal laboratory growth conditions. The interaction between these two microbes switched on a previously unrecognized PKS cluster that encoded the production of orsellinic acid, its derivative lecanoric acid, and the cathepsin K inhibitors F-9775A and F-9775B.[140] In addition to these papers, the reader's attention is also drawn to the excellent review article by Gunatilaka[141] on this subject, which, since its publication in 2006, has been cited over 100 times to date with reports showing materials isolated from plant endophytes. As a result, investigators need to consider all possible routes to novel agents.

To us, a multidisciplinary approach to drug discovery, involving the generation of truly novel molecular diversity from natural product sources, combined with total and combinatorial synthetic methodologies, and including the manipulation of biosynthetic pathways, will continue to provide the best solution to the current productivity crisis facing the scientific community engaged in drug discovery and development.

Once more, as we stated in our 2003 and 2007 reviews,[2,3] we strongly advocate expanding, not decreasing, the exploration of Nature as a source of novel active agents which may serve as the leads and scaffolds for elaboration into desperately needed efficacious drugs for a multitude of disease indications. A very recent commentary by Carter in the review journal, *Natural Products Reports* shows that such a realization might be closer than one may think.[142]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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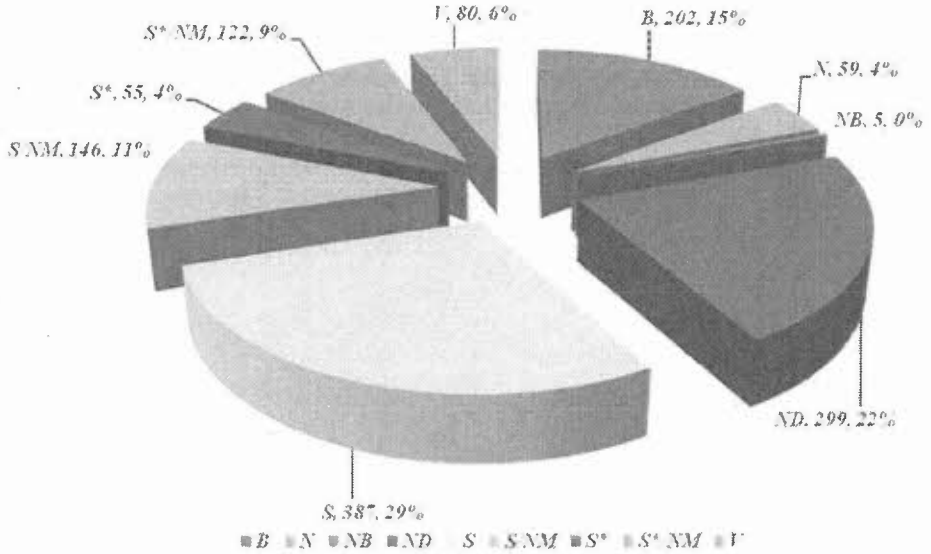


Figure 1. All New Approved Drugs; n = 1355

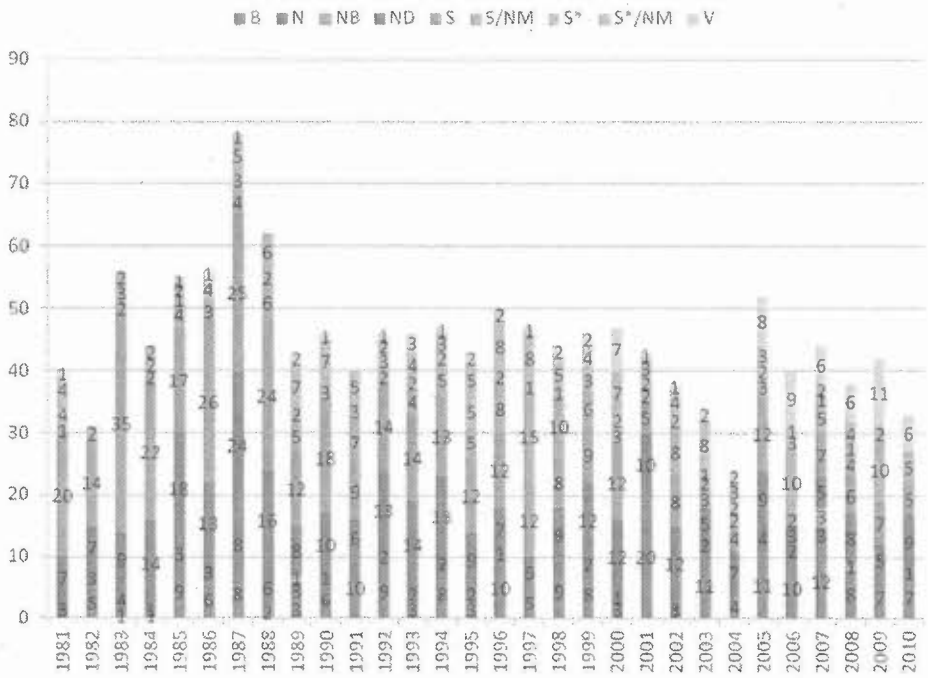


Figure 2. All New Approved Drugs by Source/Year

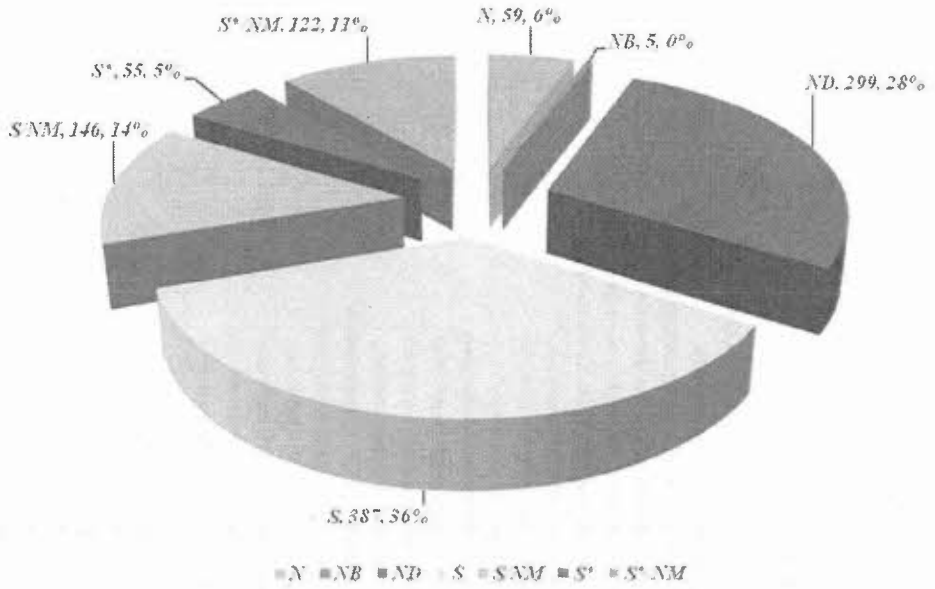


Figure 3. Source of Small Molecule Approved Drugs; n = 1073

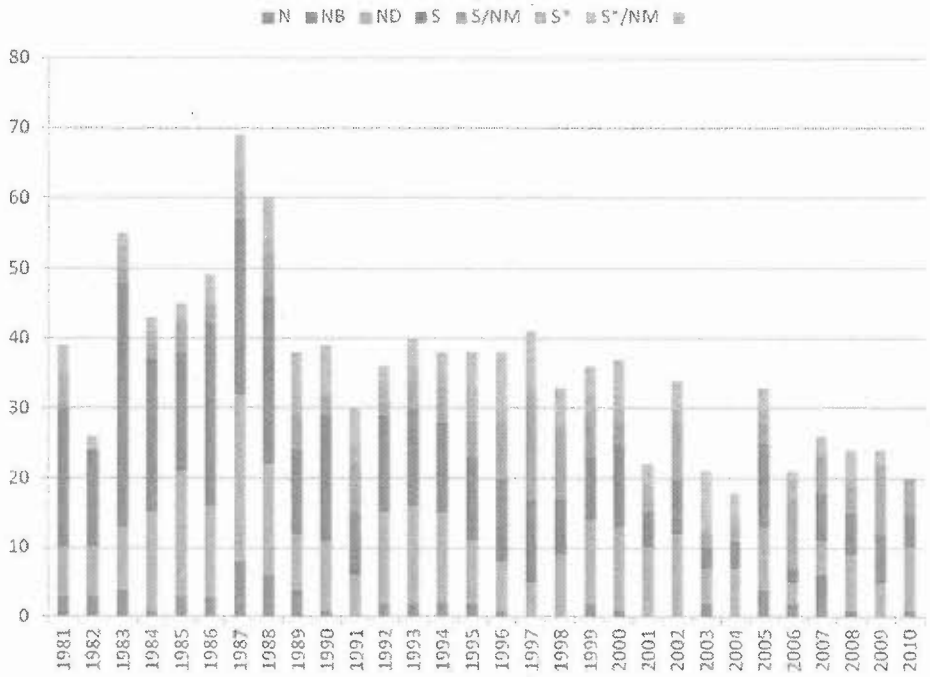


Figure 4. Sources of Small Molecule NCEs by Source/Year

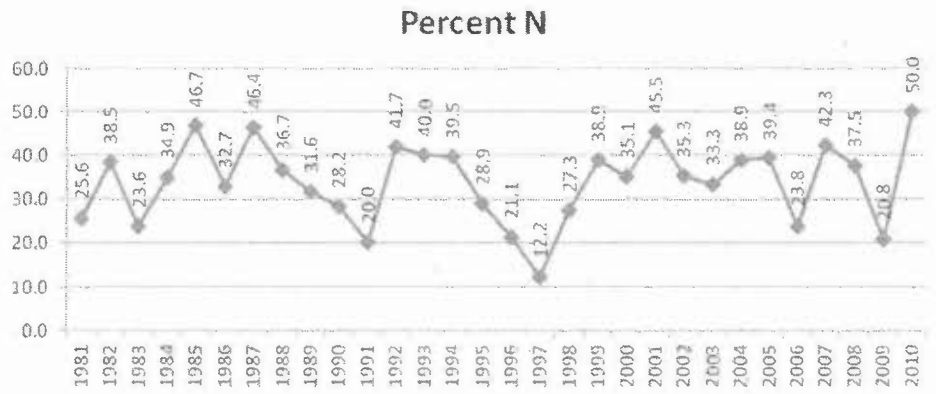


Figure 5. Percent N/NB/ND by Year, 1981 – 2010

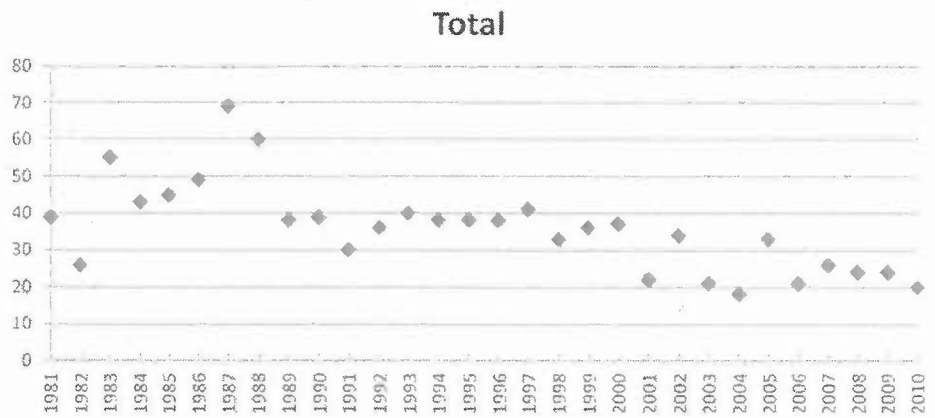


Figure 6. Total Small Molecules by Year, 1981 – 2010

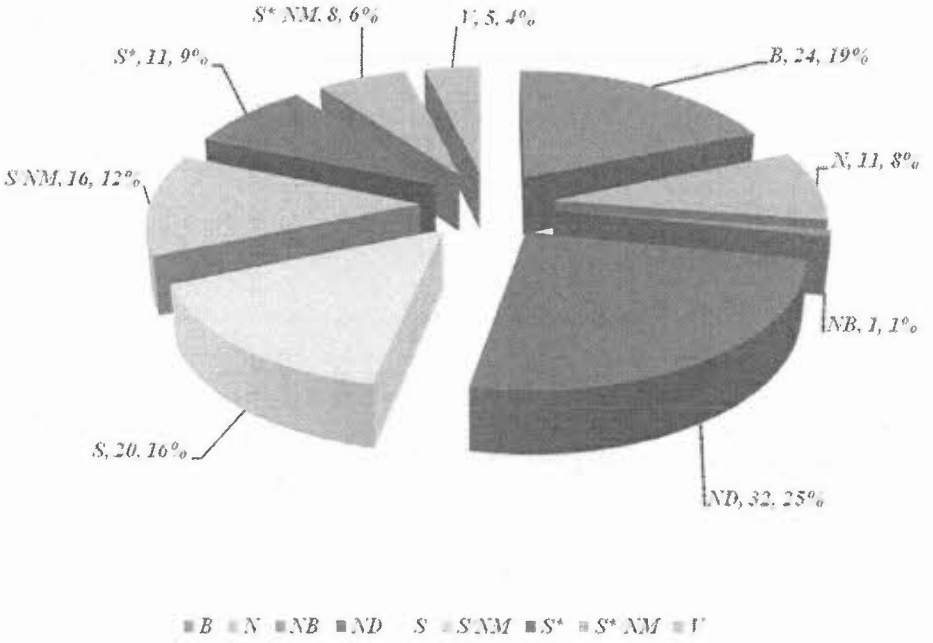


Figure 7. All Anticancer Drugs, 1981 – 2010

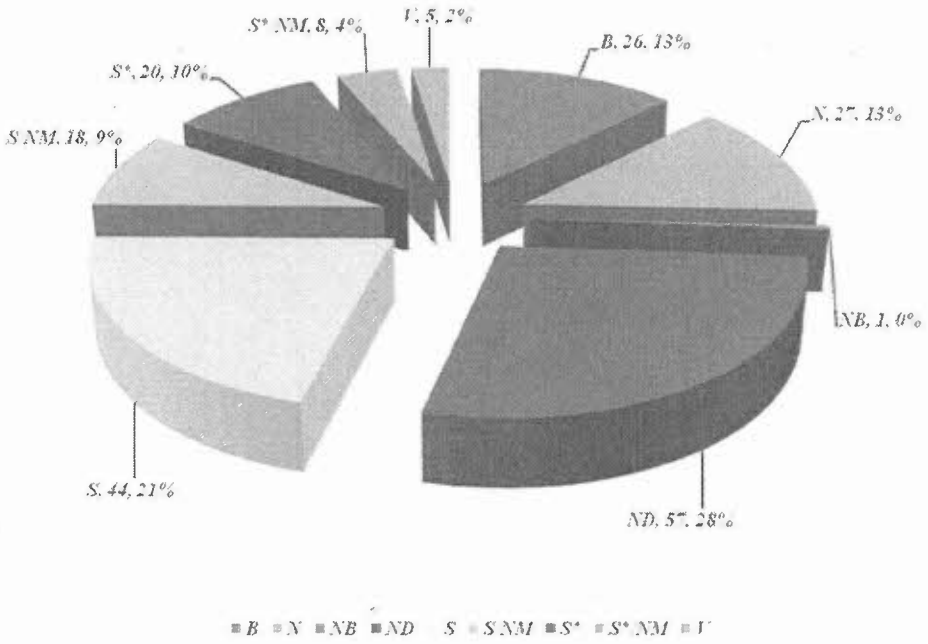
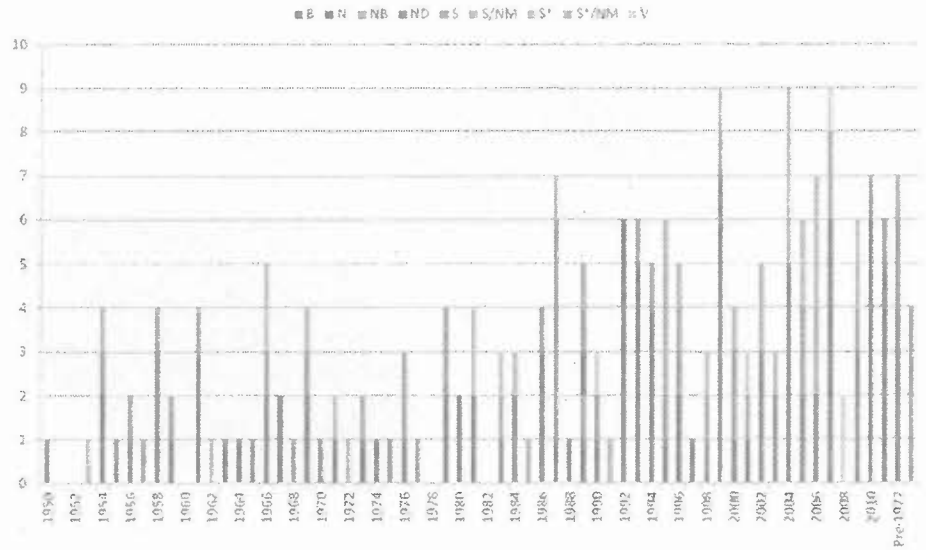


Figure 8. All Anticancer Drugs 1940s – 2010 by Source



[a] Due to space limitations, only the legend for the center "pre-1972" column shows in this plot on the RHS of "2010". The LH column legend is "pre-1970" and the RH column legend is "pre-1980".

Figure 9. All Anticancer Drugs 1940s – 2010 by Year/Source

Table 1

New Chemical Entities and Medical Indications by Source of Compound 01.01.81-12.31.2010[a]

indication	total	B	N	NB	ND	S	S/NM	S*	S*/NM	V
COPD	4						1		3	
analgesic	17		1			11	3	2		
anesthetic	5					5				
anti-Alzheimer	4		1				3			
anti-Parkinsonian	12				2	1	5		4	
antiallergic	17		1	1	4	11				
antianginal	5					5				
antiarrhythmic	17		1			14			2	
antiarthritic	17	6	1		1	3	6			
antiasthmatic	14	1			3	2	6		2	
antibacterial	118		10		67	26			1	14
anticancer	128	24	11	1	32	20	16	11	8	5
anticoagulant	19	5			13			1		
antidepressant	23					7	14		2	
antidiabetic	37	18	1		5	4	8	1		
antiemetic	11					1	2		8	
antiepileptic	15				2	9		2	2	
antifungal	29	1			3	22	3			
antiglaucoma	14				5		5	1	3	
antihistamine	13					13				
antihyperprolactinemia	4				4					
antihypertensive	79				2	28	14	2	33	
antiinflammatory	51	1			13	37				
antimigraine	10					2	1		7	
antiobesity	4				1		3			
antiparasitic	14		2		5	4		2		1
antipsoriatic	9	3		1	3			1	1	
antipsychotic	10					3	5		2	
antithrombotic	29	13	1		5	2	6		2	

J Nat Prod. Author manuscript; available in PMC 2013 July 24.

indication	total	B	N	NB	ND	S	S/NM	S*	S*/NM	V
antiulcer	34	1	1		12	20				
antiviral	110	14			4	9	2	23	10	48
anxiolytic	10					8	2			
benign prostatic hypertrophy	4		1		1	1	1			
bronchodilator	8				2				6	
calcium metabolism	20				8	9	3			
cardiotonic	13				3	2	3		5	
chelator	4					4				
contraception	9				8		1			
diuretic	6					4	2			
erythropoiesis	5	5								
gastroprokinetic	4					1	2		1	
hematopoiesis	6	6								
hemophilia	12	12								
hormone	22	12			10					
hormone replacement therapy	8				8					
hypnotic	12					12				
hypocholesterolemic	13		4		1	2	1		5	
hypolipidemic	8		1			7				
immunomodulator	4	2	1		1					
immunostimulant	11	5	3		2	1				
immunosuppressant	12	4	5		3					
irritable bowel syndrome	4					1			3	
male sexual dysfunction	4								4	
multiple sclerosis	6	3			1	1		1		
muscle relaxant	10				4	2	1	3		
neuroleptic	9					1	6		2	
nootropic	8				3	5				
osteoporosis	5	3			1	1				
platelet aggregation inhibitor	4				3		1			

J Nat Prod. Author manuscript; available in PMC 2013 July 24.

indication	total	B	N	NB	ND	S	S/NM	S*	S*/NM	V
respiratory distress syndrome	6	3	1			1	1			
urinary incontinence	5					2	3			
vulnerary	5	2			2	1				
Grand Total	1130	144	47	3	247	325	130	50	116	68

[a]**Diseases where ≤ 3 drugs approved 1981 – 2010**; 225 drugs fall into this category and are subdivided as follows: B, 58; N, 12; NB, 2; ND, 52; S, 62, S/NM. 16; S*, 5; S*/NM, 6; V, 12. The diseases covered the following; 5 α-reductase inhibitor, ADHD, CAPS, CHF, CNS Stimulant, Crohn's disease, DVT, Fabry's disease, Gaucher's disease, Hunter syndrome, Japanese encephalitis, Lambert-Eaton Myasthenic Syndrome, Lyme disease, MI, acute, MMRC, PAH, PCP/Toxoplasmosis, PNH, Pompe's disease, Turner Syndrome, abortifacient, acromelagy, actinic keratoses, adjuvant/colorectal cancer, alcohol deterrent, allergic rhinitis, anabolic metabolism, analeptic, anemia, anti sickle cell anemia, anti-smoking, antiacne, antiatherselerotic, anticonvulsant, antidiarrheal, antidote, anticmphysemic, antihyperuricemia, antihypotensive, antinarcolepsy, antinarcotic, antinauseant, antiperistaltic, antipneumococcal, antiprogestogenic, antirheumatic, antisecretory, antisepsis, antiseptic, antispasmodic, antispastic, antitussive, antityrosinaemia, antixerostomia, atrial fibrillation, benzodiazepine antagonist, β-lactamase inhibitor, blepharospasm, bone disorders, bone morphogenesis, bowel evacuant, cardioprotective, cardiovascular disease, cartilage disorders, cervical dystonia, choleretic, chronic idiopathic constipation, cognition enhancer, congestive heart failure, constipation, cystic fibrosis, cytoprotective, dementia (Alzheimer's), diabetic foot ulcers, diabetic neuropathies, digoxin toxicity, dpt, dry eye syndrome, dyslipidemia, dysuria, endometriosis, enzyme, expectorant, fertility inducer, gastroprotectant, genital warts, hematological, hemorrhage, hemostasis, hemostatic, hepatoprotectant, hereditary angioedema, homocystinuria, hyperammonemia, hyperparathyroidism, hyperphenylalaninemia, hyperphosphatemia, hyperuricemia, hypoammonuric, hypocalciuric, hypogonadism, hyponatremia, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia, immediate allergy, infertility (female), inflammatory bowel disease, insomnia, joint lubricant, lipoprotein disorders, macular degeneration, mucolytic, mucopolysaccharidosis, mucositis, myleodysplasia, narcolepsy, nasal decongestant, neuropathic pain, neuroprotective, ocular inflammation, opiate detoxification, osteoarthritis, overactive bladder, ovulation, pancreatic disorders, pancreatitis, pertussis, photosensitizer, pituitary disorders, porphyria, premature birth, premature ejaculation, progestogen, psychostimulant, pulmonary arterial hypertension, purpura fulminans, rattlesnake antivenom, reproduction, restenosis, schizophrenia, sclerosant, secondary hyperthryoidism, sedative, skin photodamage, strabismus, subarachnoid hemorrhage, thrombocytopenia, treatment of GH deficiency, ulcerative colitis, urea cycle disorders, uremic pruritis, urolithiasis, vaccinia complications, varicella (chicken pox), vasodilator, vasodilator (cerebral), vasodilator (coronary), vasoprotective, venous thromboembolism

Table 2

Antibacterial Drugs from 01.01.81 to 12.31.10 Organized Alphabetically by Generic Name within Source

generic name	trade name	year introduced	volume	page	source
carumonam	Amasulin	1988	ARMC 24	298	N
daptomycin	Cubicin	2003	ARMC 39	347	N
fosfomycin tromctamol	Monuril	1988	I 112334		N
isepamicin	Isepacin	1988	ARMC 24	305	N
micronomicin sulfate	Sagamicin	1982	P091082		N
miokamycin	Miocamycin	1985	ARMC 21	329	N
mupirocin	Bactroban	1985	ARMC 21	330	N
netilimicin sulfate	Netromicine	1981	I 070366		N
RV-11	Zalig	1989	ARMC 25	318	N
teicoplanin	Targocid	1988	ARMC 24	311	N
apalcillin sodium	Lumota	1982	I 091130		ND
arbekacin	Habekacin	1990	ARMC 26	298	ND
aspoxicillin	Doyle	1987	ARMC 23	328	ND
astromycin sulfate	Fortimicin	1985	ARMC 21	324	ND
azithromycin	Sunamed	1988	ARMC 24	298	ND
aztreonam	Azactam	1984	ARMC 20	315	ND
biapenem	Omegacin	2002	ARMC 38	351	ND
cefbuperazone sodium	Tomiporan	1985	ARMC 21	325	ND
cefcapene pivoxil	Flomox	1997	ARMC 33	330	ND
cefdinir	Cefzon	1991	ARMC 27	323	ND
cefditoren pivoxil	Meiact	1994	ARMC 30	297	ND
cefepime	Maxipime	1993	ARMC 29	334	ND
cefetamet pivoxil HCl	Globocef	1992	ARMC 28	327	ND
cefixime	Cefspan	1987	ARMC 23	329	ND
cefmenoxime HCl	Tacef	1983	ARMC 19	316	ND
cefminox sodium	Meicelin	1987	ARMC 23	330	ND
cefodizime sodium	Neucef	1990	ARMC 26	300	ND
cefonicid sodium	Monocid	1984	ARMC 20	316	ND
cefoperazone sodium	Cefobis	1981	I 127130		ND

J Nat Prod. Author manuscript; available in PMC 2013 July 24.

generic name	trade name	year introduced	volume	page	source
ceforanide	Precef	1984	ARMC 20	317	ND
cefoselis	Wincef	1998	ARMC 34	319	ND
cefotetan disodium	Yamatetan	1984	ARMC 20	317	ND
cefotiam HCl	Pansporin	1981	I 091106		ND
cefozopran HCl	Firstcin	1995	ARMC 31	339	ND
cefpimizole	Ajicef	1987	ARMC 23	330	ND
cefpiramide sodium	Sepatren	1985	ARMC 21	325	ND
cefpirome sulfate	Cefrom	1992	ARMC 28	328	ND
cefpodoxime proxetil	Banan	1989	ARMC 25	310	ND
cefprozil	Cefzil	1992	ARMC 28	328	ND
cefsoludin sodium	Takesulin	1981	I 091108		ND
ceftazidime	Fortam	1983	ARMC 19	316	ND
cefteram pivoxil	Tomiron	1987	ARMC 23	330	ND
ceftibuten	Seftem	1992	ARMC 28	329	ND
ceftizoxime sodium	Epocelin	1982	I 070260		ND
ceftobiprole medocaril	Zeftera	2008	ARMC 44	589	ND
ceftriaxone sodium	Rocephin	1982	I 091136		ND
cefuroxime axetil	Zinnat	1987	ARMC 23	331	ND
cefuzonam sodium	Cosmosin	1987	ARMC 23	331	ND
clarithromycin	Klaricid	1990	ARMC 26	302	ND
dalfopristin	Synercid	1999	ARMC 35	338	ND
dirithromycin	Nortron	1993	ARMC 29	336	ND
doripenem	Finibax	2005	DNP 19	42	ND
ertapenem sodium	Invanz	2002	ARMC 38	353	ND
erythromycin acistrate	Erasis	1988	ARMC 24	301	ND
flomoxef sodium	Flumarin	1988	ARMC 24	302	ND
flurithromycin ethylsuccinate	Ritro	1997	ARMC 33	333	ND
fropenam	Farom	1997	ARMC 33	334	ND
imipenem/cilastatin	Zienam	1985	ARMC 21	328	ND
lenampicillin HCl	Varacillin	1987	ARMC 23	336	ND
loracarbef	Lorabid	1992	ARMC 28	333	ND

J Nat Prod. Author manuscript; available in PMC 2013 July 24.

generic name	trade name	year introduced	volume	page	source
meropenem	Merrem	1994	ARMC 30	303	ND
moxalactam disodium	Shiomarin	1982	J 070301		ND
panipenem/betamipron	Carbenin	1994	ARMC 30	305	ND
quinupristin	Synercid	1999	ARMC 35	338	ND
retapamulin	Altabax	2007	ARMC 43	486	ND
rifabutin	Mycobutin	1992	ARMC 28	335	ND
rifamixin	Normix	1987	ARMC 23	341	ND
rifapentine	Rifampin	1988	ARMC 24	310	ND
rifaximin	Rifacol	1985	ARMC 21	332	ND
rokitamycin	Ricamycin	1986	ARMC 22	325	ND
roxithromycin	Rulid	1987	ARMC 23	342	ND
sultamycillin tosylate	Unasyn	1987	ARMC 23	343	ND
tazobactam sodium	Tazocillin	1992	ARMC 28	336	ND
telavancin HCl	Vibativ	2009	DNP 23	15	ND
telithromycin	Ketek	2001	DNP 15	35	ND
temocillin disodium	Temopen	1984	ARMC 20	323	ND
tigecycline	Tygacil	2005	DNP 19	42	ND
balafloxacin	Q-Roxin	2002	ARMC 38	351	S
besifloxacin	Besivance	2009	DNP 23	20	S
ciprofloxacin	Ciprobay	1986	ARMC 22	318	S
enoxacin	Flumark	1986	ARMC 22	320	S
fleroxacin	Quinodis	1992	ARMC 28	331	S
garenoxacin	Geninax	2007	ARMC 43	471	S
gatifloxacin	Tequin	1999	ARMC 35	340	S
gemifloxacin mesilate	Factive	2003	ARMC 40	458	S
grepafloxacin	Vaxor	1997	DNP 11	23	S
levofloxacin	Floxacin	1993	ARMC 29	340	S
linezolid	Zyvox	2000	DNP 14	21	S
lomefloxacin	Uniquin	1989	ARMC 25	315	S
moxifloxacin HCl	Avelox	1999	ARMC 35	343	S
nadifloxacin	Acuatim	1993	ARMC 29	340	S

J Nat Prod. Author manuscript; available in PMC 2013 July 24.

generic name	trade name	year introduced	volume	page	source
norfloxacin	Noroxin	1983	ARMC 19	322	S
ofloxacin	Tarivid	1985	ARMC 21	331	S
pazufloxacin	Pasil	2002	ARMC 38	364	S
pefloxacin mesylate	Perflacine	1985	ARMC 21	331	S
prulifloxacin	Sword	2002	ARMC 38	366	S
rufloxacin hydrochloride	Qari	1992	ARMC 28	335	S
sitafloxacin hydrate	Gracevit	2008	DNP 22	15	S
sparfloxacin	Spara	1993	ARMC 29	345	S
taurolidine	Taurolin	1988	I 107771		S
temafloxacin hydrochloride	Temac	1991	ARMC 27	334	S
tosufloxacin	Ozex	1990	ARMC 26	310	S
trovafloxacin mesylate	Trovan	1998	ARMC 34	332	S
brodimoprin	Hyprim	1993	ARMC 29	333	S*/NM
ACWY meningoccal PS vaccine	Menecvax	1981	I 420128		V
DTPw-HepB-Hib	Quinvaxem	2006	DNP 20	26	V
H. influenzae b vaccine	Hibtitek	1989	DNP 03	24	V
H. influenzae b vaccine	Prohibit	1989	DNP 03	24	V
MCV-4	Menactra	2005	DNP 19	43	V
menACWY-CRM	Menveo	2010	I 341212		V
meningitis b vaccine	MeNZB	2004	DNP 18	29	V
meningococcal vaccine	Menigetec	1999	DNP 14	22	V
meningococcal vaccine	NeisVac-C	2000	DNP 14	22	V
meningococcal vaccine	Menjugate	2000	DNP 14	22	V
oral cholera vaccine	Orochol	1994	DNP 08	30	V
pneumococcal vaccine	Prevnar	2000	DNP 14	22	V
PsA-TT	MenAfriVac	2010	I 437718		V
vi polysaccharide typhoid vacc	Typherix	1998	DNP 12	35	V

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Table 3

Antifungal Drugs from 01.01.81 to 12.31.10 Organized Alphabetically by Generic Name within Source

generic name	trade name	year introduced	volume	page	source
interferon γ-n1	OGamma100	1996	DNP 10	13	B
anidulafungin	Eraxis	2006	DNP 20	24	ND
caspofungin acetate	Cancidas	2001	DNP 15	36	ND
micafungin sodium	Fungard	2002	ARMC 38	360	ND
amorolfine hydrochloride	Loceryl	1991	ARMC 27	322	S
butoconazole	Femstat	1986	ARMC 22	318	S
ciclopirox olamine	Loprox	1982	I 070449		S
cloconazole HCl	Pilzcin	1986	ARMC 22	318	S
eberconazole	Ebernet	2005	DNP 19	42	S
fenticonazole nitrate	Lomexin	1987	ARMC 23	334	S
fluconazole	Diflucan	1988	ARMC 24	303	S
flutrimazole	Micetal	1995	ARMC 31	343	S
fosfluconazole	Prodif	2003	DNP 17	49	S
itraconazole	Sporanox	1988	ARMC 24	305	S
ketoconazole	Nizoral	1981	I 116505		S
lanoconazole	Astat	1994	ARMC 30	302	S
luliconazole	Lulicon	2005	DNP 19	42	S
naftifine HCl	Exoderil	1984	ARMC 20	321	S
neticonazole HCl	Atolant	1993	ARMC 29	341	S
oxiconazole nitrate	Oceral	1983	ARMC 19	322	S
posaconazole	Noxafil	2005	DNP 19	42	S
sertaconazole nitrate	Dermofix	1992	ARMC 28	336	S
sulconazole nitrate	Exelderm	1985	ARMC 21	332	S
terconazole	Gyno-Terazol	1983	ARMC 19	324	S
tioconazole	Trosyl	1983	ARMC 19	324	S
voriconazole	Vfend	2002	ARMC 38	370	S
butenafine hydrochloride	Mentax	1992	ARMC 28	327	S/NM
liranaftate	Zefnart	2000	DNP 14	21	S/NM
terbinafine hydrochloride	Lamisil	1991	ARMC 27	334	S/NM

Table 4

Antiviral Drugs from 01.01.81 to 12.31.10 Organized Alphabetically by Generic Name within Source

generic name	trade name	year introduced	volume	page	source
interferon α	Alfaferone	1987	I 215443		B
interferon α-n3	Alferon N	1990	DNP 04	104	B
interferon β	Frone	1985	I115091		B
immunoglobulin intravenous	Gammagard Liquid	2005	I 231564		B
interferon alfacon-1	Infergen	1997	ARMC 33	336	B
IGIV-HB	Niuliva	2009	DNP 23	16	B
	Oralgen	2007	I 415378		B
peginterferon α-2a	Pegasys	2001	DNP 15	34	B
peginterferon α-2b	Pegintron	2000	DNP 14	18	B
resp syncytial virus IG	RespiGam	1996	DNP 10	11	B
palivizumab	Synagis	1998	DNP 12	33	B
interferon α-2b	Viraferon	1985	I 165805		B
interferon α-n1	Wellferon	1986	I 125561		B
thymalfasin	Zadaxin	1996	DNP 10	11	B
enfuvirtide	Fuzeon	2003	ARMC 39	350	ND
laninamivir octanoate	Inavir	2010	I 340894		ND
peramivir	PeramiFlu	2010	I 273549		ND
zanamivir	Relenza	1999	ARMC 35	352	ND
imiquimod	Aldara	1997	ARMC 33	335	S
maraviroc	Celsentri	2007	ARMC 43	478	S
foscarnet sodium	Foscavir	1989	ARMC 25	313	S
raltegravir potassium	Isentress	2007	ARMC 43	484	S
delavirdine mesylate	Rescriptor	1997	ARMC 33	331	S
rimantadine HCl	Roflual	1987	ARMC 23	342	S
propagermanium	Serosion	1994	ARMC 30	308	S
efavirenz	Sustiva	1998	ARMC 34	321	S
nevirapine	Viramune	1996	ARMC 32	313	S
darunavir	Prezista	2006	DNP 20	25	S/NM

generic name	trade name	year introduced	volume	page	source
oseltamivir	Tamiflu	1999	ARMC 35	346	S/NM
entecavir	Baraclude	2005	DNP 19	39	S*
ganciclovir	Cymevene	1988	ARMC 24	303	S*
emtricitabine	Emtriva	2003	ARMC 39	350	S*
lamivudine	Epivir	1995	ARMC 31	345	S*
famciclovir	Famvir	1994	ARMC 30	300	S*
adefovir dipivoxil	Hepsera	2002	ARMC 38	348	S*
efavirenz	Sustiva	1988	I 157373		S*
zalcitabine	Hivid	1992	ARMC 28	338	S*
inosine pranobex	Imunovir	1981	I 277341		S*
etravirine	Intelence	2008	DNP 22	15	S*
elvucitabine	Levovir	2007	ARMC 43	466	S*
zidovudine	Retrovir	1987	ARMC 23	345	S*
telbivudine	Sebivo	2006	DNP 20	22	S*
sorivudine	Usevir	1993	ARMC 29	345	S*
valganciclovir	Valcyte	2001	DNP 15	36	S*
valaciclovir HCl	Valtrex	1995	ARMC 31	352	S*
penciclovir	Vectavir	1996	ARMC 32	314	S*
didanosine	Videx	1991	ARMC 27	326	S*
tenofovir disoproxil fumarate	Viread	2001	DNP 15	37	S*
cidofovir	Vistide	1996	ARMC 32	306	S*
stavudine	Zerit	1994	ARMC 30	311	S*
abacavir sulfate	Ziagen	1999	ARMC 35	333	S*
acyclovir	Zovirax	1981	I 091119		S*
amprenavir	Agenerase	1999	ARMC 35	334	S*/NM
tipranavir	Aptivus	2005	DNP 19	42	S*/NM
indinavir sulfate	Crixivan	1996	ARMC 32	310	S*/NM
saquinavir mesylate	Invirase	1995	ARMC 31	349	S*/NM
lopinavir	Kaletra	2000	ARMC 36	310	S*/NM
fosamprenevir	Lexiva	2003	ARMC 39	353	S*/NM

J Nat Prod. Author manuscript; available in PMC 2013 July 24.

generic name	trade name	year introduced	volume	page	source
ritonavir	Norvir	1996	ARMC 32	317	S*/NM
atazanavir	Reyataz	2003	ARMC 39	342	S*/NM
nelfinavir mesylate	Viracept	1997	ARMC 33	340	S*/NM
fomivirsen sodium	Vitravene	1998	ARMC 34	323	S*/NM
H5N1 avian flu vaccine		2007	I 440743		V
Influenza A(H1N1) monovalent		2010	I 678265		V
	ACAM-2000	2007	I 328985		V
influenza virus vaccine	Afluria	2007	I 449226		V
hepatitis A vaccine	Aimmugen	1995	DNP 09	23	V
hepatitis A and B vaccine	Ambirix	2003	I 334416		V
split influenza vaccine	Anflu	2006	DNP 20	26	V
inact hepatitis A vaccine	Avaxim	1996	DNP 10	12	V
hepatitis B vaccine	Biken-HB	1993	DNP 07	31	V
	Bilive	2005	DNP 19	43	V
hepatitis B vaccine	Bio-Hep B	2000	DNP 14	22	V
	Celtura	2009	DNP 23	17	V
	Celvapan	2009	DNP 23	17	V
	Daronix	2007	I 427024		V
hepatitis B vaccine	Engerix B	1987	I 137797		V
rubella vaccine	Ervevax	1985	I 115078		V
hepatitis B vaccine	Fendrix	2005	DNP 19	43	V
influenza virus (live)	FluMist	2003	ARMC 39	353	V
	Fluval P	2009	DNP 23	17	V
	Focetria	2009	DNP 23	17	V
hpv vaccine	Gardasil	2006	DNP 20	26	V
	Grippol Neo	2009	DNP 23	16	V
hepatitis a vaccine	Havrix	1992	DNP 06	99	V
hepatitis b vaccine	Hepacure	2000	DNP 14	22	V
anti-Hep B immunoglobulin	HepaGam B	2006	DNP 20	27	V

generic name	trade name	year introduced	volume	page	source
HN-VAC	HNVAC	2010	I 684608		V
influenza vaccine	Invivac	2004	I 391186		V
MR vaccine	Mearubik	2005	DNP 19	44	V
hepatitis b vaccine	Meinyu	1997	DNP 11	24	V
attenuated chicken pox vaccine	Mericux Varicella Vaccine	1993	DNP 07	31	V
	Optaflu	2007	I 410266		V
influenza vaccine	Optaflu	2008	DNP 22	16	V
	Pandremix	2009	DNP 23	17	V
	Panenza	2009	DNP 23	17	V
	Panflu	2008	DNP 22	16	V
VCIV	PreFluCel	2010	I 444826		V
GSK-1562902A	Prepandrix	2008	DNP 22	16	V
antirabies vaccine	Rabirix	2006	DNP 20	27	V
rotavirus vaccine	Rotarix	2005	DNP 18	29	V
rotavirus vaccine	Rota-Shield	1998	DNP 12	35	V
rotavirus vaccine	Rotateq	2006	DNP 20	26	V
rec hepatitis B vaccine	Supervax	2006	DNP 20	27	V
hepatitis a vaccine	Vaqta	1996	DNP 10	11	V
varicella virus vaccine	Varivax	1995	DNP 09	25	V
	VariZIG	2005	I 230590		V
	Vaxiflu-S	2010	I 698015		V
zoster vaccine live	Zostavax	2006	DNP 20	26	V

J Nat Prod. Author manuscript; available in PMC 2013 July 24.

Table 5

Antiparasitic Drugs from 01.01.81 to 12.01.10 Organized Alphabetically by Generic Name within Source

generic name	trade name	year introduced	volume	page	source
artemisinin	Artemisin	1987	ARMC 23	327	N
ivermectin	Mectizan	1987	ARMC 23	336	N
arteether	Artemotil	2000	DNP 14	22	ND
artemether	Artemetheri	1987	I 90712		ND
artesunate	Arinate	1987	I 91299		ND
eflornithine HCl	Ornidyl	1990	DNP 04	104	ND
mefloquine HCl	Fansimef	1985	ARMC 21	329	ND
albendazole	Eskazole	1982	I 129625		S
halofantrine	Halfan	1988	ARMC 24	304	S
lumefantrine	?	1987	I 269095		S
quinfamide	Amenox	1984	ARMC 20	322	S
atovaquone	Mepron	1992	ARMC 28	326	S*
bulaquine/chloroquine	Aablaquin	2000	DNP 14	22	S*
trichomonas vaccine	Gynatren	1986	I 125543		V

Table 6

All Antiinfective (Bacterial, Fungal, Parasitic, and Viral) Drugs (n = 270)

indication	total	B	N	ND	S	S/NM	S*	S*/NM	V
Antibacterial	118		10	67	26			1	14
Antifungal	29	1		3	22	3			
Antiparasitic	14		2	5	4		2		1
Antiviral	109	14		4	9	2	23	10	47
total	270	15	12	79	61	5	25	11	62
percentage	100	5.6	4.4	29.3	22.6	1.8	9.3	4	23

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Table 7

Small Molecule Antiinfective (Bacterial, Fungal, Parasitic, and Viral) Drugs (n = 193)

indication	total	N	ND	S	S/NM	S*	S*/NM
Antibacterial	104	10	67	26			1
Antifungal	28		3	22	3		
Antiparasitic	13	2	5	4		2	
Antiviral	48		4	9	2	23	10
total	193	12	79	61	5	25	11
percentage	100	6.2	40.9	31.6	2.6	13	5.7

J Nat Prod. Author manuscript; available in PMC 2013 July 24.

Table 8

Anticancer Drugs from 01.01.81 to 12.31.10 Organized Alphabetically by Generic Name within Source

generic name	trade name	year introduced	volume	page	source
	Rexin-G	2007	I 346431		B
131I-chTNT		2007	I 393351		B
alemtuzumab	Campath	2001	DNP 15	38	B
bevacizumab	Avastin	2004	ARMC 40	450	B
catumaxomab	Removab	2009	DNP 23	18	B
celmoleukin	Celeuk	1992	DNP 06	102	B
cetuximab	Erbitux	2003	ARMC 39	346	B
denileukin diftitox	Ontak	1999	ARMC 35	338	B
H-101		2005	DNP 19	46	B
ibritumomab	Zevalin	2002	ARMC 38	359	B
interferon α-2a	Roferon-A	1986	I 204503		B
interferon, γ-1a	Biogamma	1992	ARMC 28	332	B
interleukin-2	Proleukin	1989	ARMC 25	314	B
mobenakin	Octin	1999	ARMC 35	345	B
	BIOMAb				
nimotuzumab	EFGR	2006	DNP 20	29	B
ofatumumab	Arzerra	2009	DNP 23	18	B
panitumumab	Vectibix	2006	DNP 20	28	B
pegaspargase	Oncaspar	1994	ARMC 30	306	B
rituximab	Rituxan	1997	DNP 11	25	B
sipuleucel-T	Provenge	2010	I 259673		B
tasonermin	Beromun	1999	ARMC 35	349	B
teceleukin	Imumace	1992	DNP 06	102	B
tositumomab	Bexxar	2003	ARMC 39	364	B
trastuzumab	Herceptin	1998	DNP 12	35	B
aclarubicin	Aclacin	1981	P090013		N
angiotensin II	Delivert	1994	ARMC 30	296	N
arglabin	?	1999	ARMC 35	335	N
masoprocol	Actinex	1992	ARMC 28	333	N

generic name	trade name	year introduced	volume	page	source
paclitaxel	Taxol	1993	ARMC 29	342	N
paclitaxel nanoparticles	Abraxane	2005	DNP 19	45	N
paclitaxel nanoparticles	Nanoxel	2007	I 422122		N
pentostatin	Nipent	1992	ARMC 28	334	N
peplomycin	Pepleo	1981	P090889		N
romidepsin	Istodax	2010	DNP 23	18	N
trabectedin	Yondelis	2007	ARMC 43	492	N
solamargines	Curaderm	1989	DNP 03	25	NB
alitretinoin	Panretin	1999	ARMC 35	333	ND
amrubicin HCl	Calsed	2002	ARMC 38	349	ND
belotecan hydrochloride	Camtobell	2004	ARMC 40	449	ND
cabazitaxel	Jevtana	2010	I 287186		ND
cladribine	Leustatin	1993	ARMC 29	335	ND
cytarabine ocfosfate	Starsaid	1993	ARMC 29	335	ND
docetaxel	Taxotere	1995	ARMC 31	341	ND
elliptinium acetate	Celiptium	1983	P091123		ND
epirubicin HCl	Farmorubicin	1984	ARMC 20	318	ND
eribulin	Halaven	2010	I 287199		ND
etoposide phosphate	Etopophos	1996	DNP 10	13	ND
exemestane	Aromasin	1999	DNP 13	46	ND
formestane	Lentaron	1993	ARMC 29	337	ND
fulvestrant	Faslodex	2002	ARMC 38	357	ND
gemtuzumab ozogamicin	Mylotarg	2000	DNP 14	23	ND
hexyl aminolevulinate	Hexvix	2004	I 300211		ND
idarubicin hydrochloride	Zavedos	1990	ARMC 26	303	ND
irinotecan hydrochloride	Campto	1994	ARMC 30	301	ND
ixabepilone	Ixempra	2007	ARMC 43	473	ND
mifamurtide	Junovan	2010	DNP 23	18	ND
miltefosine	Miltex	1993	ARMC 29	340	ND
pirarubicin	Pinorubicin	1988	ARMC 24	309	ND

J Nat Prod. Author manuscript; available in PMC 2013 July 24.

generic name	trade name	year introduced	volume	page	source
pralatrexate	Folotyn	2009	DNP 23	18	ND
talaporfin sodium	Laserphyrin	2004	ARMC 40	469	ND
temsirolimus	Toricel	2007	ARMC 43	490	ND
topotecan HCl	Hycamptin	1996	ARMC 32	320	ND
triptorelin	Decapeptyl	1986	I 090485		ND
valrubicin	Valstar	1999	ARMC 35	350	ND
vapreotide acetate	Docrised	2004	I 135014		ND
vinflunine	Javlor	2010	I 219585		ND
vinorelbine	Navelbine	1989	ARMC 25	320	ND
zinostatin stimalamer	Smancs	1994	ARMC 30	313	ND
aminoglutethimide	Cytadren	1981	I 070408		S
amsacrine	Amsakrin	1987	ARMC 23	327	S
arsenic trioxide	Trisenox	2000	DNP 14	23	S
bisantrene hydrochloride	Zantrene	1990	ARMC 26	300	S
carboplatin	Paraplatin	1986	ARMC 22	318	S
flutamide	Drogenil	1983	ARMC 19	318	S
fotemustine	Muphoran	1989	ARMC 25	313	S
heptaplatin/SK-2053R	Sunpla	1999	ARMC 35	348	S
lobaplatin	Lobaplatin	1998	DNP 12	35	S
lonidamine	Doridamina	1987	ARMC 23	337	S
miriplatin hydrate	Miripla	2010	DNP 23	17	S
nedaplatin	Aqupla	1995	ARMC 31	347	S
nilutamide	Anadron	1987	ARMC 23	338	S
oxaliplatin	Eloxatin	1996	ARMC 32	313	S
plerixafor hydrochloride	Mozobil	2009	DNP 22	17	S
porfimer sodium	Photofrin	1993	ARMC 29	343	S
ranimustine	Cymerine	1987	ARMC 23	341	S
sobuzoxane	Parazolin	1994	ARMC 30	310	S
sorafenib	Nexavar	2005	DNP 19	45	S
anastrozole	Arimidex	1995	ARMC 31	338	S/NM
bicalutamide	Casodex	1995	ARMC 31	338	S/NM

J Nat Prod. Author manuscript; available in PMC 2013 July 24.

generic name	trade name	year introduced	volume	page	source
bortezomib	Velcade	2003	ARMC 39	345	S/NM
camostat mesylate	Foipan	1985	ARMC 21	325	S/NM
dasatinib	Sprycel	2006	DNP 20	27	S/NM
erlotinib hydrochloride	Tarceva	2004	ARMC 40	454	S/NM
fadrozole HCl	Afema	1995	ARMC 31	342	S/NM
gefitinib	Iressa	2002	ARMC 38	358	S/NM
imatinib mesilate	Gleevec	2001	DNP 15	38	S/NM
lapatinib ditosylate	Tykerb	2007	ARMC 43	475	S/NM
letrazole	Femara	1996	ARMC 32	311	S/NM
nilotinib hydrochloride	Tasigna	2007	ARMC 43	480	S/NM
pazopanib	Votrient	2009	DNP 23	18	S/NM
sunitinib malate	Sutent	2006	DNP 20	27	S/NM
temoporfin	Foscan	2002	J 158118		S/NM
toremifene	Fareston	1989	ARMC 25	319	S/NM
zoledronic acid	Zometa	2000	DNP 14	24	S
azacytidine	Vidaza	2004	ARMC 40	447	S*
capecitabine	Xeloda	1998	ARMC 34	319	S*
carmofur	Mifurol	1981	J 091100		S*
clofarabine	Clolar	2005	DNP 19	44	S*
decitabine	Dacogen	2006	DNP 20	27	S*
doxifluridine	Furtulon	1987	ARMC 23	332	S*
enocitabine	Sunrabin	1983	ARMC 19	318	S*
fludarabine phosphate	Fludara	1991	ARMC 27	327	S*
gemcitabine HCl	Gemzar	1995	ARMC 31	344	S*
mitoxantrone HCl	Novantrone	1984	ARMC 20	321	S*
nelarabine	Arranon	2006	ARMC 42	528	S*
abarelix	Plenaxis	2004	ARMC 40	446	S*/NM
bexarotene	Targretine	2000	DNP 14	23	S*/NM
degarelix	Firmagon	2009	DNP 22	16	S*/NM
pemetrexed disodium	Alimta	2004	ARMC 40	463	S*/NM
raltitrexed	Tomudex	1996	ARMC 32	315	S*/NM

generic name	trade name	year introduced	volume	page	source
tamibarotene	Amnoid	2005	DNP 19	45	S*/NM
temozolomide	Temodal	1999	ARMC 35	350	S*/NM
vorinostat	Zolinza	2006	DNP 20	27	S*/NM
	Cervarix	2007	I 309201		V
autologous tumor cell-BCG	OncoVAX	2008	DNP 22	17	V
bcg live	TheraCys	1990	DNP 04	104	V
melanoma theraccine	Melacine	2001	DNP 15	38	V
vitespen	Oncophage	2008	DNP 22	17	V

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Table 9

All Anticancer Drugs (1940s to 12.31.10) Organized Alphabetically by Generic Name within Source[a]

generic name	year introduced	reference	page	source
131I-chTNT	2007	I 393351		B
alemtuzumab	2001	DNP 15	38	B
aldesleukin	1992	ARMC 25	314	B
bevacizumab	2004	ARMC 40	450	B
catumaxomab	2009	DNP 23	18	B
celmoleukin	1992	DNP 06	102	B
cetuximab	2003	ARMC 39	346	B
denileukin diftitox	1999	ARMC 35	338	B
H-101	2005	DNP 19	46	B
ibritumomab	2002	ARMC 38	359	B
interferon alfa2a	1986	I 204503		B
interferon alfa2b	1986	I 165805		B
interferon, gamma-1a	1992	ARMC 28	332	B
interleukin-2	1989	ARMC 25	314	B
mobenakin	1999	ARMC 35	345	B
nimotuzumab	2006	DNP 20	29	B
ofatumumab	2009	DNP 23	18	B
panitumumab	2006	DNP 20	28	B
pegaspargase	1994	ARMC 30	306	B
Rexin-G (Trade name)	2007	I 346431		B
rituximab	1997	DNP 11	25	B
sipuleucel-T	2010	I 259673		B
tasonermin	1999	ARMC 35	349	B
teceleukin	1992	DNP 06	102	B
tositumomab	2003	ARMC 39	364	B
trastuzumab	1998	DNP 12	35	B
aclarubicin	1981	I 090013		N
actinomycin D	1964	FDA		N
angiotensin II	1994	ARMC 30	296	N
arglabin	1999	ARMC 35	335	N
asparaginase	1969	FDA		N
bleomycin	1966	FDA		N
carzinophilin	1954	Japan Antibiotics		N
chromomycin A3	1961	Japan Antibiotics		N
daunomycin	1967	FDA		N
doxorubicin	1966	FDA		N
leucovorin	1950	FDA		N
masoprocol	1992	ARMC 28	333	N

generic name	year introduced	reference	page	source
mithramycin	1961	FDA		N
mitomycin C	1956	FDA		N
neocarzinostatin	1976	Japan Antibiotics		N
paclitaxel	1993	ARMC 29	342	N
paclitaxel nanoparticles (Abraxane)	2005	DNP 19	45	N
paclitaxel nanoparticles (Nanoxel)	2007	I 422122		N
pentostatin	1992	ARMC 28	334	N
peplomycin	1981	I 090889		N
romidepsin	2010	DNP 23	18	N
sarkomycin	1954	FDA		N
streptozocin	pre-1977	Carter		N
testosterone	pre-1970	Cole		N
trabectedin	2007	ARMC 43	492	N
vinblastine	1965	FDA		N
vincristine	1963	FDA		N
solamargines	1989	DNP 03	25	NB
alitretinoin	1999	ARMC 35	333	ND
amrubicin HCl	2002	ARMC 38	349	ND
belotecan hydrochloride	2004	ARMC 40	449	ND
cabazitaxel	2010	I 287186		ND
calusterone	1973	FDA		ND
cladribine	1993	ARMC 29	335	ND
cytarabine ocfosfate	1993	ARMC 29	335	ND
dexamethasone	1958	FDA		ND
docetaxel	1995	ARMC 31	341	ND
dromostanolone	1961	FDA		ND
elliptinium acetate	1983	P091123		ND
epirubicin HCl	1984	ARMC 20	318	ND
eribulin	2010	I 287199		ND
estramustine	1980	FDA		ND
ethinyl estradiol	pre-1970	Cole		ND
etoposide	1980	FDA		ND
etoposide phosphate	1996	DNP 10	13	ND
exemestane	1999	DNP 13	46	ND
fluoxymesterone	pre-1970	Cole		ND
formestane	1993	ARMC 29	337	ND
fosfestrol	pre-1977	Carter		ND
fulvestrant	2002	ARMC 38	357	ND
gemtuzumab ozogamicin	2000	DNP 14	23	ND
goserelin acetate	1987	ARMC 23	336	ND
hexyl aminolevulinate	2004	I 300211		ND
histrelin	2004	I 109865		ND

generic name	year introduced	reference	page	source
hydroxyprogesterone	pre-1970	Cole		ND
idarubicin hydrochloride	1990	ARMC 26	303	ND
irinotecan hydrochloride	1994	ARMC 30	301	ND
ixabepilone	2007	ARMC 43	473	ND
leuprolide	1984	ARMC 20	319	ND
medroxyprogesterone acetate	1958	FDA		ND
megesterol acetate	1971	FDA		ND
methylprednisolone	1955	FDA		ND
methyltestosterone	1974	FDA		ND
mifamurtide	2010	DNP 23	18	ND
miltefosine	1993	ARMC 29	340	ND
mitobronitol	1979	FDA		ND
nadrolone phenylpropionate	1959	FDA		ND
norethindrone acetate	pre-1977	Carter		ND
pirarubicin	1988	ARMC 24	309	ND
pralatrexate	2009	DNP 23	18	ND
prednisolone	pre-1977	Carter		ND
prednisone	pre-1970	Cole		ND
talaporfin sodium	2004	ARMC 40	469	ND
temsirolimus	2007	ARMC 43	490	ND
teniposide	1967	FDA		ND
testolactone	1969	FDA		ND
topotecan HCl	1996	ARMC 32	320	ND
triamcinolone	1958	FDA		ND
triptorelin	1986	I 090485		ND
valrubicin	1999	ARMC 35	350	ND
vapreotide acetate	2004	I 135014		ND
vindesine	1979	FDA		ND
vinflunine	2010	I 219585		ND
vinorelbine	1989	ARMC 25	320	ND
zinostatin stimalamer	1994	ARMC 30	313	ND
amsacrine	1987	ARMC 23	327	S
arsenic trioxide	2000	DNP 14	23	S
bisantrene hydrochloride	1990	ARMC 26	300	S
busulfan	1954	FDA		S
carboplatin	1986	ARMC 22	318	S
carmustine (BCNU)	1977	FDA		S
chlorambucil	1956	FDA		S
chlortrianisene	pre-1981	Boyd		S
cis-diamminedichloroplatinum	1979	FDA		S
cyclophosphamide	1957	FDA		S
dacarbazine	1975	FDA		S

generic name	year introduced	reference	page	source
diethylstilbestrol	pre-1970	Cole		S
flutamide	1983	ARMC 19	318	S
fotemustine	1989	ARMC 25	313	S
heptaplatin/SK-2053R	1999	ARMC 35	348	S
hexamethylmelamine	1979	FDA		S
hydroxyurea	1968	FDA		S
ifosfamide	1976	FDA		S
lenalidomide	2005	DNP 19	45	S
levamisole	pre-1981	Boyd		S
lobaplatin	1998	DNP 12	35	S
lomustine (CCNU)	1976	FDA		S
lonidamine	1987	ARMC 23	337	S
mechlorethanamine	1958	FDA		S
melphalan	1961	FDA		S
miriplatin hydrate	2010	DNP 23	17	S
mitotane	1970	FDA		S
nedaplatin	1995	ARMC 31	347	S
nilutamide	1987	ARMC 23	338	S
nimustine hydrochloride	pre-1981	Boyd		S
oxaliplatin	1996	ARMC 32	313	S
pamidronate	1987	ARMC 23	326	S
pipobroman	1966	FDA		S
plerixafor hydrochloride	2009	DNP 22	17	S
porfimer sodium	1993	ARMC 29	343	S
procarbazine	1969	FDA		S
ranimustine	1987	ARMC 23	341	S
razoxane	pre-1977	Carter		S
semustine (MCCNU)	pre-1977	Carter		S
sobuzoxane	1994	ARMC 30	310	S
sorafenib	2005	DNP 19	45	S
thiotepa	1959	FDA		S
triethylenemelamine	pre-1981	Boyd		S
zoledronic acid	2000	DNP 14	24	S
anastrozole	1995	ARMC 31	338	S/NM
bicalutamide	1995	ARMC 31	338	S/NM
bortezomib	2003	ARMC 39	345	S/NM
camostat mesylate	1985	ARMC 21	325	S/NM
dasatinib	2006	DNP 20	27	S/NM
erlotinib hydrochloride	2004	ARMC 40	454	S/NM
fadrozole HCl	1995	ARMC 31	342	S/NM
gefitinib	2002	ARMC 38	358	S/NM
imatinib mesilate	2001	DNP 15	38	S/NM

generic name	year introduced	reference	page	source
lapatinib ditosylate	2007	ARMC 43	475	S/NM
letrazole	1996	ARMC 32	311	S/NM
nafoxidine	pre-1977	Carter		S/NM
nilotinib hydrochloride	2007	ARMC 43	480	S/NM
pazopanib	2009	DNP 23	18	S/NM
sunitinib malate	2006	DNP 20	27	S/NM
tamoxifen	1973	FDA		S/NM
temoporfin	2002	I 158118		S/NM
toremifene	1989	ARMC 25	319	S/NM
aminoglutethimide	1981	FDA		S*
azacytidine	2004	ARMC 40	447	S*
capecitabine	1998	ARMC 34	319	S*
carmofur	1981	I 091100		S*
clofarabine	2005	DNP 19	44	S*
cytosine arabinoside	1969	FDA		S*
decitabine	2006	DNP 20	27	S*
doxifluridine	1987	ARMC 23	332	S*
enocitabine	1983	ARMC 19	318	S*
floxuridine	1971	FDA		S*
fludarabine phosphate	1991	ARMC 27	327	S*
fluorouracil	1962	FDA		S*
ftorafur	1972	FDA		S*
gemcitabine HCl	1995	ARMC 31	344	S*
mercaptopurine	1953	FDA		S*
methotrexate	1954	FDA		S*
mitoxantrone HCl	1984	ARMC 20	321	S*
nelarabine	2006	ARMC 42	528	S*
thioguanine	1966	FDA		S*
uracil mustard	1966	FDA		S*
abarelix	2004	ARMC 40	446	S*/NM
bexarotene	2000	DNP 14	23	S*/NM
degarelix	2009	DNP 22	16	S*/NM
pemetrexed disodium	2004	ARMC 40	463	S*/NM
raltitrexed	1996	ARMC 32	315	S*/NM
tamibarotene	2005	DNP 19	45	S*/NM
temozolomide	1999	ARMC 35	350	S*/NM
vorinostat	2006	DNP 20	27	S*/NM
autologous tumor cell-BCG	2008	DNP 22	17	V
bcg live	1990	DNP 04	104	V
Cervarix (Trade name)	2007	I 309201		V
melanoma theraccine	2001	DNP 15	38	V
vitespen	2008	DNP 22	17	V

[a]Note that in Figure 9 there are three vertical bars corresponding to the drugs noted in the "year introduced" column above as "pre-1970", "pre-1977" and "pre-1981". The entries under these three categories are not repeated the other two, as the drugs are individually distinct entries, but their actual dates cannot be determined.

Table 10

Antidiabetic Drugs from 01.01.1981 to 12.31.2010 Organized Alphabetically by Generic Name within Source

generic name	trade name	year introduced	volume	page	source
biphasic porcine insulin	Pork Mixtard 30	1982	I 303034		B
hu neutral insulin	Insuman	1992	I 255451		B
hu insulin zinc suspension	Humulin Zn	1985	I 091584		B
human insulin Zn suspension	Humulin L	1985	I 302828		B
human neutral insulin	Novolin R	1991	I 182551		B
insulin aspart	NovoRapid	1999	DNP 13	41	B
insulin aspart/IA protamine	NovoMix 30	2001	DNP 15	34	B
insulin determir	Levemir	2004	DNP 18	27	B
insulin glargine	Lantus	2000	DNP 14	19	B
insulin glulisine	Apidra	2005	DNP 19	39	B
insulin lispro	Humalog	1996	ARMC 32	310	B
isophane insulin	Humulin N	1982	I 091583		B
mecasermin	Somazon	1994	DNP 08	28	B
oral insulin	Oral-lyn	2005	DNP 19	39	B
porcine isophane insulin	Pork Insulatard	1982	I 302757		B
porcine neutral insulin	Pork Actrapid	1998	I 302749		B
pulmonary insulin	Exubera	2005	DNP 20	23	B
soluble insulin	Velosulin BR	1986	I 091581		B
voglibose	Basen	1994	ARMC 30	313	N
acarbose	Glucobay	1990	DNP 03	23	ND
exenatide	Byetta	2005	DNP 19	40	ND
liraglutide	Victoza	2009	DNP 23	13	ND
miglitol	Diastabol	1998	ARMC 34	325	ND
triproamylin acetate	Normylin	2005	DNP 19	40	ND
glimepiride	Amaryl	1995	ARMC 31	344	S
mitiglinide calcium hydrate	Glufast	2004	ARMC 40	460	S
pioglitazone NCl	Actos	1999	ARMC 35	346	S
repaglinide	Prandin	1998	ARMC 34	329	S
alogliptin benzoate	Nesina	2010	I 405286		S/NM

generic name	trade name	year introduced	volume	page	source
epalrestat	Kinedak	1992	ARMC 28	330	S/NM
rosiglitazone maleate	Avandia	1999	ARMC 35	348	S/NM
saxagliptin	Onglyza	2009	DNP 23	13	S/NM
sitagliptin	Januvia	2006	DNP 20	23	S/NM
tolrestat	Alredase	1989	ARMC 25	319	S/NM
troglitazone	Rezulin	1997	ARMC 33	344	S/NM
vildagliptin	Galvus	2007	ARMC 43	494	S/NM
nateglinide	Starsis	1999	ARMC 35	344	S*

J Nat Prod. Author manuscript; available in PMC 2013 July 24.

IN THE HIGH COURT OF AUSTRALIA
SYDNEY REGISTRY No. S28 of 2015

BETWEEN: **YVONNE D'ARCY**
Appellant

and

MYRIAD GENETICS INC
First Respondent

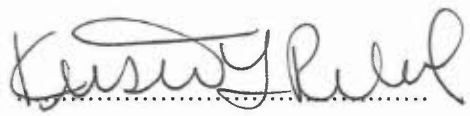
GENETIC TECHNOLOGIES LIMITED ABN 17 009 212 328
Second Respondent

EXHIBIT SMK-2

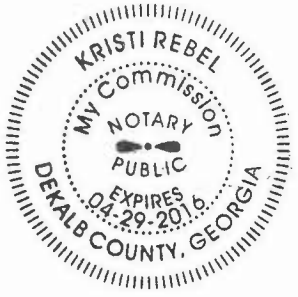
This is the exhibit marked **Exhibit SMK-2** produced and shown to Sherry M. Knowles at the time of swearing her affidavit this 11 March 2015.

Press release from The Tufts Center for the Study of Drug Development dated November 18, 2014

Before me



Kristi L. Rebel, Notary Public



Tufts Center for the Study of Drug Development

Tufts University

News

November 18, 2014

Cost to Develop and Win Marketing Approval for a New Drug Is $2.6 Billion

BOSTON – Nov. 18, 2014 – Developing a new prescription medicine that gains marketing approval, a process often lasting longer than a decade, is estimated to cost $2,558 million, according to a new study by the Tufts Center for the Study of Drug Development.

The $2,558 million figure per approved compound is based on estimated:

- Average out-of-pocket cost of $1,395 million
- Time costs (expected returns that investors forego while a drug is in development) of $1,163 million

Estimated average cost of post-approval R&D—studies to test new indications, new formulations, new dosage strengths and regimens, and to

monitor safety and long-term side effects in patients required by the U.S. Food and Drug Administration as a condition of approval—of $312 million boosts the full product lifecycle cost per approved drug to $2,870 million. All figures are expressed in 2013 dollars.

The new analysis, which updates similar Tufts CSDD analyses, was developed from information provided by 10 pharmaceutical companies on 106 randomly selected drugs that were first tested in human subjects anywhere in the world from 1995 to 2007.

"Drug development remains a costly undertaking despite ongoing efforts across the full spectrum of pharmaceutical and biotech companies to rein in growing R&D costs," said Joseph A. DiMasi, director of economic analysis at Tufts CSDD and principal investigator for the study.

He added, "Because the R&D process is marked by substantial technical risks, with expenditures incurred for many development projects that fail to result in a marketed product, our estimate links the costs of unsuccessful projects to those that are successful in obtaining marketing approval from regulatory authorities."

In a study published in 2003, Tufts CSDD estimated the cost per approved new drug to be $802 million (in 2000 dollars) for drugs first tested in human subjects from 1983 to 1994, based on average out-of-pocket costs of $403 million and capital costs of $401 million.

The $802 million, equal to $1,044 million in 2013 dollars, indicates that the cost to develop and win marketing approval for a new drug has increased by 145% between the two study periods, or at a compound annual growth rate of 8.5%.

According to DiMasi, rising drug development costs have been driven mainly by increases in out-of-pocket costs for individual drugs and higher failure rates for drugs tested in human subjects.

Factors that likely have boosted out-of-pocket clinical costs include increased clinical trial complexity, larger clinical trial sizes, higher cost of inputs from the medical sector used for development, greater focus on

targeting chronic and degenerative diseases, changes in protocol design to include efforts to gather health technology assessment information, and testing on comparator drugs to accommodate payer demands for comparative effectiveness data.

Lengthening development and approval times were not responsible for driving up development costs, according to DiMasi.

"In fact," DiMasi said, "changes in the overall time profile for development and regulatory approval phases had a modest moderating effect on the increase in R&D costs. As a result, the time cost share of total cost declined from approximately 50% in previous studies to 45% for this study."

The study was authored by DiMasi, Henry G. Grabowski of the Duke University Department of Economics, and Ronald W. Hansen at the Simon Business School at the University of Rochester.

ABOUT THE TUFTS CENTER FOR THE STUDY OF DRUG DEVELOPMENT

The Tufts Center for the Study of Drug Development (http://csdd.tufts.edu) at Tufts University provides strategic information to help drug developers, regulators, and policy makers improve the quality and efficiency of pharmaceutical development, review, and utilization. Tufts CSDD, based in Boston, conducts a wide range of in-depth analyses on pharmaceutical issues and hosts symposia, workshops, and public forums, and publishes Tufts CSDD Impact Reports, a bi-monthly newsletter providing analysis and insight into critical drug development issues.

--end--

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Business Communication Strategies

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For the backgrounder and slides for the Tufts CSDD Cost Study, click here.

- News
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Privacy Policy

IN THE HIGH COURT OF AUSTRALIA
SYDNEY REGISTRY

No. S28 of 2015

BETWEEN:

YVONNE D'ARCY
Appellant

and

MYRIAD GENETICS INC
First Respondent

GENETIC TECHNOLOGIES LIMITED ABN 17 009 212 328
Second Respondent

EXHIBIT SMK-3

This is the exhibit marked **Exhibit SMK-3** produced and shown to Sherry M. Knowles at the time of swearing her affidavit this 11 March 2015.

U.S. Patent No. 3,590,028

Before me

.....
Kristi L. Rebel, Notary Public

KRISTI REBEL
My Commission
NOTARY
PUBLIC
EXPIRES
04-29-2016
DEKALB COUNTY, GEORGIA

United States Patent Office

3,590,028

Patented June 29, 1971

1

3,590,028

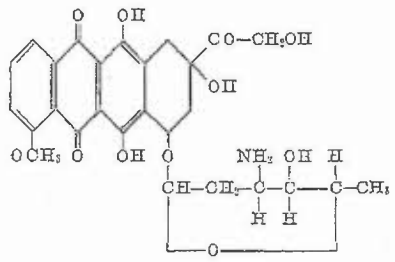
ADRIAMYCIN DERIVATIVES

Federico Arcamone, Milan, Giuseppe Cassinelli, Rivanazzano, Pavia, and Aurelio di Marco and Marcello Gaetani, Milan, Italy, assignors to Società Farmaceutici Italia, Milan, Italy

No Drawing. Filed Apr. 18, 1968, Ser. No. 722,221
Claims priority, application Italy, Apr. 18, 1967, 15,056/67
Int. Cl. C07c 47/18, 95/04
U.S. Cl. 260—210 7 Claims

ABSTRACT OF THE DISCLOSURE

Described is the antibiotic "adriamycin" and its derivatives. "Adriamycin" has the formula



and is prepared by aerobic fermentation of mutant F.I. 106 of *Streptomyces peucetius*. The compounds show antitumoral activity on some mouse and rat tumors.

Our invention relates to a new antibiotic substance and its derivatives which are particularly useful in therapy as antitumoral products and to a process for the preparation thereof. More particularly our invention has as its object a new antibiotic of the indicator type, which we call "adriamycin" or antibiotic "B–106 F.I.," its salts, its hydrolytic degradation products, and a biosynthesis process for the preparation thereof by the use of a new microorganism. The new microorganism used in the process of the present invention has been obtained by mutageneous treatment of *Streptomyces peucetius* described in British Pat. 1,003,383, U.S. patent application Ser. No. 404,550 and in the Giorn. Microbiol. vol. 11, 1963, pp. 109–118. The new strain thus obtained has been given the code F.I. 106 of the Farmitalia microbiological collection and has been called *Streptomyces peucetius* var. *caesius*.

S. peucetius var. *caesius* has been deposited at the Institute of Microbiology of the Rutger University (U.S.A.) receiving the index number I.M.R.U. 3920 and at the Institute of Plant Pathology of the University of Milan (Italy) receiving the index number I.P.V. 1946.

The new microorganism has the following microscopic, macroscopic and biochemical properties:

MICROSCOPIC PROPERTIES

The vegetative mycelium on the usual culture media shows thin hyphae (0.5–0.9μ thick) more or less long and branched. The ramifications form thicker hyphae (1.1–1.6μ thick), the conidiophores are often collected in fasciculated forms ending in hooks. The conidia are spherical with a diameter between 1.8 and 3.3μ, first disposed in little chains, then free. Under the electronic microscope, the conidia appear nearly spherical, of irregula contours with a warty surface.

2

MACROSCOPIC PROPERTIES

In Table 1 are given the cultural properties noticed on the indicated media, in which the microorganism is grown at 28° C. observations being made at the 3rd, 8th, 15th. 21st and 30th day after inoculation.

BIOCHEMICAL PROPERTIES

Gelatin: slow and partial hydrolysis
Nitrates: no reduction to nitrites
Production of hydrogen sulfide: positive
Milk: no peptonization; no coagulation
Starch: very slow and slight hydrolysis
Maltose, xylose, mannose, mannitol, glycerol, glucose, saccharose, trehalose, raffinose, fructose are utilized.
Lactose, adonitol, ramnose, sorbitol, arabinose, esculine and mesoinositol are not utilized.
Antibiotics: in liquid submerged culture it produces substances having antibiotic and antitumoral activity.

CLASSIFICATION OF THE MUTANT F.I. 106

The mutant F.I. 106 has the following taxonomic position. In the classification system of Pridham et al. (Appl. Microbiol. 6, p. 52 1958) the microorganism belongs to the section *Retinaculum apertum*, series Red. In the classification system of Baldacci (Giorn. Microbiol. 6, p. 10, 1958) the microorganism belongs to the series *Albosporeus*; and in the system of Waksman (The Actinomycetes, Vol. II, p. 129, 1961) the microorganism belongs to the series Ruber. A comparison between the characteristics of the microorganism F.I. 106 and those of the species belonging to the cited systematic groups (Taxa) has shown that none of the latter has characteristics corresponding to those of F.I. 106.

In Table II are given these comparison data concerning the species producing substances similar to those studied. In this table, *S. cinereoruber*, *S. cinereoruber* var. *fructofermentans*, *S. caespitosus* and *S. antibioticus* have also been included even though they are not part of the above cited Taxa. There is also a list of the differences from the species which do not produce substances of the studied type.

Our microorganism differs from the species *S. albosporeus* (Waksman: The Actinomycetes, Vol. II, p. 171, 1961) because the latter does not produce soluble pigments, reduces nitrates and does not produce H_2S; from *S. cinnamomensis* (Waksman: The Actinomycetes, Vol. II, p. 195, 1961) and from *S. fradiae* (Waksman: The Actinomycetes, Vol. II, p. 211, 1961) in the color of the vegetative mycelium and aerial mycelium; from the species *S. ruber* (Waksman: The Actinomycetes, Vol. II, p. 271, 1961) because the latter coagulates the milk, does not produce soluble pigments and does not produce H_2S; from *S. rubescens* (Waksman: The Actinomycetes, Vol. II, p. 271, 1961) in the color of the aerial mycelium and because *S. rubescens* does not form any soluble pigments and does not produce hydrogen sulfide; from *S. oidiosporus* (Waksman; The Actinomycetes, Vol. II, p. 251, 1961) because the latter dotes not reduce nitrates and does not peptonize milk. Moreover, *S. oidiosporus* does not produce soluble pigments.

It is concluded that the mutant F.I. 106 of *S. peucetius* is different from all the species producing similar substances and more generally, it is different from all the species belonging to the systematic subgeneric groups to which the strain itself belongs. Particularly, the strain F.I. 106 differs from the parent strain *S. peucetius* which produces daunomycin (British Pat. 1,003,383) because it forms a vegetative mycelium more intensely red colored, an aerial mycelium which sometimes assumes blue-green turquoise tonality and lastly because it produces the antibiotic adriamycin.

TABLE I

Cultural properties of the mutant F.I. 106 of *S. peucetius*

Medium	Growth	Aerial mycelium	Vegetative mycelium	Soluble pigments
Agar malt yeast extract (according to Hesseltine et al. 1954).[1]	Little confluent colonies with wrinkled folds, hard, relieved, abundant.	Very scanty, smooth pale pink colored, absence of spirals and verticils.	Abundant, yellowish then yellow-reddish.	Intense, first yellow-red then brown-red.
Bennet agar	Scanty, single yellowish little colonies.	Absent	Scanty, first yellowish then orange.	Absent.
Emerson agar	Moderate, little confluent colonies.	do	Moderate, first pale pink then reddish.	Reddish-clear brown.
Agar potato (according to Hessoltine et. al 1954).[1]	Abundant in smooth regular patina.	Abundant, first pink, then attenuate blue-green turquoise hook-ended and then ball-ended hyphae.	Abundant, flesh colored. Hard smooth patina.	Intense, first yellow-reddish then from strong orange to light red.
Agar peptone plus potassium nitrate.	Abundant, in confluent little colonies.	Absent	Abundant, colorless	Absent.
Agar Czapeck	Abundant in confluent little colonies.	Scanty, first dirty white then attenuate blue-green turquoise, slightly cottony hook-ended or ball-ended hyphae.	Abundant, pale pink colored.	Do.
Asparagine glucose agar	Scanty, in isolated little colonies.	Scanty, whitish rose. Very broken mycelium short without apical hooks.	Scanty, colorless	Do.
Glycerine-glycine agar	Abundant, in smooth, hard patina.	Absent	Abundant, from yellow to orange.	Do.
Starch agar	Scanty, in single little colonies.	do	Scanty, colorless then yellowish rose.	Do.
Gelatin	Moderate, in surface	do	Moderate, from colourless to yellowish.	Abundant, brown dark black.
Milk	Scanty	do	Scanty, ring formed surface pink-salmon colored.	Scanty, pink.

[1] Hesseltine et al.: Ann. N.Y. Acad. Sci., 1954, 60, pp. 136–151.

TABLE II

Comparison between the mutant F.I. 106 of *S. peucetius* and species producing substances similar to the antibiotic adriamycin

	Mutant F.I. 106	*S. purpurascens*	*S. bobiliae*	*S. cinereo ruber*	*S. cerulco rubidus*
Sporophores	Straight or hooked-like	Spirally	Spirally	Straight or hooked-like	Biverticilly spirally.
Spores	Nearly round, warty, 1.8×3.8μ.	Oval, spiny, 0.8–1μ per 0.4–0.5μ		Oval, smooth, 0.7–1μ per 0.9–2μ,	Oval, spiny, 0.6–0.9×0.8–1.2μ.
Vegetative mycelium	From yellow-red to intense red.	Red	Coral-red	Yellow-red brown	Yellow-red brown.
Aerial mycelium	White-rose, sometimes attenuate blue-green turquoise.	White-rose	White	Ash-grey	Blue turquoise.
Reduction of:					
Nitrates	−	/	+	/	/.
Milk (pep. coag.)	−	−	+	+	/.
L-xylose	+	+	+	+	+.
L-arabinose	−	+	+	+	+.
L-ramnose	−	+	+	−	+.
Fructose	+	+	+	−	+.
Saccharose	+	+	+	−	+.
Lactose	−	+	+	+	+.
Raffinose	−	+	+	−	+.
D-mannite	+	+	−	−	+.
D-sorbite	−	−	−	−	+.
Produced antibiotics	Adriamycin	Rodomycin	Cynerubin	Rodomycin	Rubidomycin.

	S. cinereo ruber var. *fructofermentans*	*S. caespitosus*	*S. nivecruber*	*S. galilaeus*	*S. nogalater* var. *nogalater*
Sporophores	Straight or hooked-like	Verticilly	Spirally	Spirally	Straight or hooked-like.
Spores	Oval, smooth, 0.7–1μ per 0.9–2μ.	Oval, smooth, 0.5–1.5μ per 0.3–0.5μ.	Smooth	Smooth	More or less spherical, smooth.
Vegetative mycelium	Yellow-red brown	From cream to brown to yellow-reddish.	Carmine-red	Carmine-red	Orange-red.
Aerial mycelium	Ash-grey	White yellowish grey	Whitish	From white to ash-grey	Grey.
Reduction of:					
Nitrates	/	+	/	/	−.
Milk (pep. coag.)	+	+	−	+	+.
L-xylose	+	−	/	/	+.
L-arabinose	+	−	/	/	+.
L-ramnose	+	−	/	/	+.
Fructose	+	+	/	/	+.
Saccharose	+	+	/	/	−.
Lactose	+	/	/	/	+.
Raffinose	−	−	/	/	+.
D-mannite	+	−	/	/	+.
D-sorbite	+	+	/	/	+.
Produced antibiotics	Cynerubin	Mithomycin	Cynerubin	Cynerubin	Nogalamycin.

	S. antibioticus	*S.a.* 1165	*S.a.* 220	*S. doa* 1205
Sporophores	Straight	Not described	Not described	Made as spirals.
Spores	Smooth, spheric	do	do	Not described.
Vegetative mycelium	Yellow-cream	do	do	Brick-red vinous-red.
Aerial mycelium	From white to mouse grey	do	do	Red-grey.
Reduction of:				
Nitrates	/	/	/	/.
Milk (pep. coag.)	+	/	/	+.
L-xylose	/	/	/	/.
L-arabinose	/	/	/	/.
L-ramnose	/	/	/	/.
Fructose	/	/	/	/.
Saccharose	/	/	/	/.
Lactose	/	/	/	/.
Raffinose	/	/	/	/.
D-mannite	/	/	/	/.
D-sorbite	/	/	/	/.
Produced antibiotics	Cynerubin	Akiavin	Rutilantin	Pyrromycin.

+=positive reaction.
−=negative reaction.
For S.a. 1165 and S.a. 220 see Asheshov et al. Antibiotics and Chemotherapy, 1954, 4, 389
For *S. doa* 1205 see Brockmann, Chem. Ber., 1959, 92, 1880.
/=data are lacking.

The mutant F.I. 106 may be stored by lyophilization using as suspending medium milk or milk serum, or by collecting and maintaining the spores in a sterile substrate. It may also be stored by successive cultivations on a solid medium containing glucose or another suitable sugar and complex substances containing nitrogen (yeast extract, peptone, or hydrolyzed casein). The medium may besides contain some salts among which magnesium sulphate and potassium phosphate are particularly important.

The production of the antibiotic is carried out by usual and well know methods and consists in cultering the mutant F.I. 106, in a previously sterilized liquid cultural medium under aerobic conditions at from 25° to 37° C. (preferably at 28° C.) over a period from 3 to 7 days (preferably 5 days) at a pH which initially is from 6.5 to 7.0 and at the end of the fermentative process is of from 7.5 to 8.0. The cultural medium consists of a carbon and a nitrogen source and mineral salts. The carbon source may for example be starch, dextrin, glucose, glycerin, mannite, maltose, corn steep liquor, distillers solubles, soyabean oil or soyabean meal. The nitrogen source besides the above mentioned complex substances containing nitrogen may be for example dry yeast, meat peptone, or casein. Good results are even obtained by using ammonium salts such as ammonium nitrates, ammonium sulphates, diammonium phosphates. The mineral salts useful for the production of the antibiotic may vary according to the medium employed. In a medium containing complex substances such as various meals and fermentation residues, the addition of calcium carbonate and sodium or potassium phosphates have proved useful. In media containing glucose, yeast or ammonium salts, much higher additions of mineral salts such as potassium, magnesium, iron, zinc, manganese, copper and salts are necessary. The fermentation may be carried out in Erlenmeyer flasks or in laboratory or industrial fermenters of various capacity. The quantity of adriamycin present in the broths may be evaluated by the following method. The culture is filtered with the help of 2% Hyflo Supercel (registered trademark). The broth filtered is adjusted to pH 8.6 with 1 N sodium hydroxide solution, and is extracted twice with a 9:1 chloroform-methanol mixture. The extract is washed with water, then concentrated to dryness in vacuo. The residue is taken up with methyl alcohol and then chromatographed over whatman MM No. 3 paper buffered with M/15 phosphate buffer at pH 5.4, employing as an eluant a 7:1:2 propanol-ethyl acetate-water mixture. The red-colored part corresponding to Rf of adriamycin is eluted with a 9:1 methanol-water mixture and the quantity of adriamycin present in the filtered broth is evaluated by spectrophotometrically checking a sample of the eluate at the wavelength of 495 mμ and compared with a sample of adriamycin of which the titer is known.

The quantity of adriamycin present in the mycelium is evaluated in the following manner. The mycelium is extracted with a 4:1 acetone-0.1 N sulphuric acid mixture. The extract is neutralized and concentrated under reduced pressure to ⅓ of the original volume. The concentrate is adjusted to pH 8.6 with 1 N sodium hydroxide solution, then extracted twice with a 9:1 chloroform-methanol mixture. The extract is washed with water, then concentrated to dryness in vacuo. The content of adriamycin is determined on a sample of the residue, using the same method as described above.

In order to isolate adriamycin, the antibiotic may be extracted with a suitable solvent either from the culture broth "in toto" without filtering the mycelium mass or from the mycelium and the culture liquid previously separated by filtration. When carrying out the extraction separately, it is preferred to operate as follows. At the end of the fermentation, an adsorbent siliceous material, such as Supercel, is added to the culture broth. The mixture is filtered and both the filtration cake and the filtrate are treated separately. Most of the antibiotic is found in the filtration cake which consists of the mycelium mixed with the adsorbent siliceous materials. This cake is pulped and stirred in an organic solvent. Suitable solvents are alcohols, such as methanol, ethanol, butanol, ketones such as acetone, methylethylketone; halogenated hydrocarbons such as chloroform, methylene chloride or aqueous solutions of organic or inorganic acids; such as acetic acid, hydrochloric acid, sulphuric acid. Advantageously, mixtures of organic solvents, such as alcohols and water-miscible ketones and aqueous solutions of inorganic acids may be used. Generally a mixture of acetone/0.1 N sulphuric acid in a ratio of from 7:1 to 3:1, preferably 4:1, is employed.

From the filtered broth, previously made alkaline to pH 8.5–9.0, the antibiotic may be extracted with water-immiscible organic solvents of the group of alcohols, ketones and halogenated lower aliphatic hydrocarbons such as amyl alcohol, butyl alcohol, methyl-isobutyl-ketone, methylene chloride, chloroform and mixtures thereof. Another method of extracting the filtered broth is to pass the broth itself through chromatographic column containing cationic carboxylic exchange resin (Amberlite IR 50 type) in acid form and eluting the product with an aqueous methanol solution of sodium chloride.

The organic extracts of the broth and of the mycelium are collected, neutralized, mixed with water, then concentrated under reduced pressure. The aqueous concentrate is adjusted to pH 3 with 1 N hydrochloric acid, then extracted with chloroform. The extract containing various impurities is removed while the aqueous layer is adjusted to pH 8.5–9.0 and extracted with a 9:1 chloroform-methanol mixture. The extract is washed with water, dried over anhydrous sodium sulphate, then concentrated to small volume under reduced pressure. From the concentrate, on addition of ethyl ether, a crude product containing as principal component adriamycin as a free base is obtained.

In order to purify adriamycin from various water- and lipo-soluble pigments countercurrent distribution or column chromatography may be used. In the first case, a 2:2:1 chloroform-methanol M/15 phosphate buffer mixture at pH 5.4 may be used. Better results are obtained employing chromatography over a column of cellulose buffered with a phosphate at pH 5.4 and using as eluting agent a propanol-ethyl acetate-water (7:1:2) mixture. The fractions containing adriamycin are collected and concentrated after addition of water. The aqueous concentrate is adjusted to pH 8.6 with 1 N sodium carbonate, then extracted with chloroform. The chloroform solution is dried over anhydrous sodium sulphate and then concentrated to a small volume. By adding anhydrous methanol containing hydrochloric acid, adriamycin hydrochloride is obtained as orange-red colored thin needles, which on recrystallization from anhydrous ethyl alcohol, yields orange-red needles melting at 204–205° C. (with decomposition). It is optically active $[\alpha]_D^{23°} = +248° \pm 2°$ (c.=0.1 in methanol).

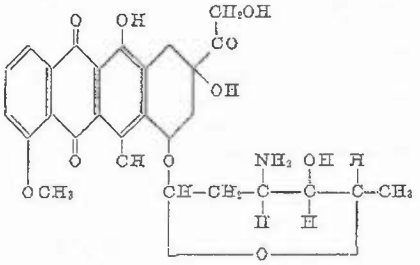
Elemental analysis of a purified adriamycin hydrochloride sample gives the following (percent): C=54.36, H=5.43, N=2.37, Cl=6.42.

The empirical formula corresponds to $C_{27}H_{29}NO_{11} \cdot HCl$ and the molecular weight is 579.98. The adriamycin hydrochloride is soluble in water, methanol and aqueous alcohols but is insoluble in acetone, benzene, chloroform, ethyl ether and petroleum ether. The alcoholic solutions of the antibiotic give characteristic coloring with metallic salts: crimson red with magnesium salts, crimson red with calcium salts, and dark red with lead salts. At an alkaline pH, a turning point to violet color and precipitation of pigmented substances is observed. Aqueous solutions of adriamycin hydrochloride are yellow-orange at acid pH, red-orange at a neutral pH and violet-blue at a pH higher than 9. The spectrum in U.V.

and in the visible ranges in methanol is characterized by the following maxima:

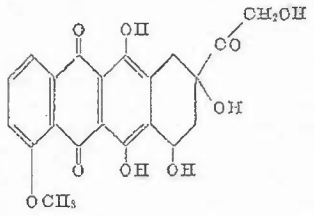
at 233 mμ ($E^{1\%}_{1cm.}$=673)
at 252 mμ ($E^{1\%}_{1cm.}$=450)
at 288 mμ ($E^{1\%}_{1cm.}$=159)
at 479 mμ ($E^{1\%}_{1cm.}$=219)
at 496 mμ ($E^{1\%}_{1cm.}$=217)
at 529 mμ ($E^{1\%}_{1cm.}$=118)

In the I.R. spectrum bands of the following wavelengths are noted: (in μ): 3.00, 3.44, 5.80, 6.17, 6.31, 6.55, 7.05, 7.78, 8.11, 8.24, 9.00, 9.35, 10.10, 10.98, 11.50, 12.68, 13.12, 14.60. Adriamycin has the following structural Formula I:



(I)

The antibiotic is a base to form salts with inorganic and organic acids. The color change observed from red to blue-violet at pH ~9 is due to the salification of the phenolic hydroxyl-groups. Acids split the glycosidic bond. For example, heating adriamycin to 100° C. in 0.5 N mineral acids for one hour, gives a red-colored aglycone, insoluble in water (adriamycinone) and a water-soluble, basic, reducing fraction (daunosamine). Adriamycinone has the following structural Formula II:



(II)

the corresponding empirical formula is $C_{21}H_{18}O_9$. It melts at 223–224° C.; $[\alpha]_D$=+156° (c.=0.1 in dioxane).

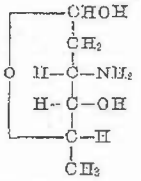
The spectrum in the U.V. and in the visible ranges shows maxima at the following wavelengths:

$\lambda_{max.}$ (mμ):	$E^{1\%}_{1cm.}$
233	887
251	631
288	211
478	282
495	290
528	173

In the I.R. spectrum, the following absorption bands are noted (in μ): 2.90, 3.42, 5.79, 6.18, 6.34, 6.92, 7.08, 7.26, 7.42, 7.80, 7.90, 8.05, 8.29, 8.43, 8.72, 8.93, 9.30, 9.88, 10.10, 10.86, 12.32, 12.75, 13.16, 13.70, 14.40. The mass spectrum of adriamycinone shows the following tops: m/e 414 (M), 378 (M–$2H_2O$), 347 (M–$2H_2O$—CH_2OH).

The pentaacetate of adriamycinone (prepared by treatment of adriamycinone with acetic anhydride and pyridine) has the empirical formula $C_{31}H_{28}O_{14}$, melting at 164–166° C.; $[\alpha]_D$=—94° (c.=0.1 chloroform) and shows the following mass spectrum: m/e 624 (M), 582 (M–CH_2CO), 540 (M–$2CH_2CO$), 480 (M–$2CH_2CO$—CH_3COOH), 420 (M–$2CH_2CO$—$2CH_3COOH$), 378 (M–$3CH_2CO$ — $2CH_3COOH$), 347 (M – $3CH_2CO$ — $2CH_3COOH$—CH_2OH).

The water-soluble fraction (daunosamine) consists of a reducing aminosugar having the following structure III:



(III)

Daunosamine hydrochloride melts at 168° C. (with decomposition); $[\alpha]_D$=—54.5° (in water); N-benzoylderivative melts at 154–156° C.

Chromatography of adriamycin hydrochloride and its aglycone in comparison with daunomycin and daunomycinone

Paper chromatography.—Whatman paper No. 1 buffered with M/15 phosphate buffer at pH 5.4, descending development for 16 hours at room temperature.

Solvent A: Butanol saturated with M/15 phosphate buffer at pH 5.4;
Solvent B: Propanol ethyl acetate water (7:1:2).

Thin layer chromatography. — Kieselgel G layer (Merck) buffered with 1% oxalic acid in water. The chromatogram was run at 10 cm. at room temperature.

System C: methylene chloride methanol (100:15);
System D: n-butanol-acetic acid-water (4:1:5) upper phase;
System E: benzene-ethyl acetate petroleum ether boiling at 80–120° C. (80:50:20);
System F: benzole-ethyl formate-formic acid (50:50:1).

	Chromatography on—					
	Paper			Thin layer		
System	A	B	C	D	E	F
Compound:						
Adriamycin Rf	0.10	0.25	0.17	0.33	0.00	0.00
Daunomycin hydrochloride Rf	0.20	0.50	0.35	0.40	0.90	0.00
Aglycone of adriamycin (Adriamycinone) Rf	0.30	0.65	0.93	0.80	0.10	0.25
Daunomycinone Rf	0.75	0.85	0.95	0.85	0.15	0.40

The acid addition salts of adriamycin are obtained by reacting the base with non-toxic organic and inorganic acids, such as hydrochloric acid, sulphuric acid, acetic acid, propionic acid, valerianic acid, palmitic acid, oleic acid, citric acid, succinic acid, mandelic acid, glutamic acid, and pantothenic acid. Neutral salts are obtained by reaction of the corresponding acid with the free base, which is obtained by extraction of an aqueous solution of the hydrochloride at pH 8.6 with organic water-immiscible solvents, such as butanol and chloroform. By evaporation of the organic solvent, the antibiotic adriamycin is obtained in the form of free base. The salts may be also obtained by double exchange of the salts, for example, adriamycin pantothenate is obtained from adiamycin sulphate with calcium pantothenate. Although the antibiotic adriamycin has a remarkable bacteriostatic activity against several microorganisms (see Table 3), it has proved particularly useful as an antitumoral.

TABLE 3

Antibiotic activity of adriamycin hydrochloride

Strains	Medium	DLM μg./ml.
Staph. aureus ep. 209 P	Meat broth	12.5
B. subtillis	do	6.25
S. faecalis	do	50
S. abortivo equina	do	50
S. coli B	do	3
Sh. flexneri	do	>50
C. albicans	Sabouraud	>50

The antibiotic shows a marked inhibitory effect on tumor growth in ascitic form, in which an immediate con-

tact of the antibiotic and the neoplastic cells is achieved. A good inhibiting effect is observed also in solid tumors where the activity is different according to the administration route and to the dose. The antitumoral activity of adriamycin gives better results in efficacy and duration than daunomycin also in these tests.

PHARMACOLOGY

Study of the antitumoral activity of the antibiotic adriamycin

The study of the antitumoral activity of the antibiotic adriamycin obtained from Streptomyces F.I. 106 has been carried out on some mouse and rat tumors both in the solid and ascitic form.

(1) Ascitic tumors.—Activity tests have been carried out on mice bearing Ehrlich ascitic carcinoma and treated intraperitoneously with solutions of the antibiotic, at different concentrations, for 5 consecutive days starting from the same day following the tumor implantation. Table 4, where the obtained results are summarized, shows that the antibiotic under examination, administered in equal doses of 1.75 and 2.50 mg./kg./day, has a remarkable inhibitory effect on the ascitic tumor growth and has increased considerably the average survival rate of the treated animals.

TABLE 4

Ehrlich ascitic carcinoma

Lots of 10 animals	Dose, mg./kg./day	Body weight change, grams (days after implantation) 6	12	Average survival time,
Controls		+7.5	+13.9	14
Adriamycin	1.75	−0.5	+3.8	33.8
	2.50	−1.8	+0.9	34.6

The results have been confirmed by a successive experiment in which the antibiotic has been administered at the doses of 1.25 and 2.50 mg./kg./day (Table 5).

TABLE 5

Ehrlich ascitic carcinoma

Lots of 10 animals	Dose, mg./kg./day	Body weight change, grams (days after implantation) 6	12	Average survival time, days
Controls		+7.6	+13.2	17.8
Adriamycin	1.25	−0.6	+4.6	31.8
	2.50	−0.9	−4.3	51.2

A comparison of the results obtained, under the same experimental conditions, on mice bearing Ehrlich ascitic carcinoma, with the antibiotics daunomycin and adriamycin in respect to control mice shows that the latter is a more active product. From Table 6, it is seen that the values of the ratio indicating the increase of the survival time in the treated mice as compared to the control mice for the same doses are higher with adriamycin.

TABLE 6

Ratio of the average survival time of mice bearing Ehrlich ascitic carcinoma (each value shows the average of the obtained results in groups of 10 animals per group)

Dose, mg./kg./day	2.50
Daunomycin	1.8
Adriamycin	2.8

The antimitotic effect of adriamycin has been shown in tests carried out on mice bearing ascitic tumors in logarithmic growth stage (5th day). These animals have been treated intraperitoneously with only one administration of adriamycin of 2 mg./kg. The examination of the smears of the neoplastic exudate drawn before and at different intervals of time after the treatment (2, 4, 8, 24, 32 and 48 hours) shows that the antibiotic causes a very quick and complete stopping the multiplicative activity of the tumor which lasts until the 32nd hour. 48 hours after the treatment, numerous cells are noticed in mitosis, but their morphology is constantly altered.

(2) Solid tumors.—The test of activity on solid tumors have been carried out with sarcoma 180 in the mouse and with Oberling-Guérin-Guérin myeloma in the rat.

(a) Sarcoma 180: mice grafted with a fragment of neoplastic tissue have been treated by subcutaneous route, for 8 days, starting from the day following the tumor implantation. The antibiotic has been administered in solution at different concentrations, corresponding to following doses in mg./kg./day: 7, 5, 3.5, 2.5 and 1.75. At the 11th day, all the animals have been slaughtered and their tumors removed and weighed. The results are reported in Tables 7 and 8.

TABLE 7

Lot	Dose, mg./kg./day	Body weight change, g. Gross	Net	Tumor weight, g.	Percent inhibition	Mortality
Controls		+4.73	+0.86	3.922		0/10
Adriamycin	7	−5.98	−6.22	0.239	93.9	6/10
	3.50	−2.31	−3.01	0.696	82.3	0/10
	1.75	+3.09	+1.10	1.988	49.3	0/10

TABLE 8

Sarcoma 180

Lot	Dose, mg./kg./day	Body weight change, g. Gross	Net	Tumor weight, g.	Percent inhibition	Mortality
Controls		+5.65	+3.19	2.461		0/10
Adriamycin	5	−4.27	+4.61	0.239	90.2	0/10
	2.50	−1.60	−2.26	0.656	73.4	0/10
Daunomycin	5	+0.85	−0.18	1.029	58.2	0/10
	2.50	+1.52	−0.23	1.745	29.1	0/10

From the 2 tables, it is seen that the antibiotic has caused a marked inhibition of the tumor growth at all doses used. A notable mortality of the treated animals has been verified only with a higher dosage (7 mg./kg./day). Tests carried out in parallel, under the same experimental conditions, with the antibiotic daunomycin (see Table 8) have made it possible to draw dose-effect graphs of the two products and to carry out a comparison of the activity. It is clearly seen that under the same experimental conditions, adriamycin has a higher activity than daunomycin on this kind of tumor. The result is even more evident, if the inhibiting doses 50 (ID50) are considered:

	Mg./kg.
Daunomycin	About 3.3
Adriamycin	About 1.5

Tests of subacuate toxicity carried out on healthy mice with adriamycin administered by subcutaneous route, for 8 days, at does variable from 10 to 1.25 mg./kg. gave the following results .

TABLE 9

Subacute toxicity of the adriamycin on mouse

Dose, mg./kg./day	Percent mortality in days 10th	15th
10	100	100
8.33	70	100
6.67	40	80
5	0	0
2.50	0	0
1.25	0	0

From the above data, it is calculated graphically that the lethal dose 10 (LD_{10}) is equal to 6.4 mg./kg. From the diagram, one can also deduce that the inhibition dose 90 (ID_{90}) of adriamycin is 5 mg./kg. With these data it is possible to calculate, according to Skipper (Cancer

Chemotherapy Report, 17, 1, 1962), the therapeutical index of adriamycin, which is

$$T.I.=\frac{LD_{10}}{ID_{90}}=\frac{6.4}{5}=1.28$$

Under the same experimental conditions, the therapeutical index of daunomycin is 0.67. It is useful to note that from the above-mentioned work of Skipper, under the same experimental conditions, the therapeutical index of other antitumoral antibiotics already in use (actinomycin, mitomycin, actinobolin, actidione) is lower than 1.

(b) Oberling-Guérin-Guérin myeloma: Wistar rats grafted with a fragment of tumor tissue have been treated by intravenous route for 8 days, starting from the day following the tumor implantation. At the 12th day of the experiment the surviving animals had been destroyed and the tumors were removed and weighed. Table 10 shows the antibiotic to be effective also against this type of tumor. Under these experimental conditions the ID_{50} of the adriamycin is about 2 mg./kg.

TABLE 10

Oberling-Guérin-Guérin myeloma

Lot	Dose, mg./kg./day	Body weight change, grams: Gross	Body weight change, grams: Net	Mortality	Tumor weight, g.	Percent inhibition
Controls		+15.7	+3.2	0/10	12.447	
Adriamycin	0.625	+9.2	−1.0	3/10	10.253	17.7
	1.25	+25.2	+14.6	0/10	10.649	14.5
	2.50	−1.3	−5.6	1/10	4.295	65.5

The following examples serve to illustrate the invention without limiting it.

EXAMPLE 1

Two 300 ml. Erlenmeyer flasks, each containing 60 ml. of the following culture medium for the vegetative phase, were prepared: peptone 0.6%; dry yeast 0.3%; hydrated calcium carbonate 0.2%; magnesium sulphate 0.01%; after sterilization was 7.2. Sterilization has been effected by heating in autoclave to 120° C. for 20 minutes. Each flask was inoculated with a quantity of mycelium of the mutant F.I. 106 corresponding to ⅛ of a suspension in sterile water of the mycelium of a 10-days old culture grown in a big test tube on the following medium: saccharose 2%; dry yeast 0.1%; bipotassium phosphate 0.2%; sodium nitrate 0.2%; magnesium sulphate 0.2%; agar 2%; tap water up to 100%. The flasks were then incubated at 28° C. for 48 hours on a rotary shaker with a stroke of 30 mm. at 220 r.p.m. 2 ml. of a vegatative medium thus grown were used to inoculate 300-ml. Erlenmeyer flasks with 60 ml. of the following medium for the productive phase: glucose 6%; dry yeast 2.5%; sodium chloride 0.2%; bipotassium phosphate 0.1%; calcium carbonate 0.2%; magnesium sulphate 0.01, ferrous sulphate 0.001%; zinc sulphate 0.001%; copper sulphate 0.001%; tap water to 100%. The glucose was previously sterilized separately at 110° C. for 20 minutes. The resulting pH was 7. This was sterilized at 120° C. for 20 minutes and incubated at 28° C. under the same conditions of stirring, as for the vegetative media. The maximum concentration of the antibiotic was reached on the 6th day of fermentation. The quantity of adriamycin produced at this time corresponds to a concentration of 15 μg./ml.

EXAMPLE 2

The operation was as in Example 1 with the difference that the inoculation culture was grown on the following solid medium: 200 g. of peeled potatoes were boiled for 20 minutes in 500 ml. of water. The volume was brought up to its original value and filtered through gauze. 2% of glucose, 0.1% of Difco yeast extract and 2% of agar were added. The volume was brought to 1000 ml. The resulting mixture was sterilized at 120° C. for 20 minutes and pH 6.8–7.0. The maximum concentration of adriamycin 12 μg./ml. was reached at the 140th hour.

EXAMPLE 3

The operation was as in Example 2 with the difference that the vegetative and productive media had the following compositions:

Vegetative medium.—Starch 3%; calcium carbonate 0.4%; distillers solubles 0.3%; ammonium sulphate 0.1%; casein 0.5%; bipotassium phosphate 0.01; in tap water up to 100%. The pH, after sterilization in an autoclave at 120° C. for 20 minutes, was 7.

Productive medium.—Starch 5%; calcium carbonate 0.8%; corn steep liquor 0.6%; casein 0.5%; ammonium sulphate 0.1%; bipotassium phosphate 0.01%. The pH after sterilization, carried out as for the vegetative phase, was 7. The maximum production was achieved at the 7th day with 6.5 μg./ml.

EXAMPLE 4

A culture of the mutant F.I. 106 on a solid medium as in Example 2 was inoculated into 500 ml. of the liquid medium of the vegetative phase in Example 1, contained in a 2000 ml. Pyrex glass flask. The resulting mixture was incubated at 28° C. for 48 hours on a rotary shaker with a stroke of 3.5 mm. at 120 r.p.m. 100 ml. of the culture broth so obtained was then inoculated in 3000 ml. of the same liquid medium contained in a 5-liter neutral glass fermenter, provided with a screw-stirrer, an inlet tube for bubbling in air ending under the screw-stirrer, a breakwater device, a tube for inoculation, an air outlet tube, temperature checking equipment and a device for intermittent or continuous additions under sterile conditions. Growth was carried out at 28° C. with an aeration rate of 3 liters per minute and under stirring at a rate of 400 r.p.m. After 24 hours, 300 ml. of the broth culture thus grown were inoculated into 6 liters of the productive medium in Example 1 contained in a 10-liter neutral glass fermenter as described above. Fermentation was carried out at a stirring rate of 350 r.p.m. and with an aeration rate of 5 liters per minute, foaming being checked by adding small quantities of silicone antifoaming agent. The highest production obtained in 150 hours of fermentation corresponded to a 6 μg./ml. concentration of adriamycin.

EXAMPLE 5

With a culture obtained as in Example 1, a 2000-ml. flask was inoculated with 500 ml. of medium of the following composition: peptone 0.6%; granulated dry yeast 0.5%; calcium nitrate 0.05%, in tap water to 100%. The medium was stirred on a rotary shaker for 48 hours at 28° C. By means of the culture thus obtained, an 80-liter fermenter was inoculated with 50 liters of the medium. This medium was stirred at 230 r.p.m. and aerated with an airflow of 0.7 liter/liter of the medium/minute at 27° C. After 4–5 hours, the culture broth was used to sow 500 liters of culture medium in an about 800-liter fermenter. The fermentation medium has the following composition: glucose 7%; chick-pea meal 6.65%; calcium carbonate 0.2%; sodium chloride 0.2%; bipotassium phosphate 0.1%; magnesium sulphate heptahydrate 0.02%; ferrous sulphate heptahydrate 0.00068%; manganese sulphate heptahydrate 0.001%; copper sulphate 0.002%; in tap water to 100%. The medium was sterilized at 120° C. for 30 minutes, cooled to 27° C. and after inoculation, stirred at 250 r.p.m. and aerated with an air flow of 0.4 liter/liter of medium/minute. After 145 hours, the culture broth contained 6.5 μg./ml. of adriamycin.

EXAMPLE 6

60 liters of culture liquid, resulting from the fermentation obtained according to Example 4, were filtered from the mycelium through Supercel to yield a cake and

a filtrate which were extracted separately. The cake was suspended in acetone diluted with 0.1 N aqueous sulphuric acid (4:1) and stirred for 2 hours. The liquid was filtered off and the cake was further stirred twice. The extracts obtained were combined, neutralized and the acetone was evaporated off in vacuo. The concentrate, which contains about 0.25 g. of adriamycin, was acidified to pH 3 with 1 N hydrochloric acid, and then extracted with chloroform which removed part of the impurities. The aqueous phase was adjusted to pH 8.6 with 1 N sodium hydroxide and then extracted with a chloroform-methanol (9:1) mixture. The operation was repeated until the aqueous phase became colorless. The methanol-chloroform extracts were washed with water at pH 8.6, then dried over anhydrous sodium sulphate, filtered and concentrated to a small volume under reduced pressure. Adriamycin in the form of free base precipitated upon addition of ethyl ether. 1.50 g. of crude product was obtained which contained about 0.2 g. of adriamycin. The filtered broth was adjusted to pH 8.6 with 1 N sodium hydroxide and extracted with a chloroform-methanol (9:1) mixture. The operation was repeated twice. The methanol-chloroform extracts were washed with water at pH 8.6 and re-extracted with 0.01 N hydrochloric acid until the aqueous phase assumed a red color. The chloroform phase was removed. The aqueous phase was filtered, adjusted to pH 8.6 with 1 N sodium hydroxide, and extracted with a chloroform-methanol (9:1) mixture. The extract, which at this point contained besides various impurities, 0.15 g. of adriamycin, was washed with water at pH 8.6, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to a small volume. By adding 10 volumes of ethyl ether, precipitation of 1.00 g. of a crude product containing 0.12 g. of adriamycin was obtained. In total 0.320 g. of adriamycin in the form of crude base were obtained.

EXAMPLE 7

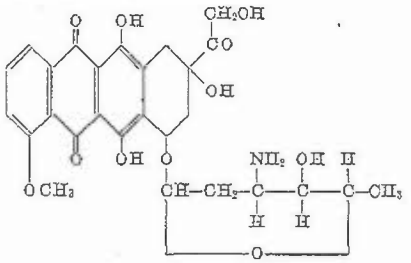
0.500 g. of crude product containing about 15% of adriamycin were dissolved in 10 cc. of M/15 buffer phosphate at pH 5.4. The solution was adsorbed on 10 g. of cellulose powder (whatman CF 11). The mixture was dried overnight in vacuo over anhydrous calcium chloride, put in a glass chromatographic column (100 cm. high and 4 cm. in diameter) containing 225 g. of cellulose powder (whatman CF 11) previously buffered with M/15 buffer phosphate at pH 5.4, and dried in vacuo over anhydrous calcium chloride. Elution was effected with a propanol-ethyl acetate-water (7:1:2) mixture and 25 ml. fractions were collected with an automatic collector. The various fractions were examined by chromatography over whatman paper No. 1, buffered at pH 5.4, using as eluting agent the same mixture as was employed to elute the column. Fractions 40–60 contain adriamycin and were combined and concentrated to 50 ml. Salts were precipitated and filtered off. 200 ml. of water were added to the filtrate and the pH of the solution was adjusted to 7 with 1 N sodium hydroxide. The resulting solution was concentrated under reduced pressure to 50 ml. The concentrate was adjusted to pH 8.6 and extracted with chloroform. The extraction was repeated three times. The chloroform extracts were then combined and washed with water adjusted to pH 8.6, and then with water. They were dehydrated over anhydrous sodium sulphate, filtered and the filtrate was concentrated under reduced pressure to 5 ml. 0.15 ml. of a 1 N solution of anhydrous hydrochloric acid in methanol were added and cooled. After a few minutes, a crystalline precipitate of adriamycin hydrochloride was formed. This was filtered off and washed with cold chloroform and anhydrous ethyl ether. 50 mg. of the product were obtained which was recrystallized from ethanol. In this manner 35 mg. of a pure product melting at 204–205° C. are obtained. From the mother liquor, a further 15 mg. of an amorphous product of 90% purity were recovered.

EXAMPLE 8

0.077 g. adriamycin hydrochloride were dissolved in 4 ml. of 0.5 N hydrochloric acid and heated for 1 hour at 100° C. A dark red amorphous precipitate was obtained which was collected by filtration after cooling. The product, washed with water to neutrality of the washings, was dried overnight in vacuo over potassium hydroxide and for 6 hours over phosphoric anhydride at 56° C. Thus 47 mg. of aglycone of adriamycin are obtained melting at 223°–224° C., $[\alpha]_D = +156°$ (dioxane) having the formula $C_{21}H_{18}O_9$. After precipitation of the aglycone, the almost colorless aqueous acid solution contains a compound which reduces Fehling's solution and gives a positive reaction with ninhydrin. The solution was neutralized (pH 6), passing through a Dowex exchange resin 1x8 (in bicarbonate form). The resin was filtered and the filtrate lyophylized. The white residue consists of an aminosugar which has the same properties as daunosamine hydrochloride. By paper chromatography with the mixed solvents: butanol-acetic acid-water (4:1:1) and (4:1:5); butanol-pyridine-water (6:4:3), and by thin layer Alusil chromatography using as solvent a propanol-ethyl acetate-water-25% aqueous ammonia (6:1:3:1) mixture, the amino-sugar did not separate from daunosamine. The product may be revealed with the ninhydrin reagent and with aniline phthalates over paper and with anisaldehyde and sulphuric acid on thin layers.

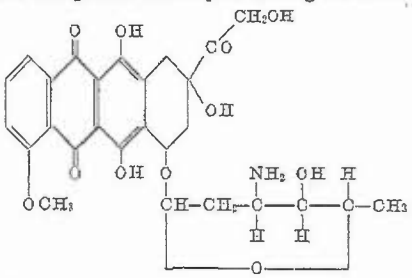
We claim:

1. A new antibiotic selected from the group consisting of adriamycin, having the formula:



its aglycone and its non-toxic pharmaceutically acceptable organic and inorganic acid salts.

2. The compound adriamycin having the formula



3. The pharmaceutically acceptable acid addition salts of the compound of claim 2.

4. The hydrochloride of the compound of claim 2.

5. The sulphate of the compound of claim 2.

6. The pantothenate of the compound of claim 2.

7. The aglycone of the compound of claim 2.

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LEWIS GOTTS, Primary Examiner

J. R. BROWN, Assistant Examiner

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