Natural Products and their Analogues as Efficient Anticancer Drugs

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Abstract: Every year about 7 million people die from of various types of cancer, making this disease responsible for 12.5% of deaths worldwide. Consequently, there is an overwhelming demand to develop new, more potent and effective, anticancer drugs. Natural products represent the most valuable source with inexhaustible reserves, in which the researchers work could identify novel active agents that may serve as the leads and scaffolds for transformation into desperately needed efficacious drugs. This paper is devoted to reviewing some of the most outstanding achievements in the application of natural products as models and precursors for anticancer agents published in the post 2000 literature. Particular emphasis is placed on the chemical modification of active principles isolated from natural products, in hope of obtaining the desirable derivatives for the treatment of different types of cancer, including pancreatic, gastric, breast, colon cancers and leukemia.

Key Words: Anticancer, natural products, pancreatic cancer, gastric cancer, breast cancer, colon cancer, leukemia.

1. INTRODUCTION

There is a great upsurge directed towards using natural products in many spheres of human life, such as: mining and engineering natural product biosynthetic pathways [1-8], metabolic engineering for drug discovery and development [9-17], natural products biosynthesis in microbial cells [18-23], antibiotic production [24], and use as redoubtable weapons in the treatment and/or prevention of various types of cancer [25-30]. Between 5 and 10 % of all cancers are caused by a defect in a single gene - these cancers are inherited and run within families. But in the vast majority of cases, tumors have much more complicated causes. Continuous series of accumulating genetic changes drive tumor progression. Over our lifetime, we gather damage to genes (mutations), and eventually these mutations interfere with the normal function of the cell and can turn a healthy cell into a cancerous one. Due to this continuous change and damage which occur in the human body over generations, there is a crucial need to develop new effective and highly specific "arms" able to successfully combat with the cancer cells. Natural products are a promising and inexhaustible source in which to identify the best candidates for anticancer purposes [25-30]. To better understand the huge impact of natural products on pharmaceuticals, it is worth mentioning that among 155 small molecules used as chemotherapeutics, 73 are directly extracted from natural products and an additional 40 are derivatives or synthetic natural product mimics [31]. Surprisingly, combinatorial chemistry is responsible for only one *de novo* new chemical entity reported in the public domain and approved for drug use in the last 20 years. Table **1** shows the anticancer drugs introduced since 2000, with their generic and the trade name.

Despite their potential to be effective cancer chemotherapeutic agents, natural products often face some major limitations, because of their poor solubility in biological fluids, undesirable pharmacokinetic properties, neurotoxicity in animal studies. Therefore, there is a major need to synthesize various analogues, to bypass these limitations, that is, to enhance pharmacological activity, improve pharmacokinetic properties, or reduce unwanted side effects.

In this review, surveying the literature post 2000, we emphasize successful studies on the chemical modification of the isolated active principle of some natural products with a view to their use as anticancer agents.

2. NATURAL PRODUCTS AND THEIR ANALOGUES AGAINST PANCREATIC CANCER

Pancreatic cancer is the fifth most deadly cancer in the U.S. Only 10% of patients are eligible for surgery [32], whereas less than 20% respond to "gemcitabine", the most common drug used in this kind of cancer [33-35].

Terpenes are among the largest classes of secondary metabolites in the plat world, with more than 55,000 substances being isolated [36, 37]. This number is expected to double over the next ten years. Both acyclic and monocyclic terpenoids are known to inhibit not only the development of pancreatic cancer, but also mammary, liver, skin, lung, colon prostate carcinomas [38-40]. Fig. (**1**) shows those terpenoids which are effective in pancreatic cancer.

The mechanism of the terpene antitumor effects is the inhibition of posttranslational isoprenylation of proteins regulating the growth of cells [41].

Recent work describing the synthesis and biological activity of pentapeptide derivatives based on the natural product sansalvamide A (San A) as potential therapies for pancreatic cancers has drawn attention to this new compound class [42-48]. San A, compound **6**, Fig. (**2**) is a pentadepsipeptide, composed of four L-amino acids and one hydroxyl acid, that exhibits antitumor activity. It was discovered by Fenical and *co-workers* in a marine fungus of the genus *Fusarium* [49].

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Table 1. Anticancer Drugs Introduced Since 2000

Generic Name	Trade Name	Year Introduced
Derived from peptide or protein isolated from an organism/cell line or produced by biotechnological means		
H-101	No trade name given	2005
Alemtuzumab	Campath	2001
Bevacizumab	Avastin	2004
Cetuximab	Erbitux	2003
Ibritumomab	Zevalin	2002
Tositumomab	Bexxar	2003
Natural products or derived from natural products		
Paclitaxel nanoparticles	Abraxane	2005
Amrubicin HCl	Calsed	2002
Belotecan hydrochloride	Camtobell	2004
Fulvestrant	Faslodex	2002
Gemtuzumab ozogamicin	Mylotarg	2000
Hexyl aminolevulinate	Hexvix	2004
Talaporfin sodium	Laserphyrin	2004
Vapreotide acetate	Docrised	2004
Bortezomib	Velcade	2003
Erlotinib hydrochloride	Tarceva	2004
Gefitinib	Iressa	2002
Imatinib mesilate	Gleevec	2001
Sunitinib malate	Sutent	2006
Temoporfin	Foscan	2002
Zoledronic acid	Zometa	2000
Azacytidine	Vidaza	2004
Clofarabine	Clolar	2005
Decitabine	Dacogen	2006
Nelarabine	Arranon	2005
Abarelix	Plenaxis	2004
Bexarotene	Targretine	2000
Pemetrexed disodium	Alimta	2004
Tamibarotene	Amnoid	2005
Vaccine		
Melanoma theraccine	Melacine	2001
Totally synthetic drug		
Arsenic trioxide	Trisenox	2000
Sorafenib	Nexavar	2005

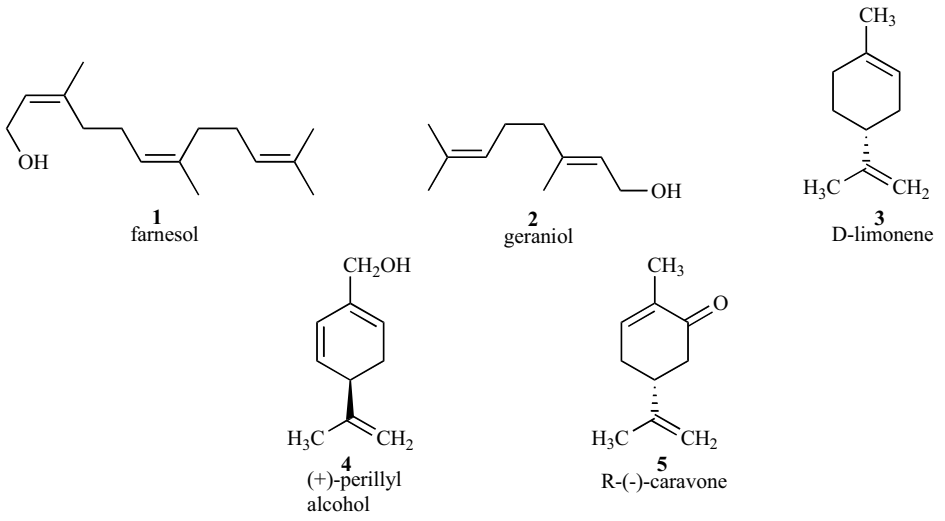


Fig. (1). Examples of terpenoids with effective action in pancreatic cancer.

McAlpine and co-workers [50] have recently reported the synthesis of five San A-based decapeptides, Fig. (**3**).

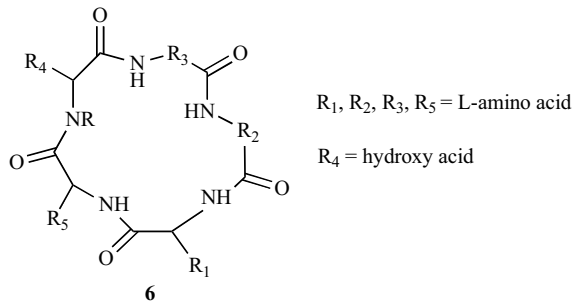


Fig. (2). Chemical structures of Sansalvamide A (San A) derivatives.

These derivatives were tested against two pancreatic cancer cell lines, PL-45 and BxPC3, showing that **11** (Fig. **3**) is extremely cytotoxic against both pancreatic cancer cell lines. The IC50 (compound concentration required to reduce cell proliferation by 50%) values for **11** (Fig. **3**) are sub-nanomolar for both cancer cell lines. Further, **11** (Fig. **3**) is 33-fold less potent against normal skin fibroblasts than against cancer cell lines, thus demonstrating differential selectivity. In addition, **11** (Fig. **3**) displayed up to 43- fold improved cytotoxicity against PL-45 than gemcitabine. The structural differences between **7** – **10** and compound **11** are hardly noticeable. Compound **11** has four D-amino acids in position 2 and 3, respectively. Both **7** and **8** have two D-amino acids in position 3 and 5 respectively, while **9** and **10** have also four D-amino acids like **11**, but linked in different positions: 1 and 5 (in **9**) and 4 and 5 (in **10**) respectively. Compound **11**, due to its specific conformation is able to reach a key biological target inside the cell or on the cell surface, unlike the other four decapeptides derivatives.

3. NATURAL PRODUCTS AND THEIR ANALOGUES AGAINST GASTRIC CANCER

Most (85%) cases of gastric cancer are adenocarcinomas that occur in the lining of the stomach (mucosa). Approximately 40% of cases develop in the lower part of the stomach (pylorus); 40% develop in the middle part (body); and 15% develop in the upper part (cardia). In about 10% of cases, the cancer develops in more than one part of the organ. Gastric cancer can spread (metastasize) to the esophagus or the small intestine, and can extend through the stomach wall to nearby lymph nodes and organs (e.g., liver, pancreas, colon). It also can metastasize to other parts of the body (e.g., lungs, ovaries, bones). Gastric cancer occurs twice as often in men and it is more common in people over the age of 55. Fig. (**4**) shows some of the new products used as antigastric cancer agents.

Yashiro *et al.* have described the use of Tranilast (compound **13**, Fig. **4**) in decreasing the production of matrix metallo-proteinase-2 (MMP-2) and in transforming the growth factor-β1 (TGF-β1) from fibroblasts, with the effect of suppressing the invasional ability of gastric cancer cells [51]. Tranilast is a derivative of anthranilic acid and shows good results as well as antiproliferative activity against cultured leiomyoma cells. Other anthranilic acid derivatives were synthesized recently by Congiu *et al.* [52], starting from anthranilic acid in reaction with trifluoroacetylvinnyl ether in refluxing acetonitrile solution to yield the corresponding acid, which was further converted into 1,1,1-trifluorohexadienone derivate by reaction with an excess of N,N-dimethylformamide dimethylacetal (DMF-DMA) in refluxing toluene, then treated with ammonium acetate in hot DMF, hydrolysis of the obtained product in 10% aqueous sodium hydroxide and refluxing in ethanol in the presence of thionyl chloride. The obtained ethyl esters were converted to the ester derivatives by reaction with flufenamic acid. The most effective compounds were found to be the 2-methoxyphenyl, 2-chlorophenyl, and pyridine-3-yl esters.

Duryne (compound **14**, Fig. **4**) was first isolated in 1987 from the marine sponge *Cribrochalina dura* by Wright *et al.* [53]. The geometry of the central C^{15}=$C^{15'}$ olefin and the absolute stereochemistry of the chiral centers were very recently a subject of intense scientific debate [54, 55], until Gung and Omollo [56] through the total syntheses of both (*Z*) and (*E*) isomers and the subsequent enzymatic resolution of each racemic mixture, were able to identify natural duryne as (15*Z*) and (3*S*, 28*S*), as shown in Fig. (**4**).

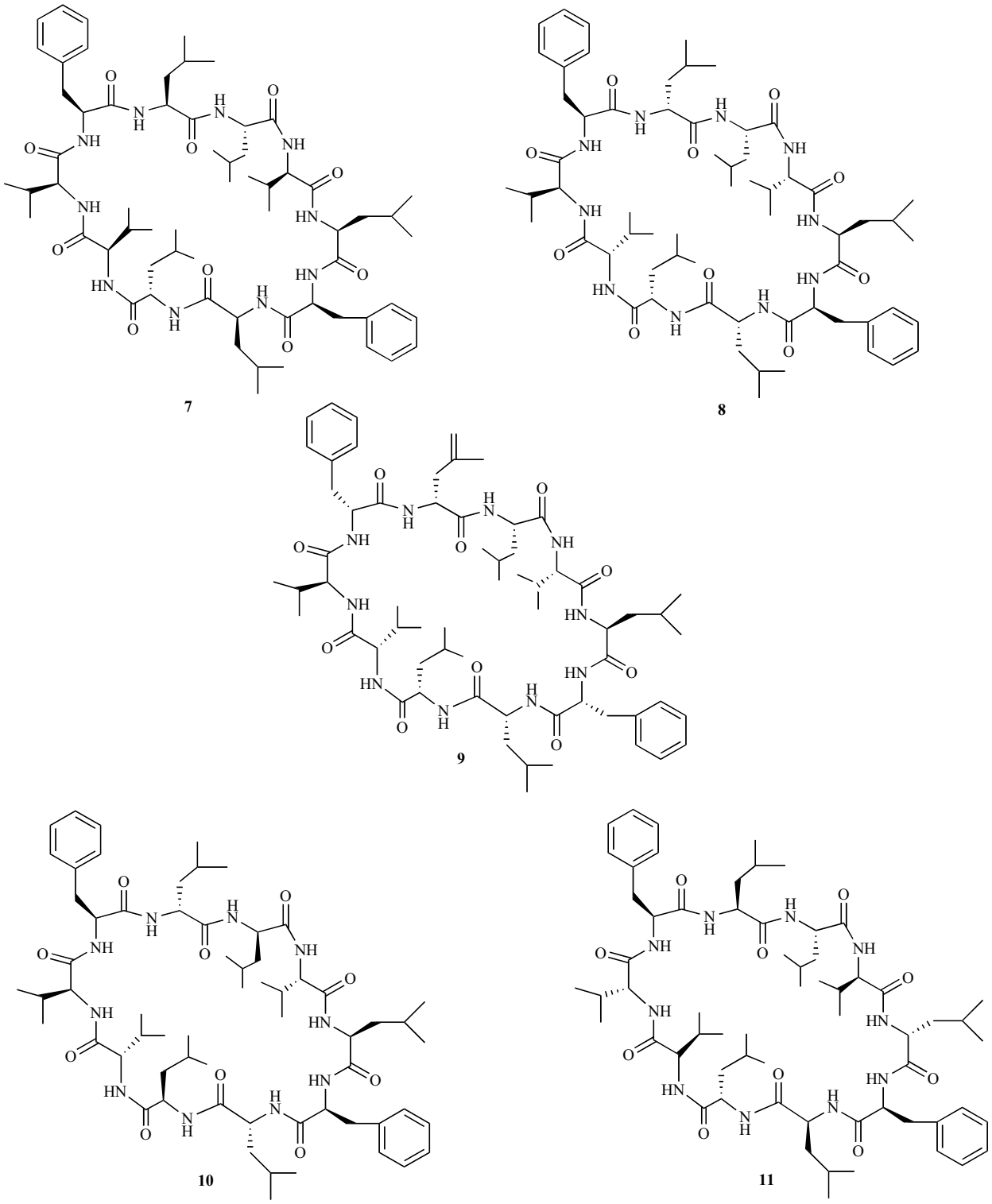
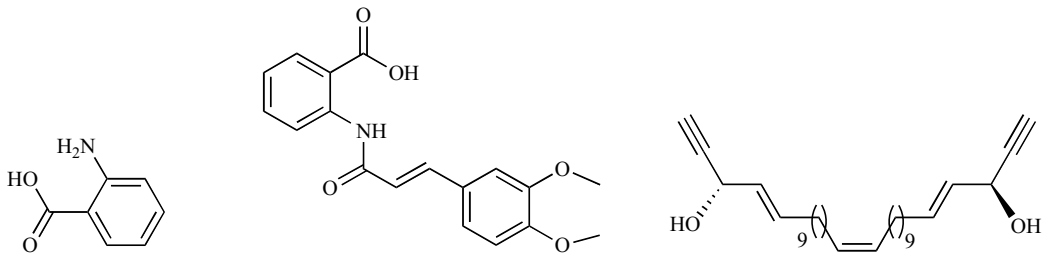


Fig. (3). Structures of five decapeptide derivatives.

4. NATURAL PRODUCTS AND THEIR ANALOGUES AGAINST BREAST CANCER

Adenocarcinoma of the breast cancer is the most common cancer in women. In the USA alone, about 180,000 women will be found to have invasive breast cancer in 2007. More than 40,000 are expected to die from it every year. Tamoxifen is the most widely used drug for the treatment of breast cancer [57]. It competitively binds to estrogen receptors on tumors, producing a nuclear complex that decreases DNA synthesis and inhibits estrogen effects. The drug's pharmacologic properties are related to its ability to compete with estrogen for estrogen receptors in breast tissue and to inhibit the stimulatory effect of estrogen for tumor growth.



12
anthranilic acid

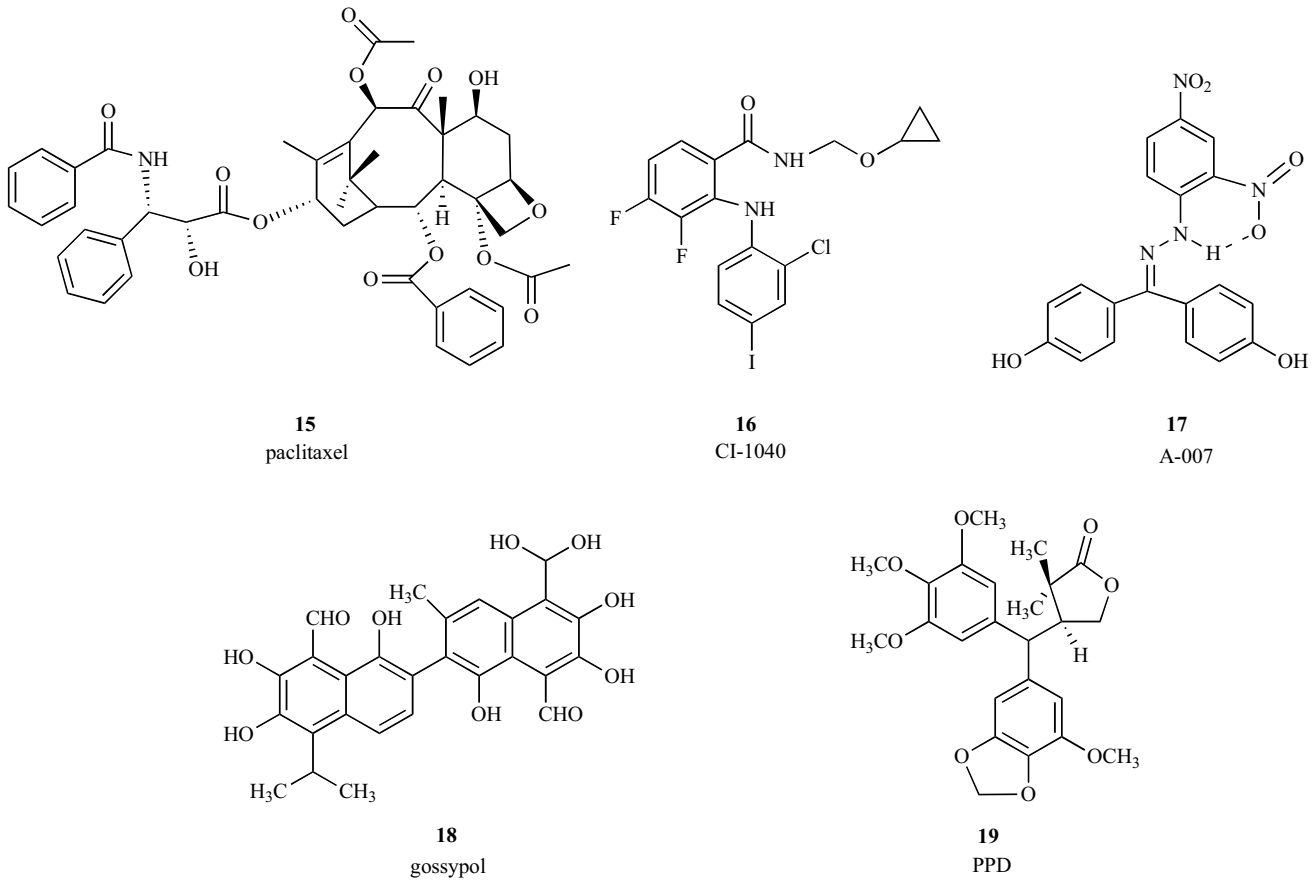
13
Tranilast

14
(S)-(+)-Duryne

Fig. (4). New products used as antigastric cancer agents.

However, tamoxifen faces major drawbacks – it is being responsible for the stimulating uterine growth, which turns into uterine cancer. Furthermore, up to 35% patients are intrinsically resistant to tamoxifen. Consequently, there is an ongoing need for breast cancer drugs with greater efficacy and fewer side effects. Fig. (**5**) shows some compounds originating from natural sources, as valuable precursors for breast cancer drugs, which eventually may fulfill these requirements.

Paclitaxel, (compound **15**, Fig. **5**) a complex diterpenoid originated from bark of Pacific yew trees, is effective against breast and ovarian cancer [58]. It stops the division of cancer cells, binds tubulin heterodimers and stabilizes microtubule assembly [59]. CI-1040, (compound **16**, Fig. **5**) an anthranilamide derivative has been demonstrated in preclinical models to possess antitumor activity [60]. Its activity is correlated with inhibition of mitogen-activated protein kinase cascade pathway. 4,4'-Dihydroxybenzophenone-2,4-dinitrophenylhydrazone (A-007) (compound **17**, Fig. **5**) has been very recently introduced and objective responses were seen in advanced breast cancer, and also in non-Hodgkin's lymphoma (NHL). Morgan *et al.* [61] have synthesized 36 analogues of A-007 and tested them on fresh human cancer tissues. It was reported that when both electron-withdrawing and donating groups (amino *vs* methoxy) were substituted



15
paclitaxel

16
CI-1040

17
A-007

18
gossypol

19
PPD

Fig. (5). Compounds originated from natural sources, as precursors for breast cancer drugs.

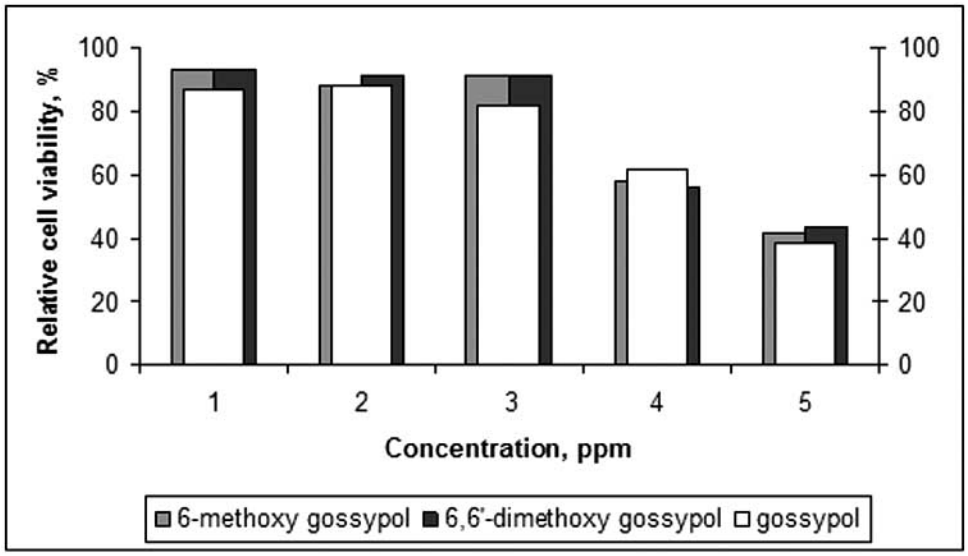


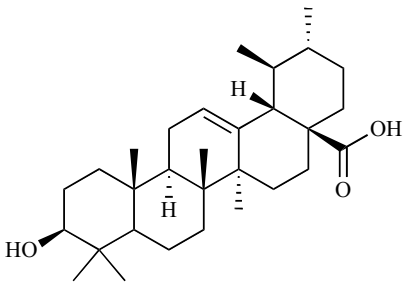
Fig. (6). Growth inhibition of the cancer cell line MCF-7 incubated with 6-methoxygossypol, 6,6'-dimethoxygossypol, and gossypol. (Adapted from ref. [70]).

into the meta-5-position of **17**'s dinitrophenyl ring an impressive activity was observed [61]. For example, a chlorine inserted in the 5-position (ortho and para to the 2- and 4-nitro groups, respectively) of the phenylhydrazone moiety improved activity by almost 800 fold *vs* **17** [61]. Gossypol (compound **18**, Fig. **5**) is a polyphenolic compound of cotton plants. Initially, research was focused on gossypol because of its contraceptive effects [62, 63], though later it was reported to also possess anticancer activity, antiviral [64, 65], antiamoebic [66], and antiprotozoan effects [67-69]. Gossypol and some of its derivatives (6-methoxygossypol, and 6,6'-dimethoxygossypol) isolated from the root bark of *Gossypium barbadense* (See Island cotton) were very recently [70], subjects of several bioassays, including free radical scavenging activity, reducing power, DNA damage prevention capability, and cancer and trypanosomal cell growth inhibition. Gossypol and its derivatives exhibited similar dose-dependent growth inhibition against the MCF-7 cell line, [70] Fig. (**6**). The IC_{50} values are around 10 ppm for 6-methoxygossypol and 6,6'-dimethoxygossypol. A much higher value of IC_{50} for gossypol (> 10 ppm) was observed in the presence of serum proteins [70].

PPD, (compound **19**, Fig. **5**) a secolignan extracted from *Peperomia pellucida* Kunth (Piperaceae), a traditional Chinese herb, was reported to be a potent inhibitor of cancer cell proliferation [71]. The IC_{50} (μM) against the cell line MCF-7 is reported to be 3.8 ± 1.1 and the growth inhibition (%) at a 20 μM concentration reached almost 92 % [71].

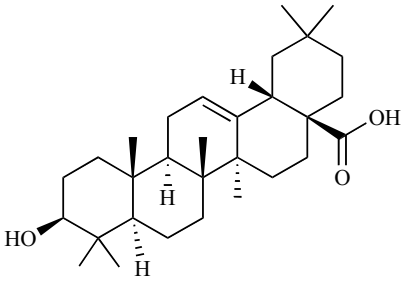
5. NATURAL PRODUCTS AND THEIR ANALOGUES AGAINST THE LEUKEMIA CELL GROWTH

Leukemia is a cancer of the blood or bone marrow and is characterized by an abnormal proliferation (production by multiplication) of blood cells, usually white blood cells (leukocytes). There are four common types of leukemia: chronic lymphocytic leukemia *(chronic lymphoblastic leukemia, CLL)* accounts for about 7,000 new cases of leukemia each year. Most often, people diagnosed with the disease are over age 55. It almost never affects children. Chronic myeloid leukemia *(chronic myelogenous leukemia, CML)* accounts for about 4,400 new cases of leukemia each year. It affects mainly adults. Acute lymphocytic leukemia *(acute lymphoblastic leukemia, ALL)* accounts for about 3,800 new cases of leukemia each year. It is the most common type of



20

ursolic acid



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oleanolic acid

Fig. (7). Plant triterpenes used to reduce leukemia cell growth.

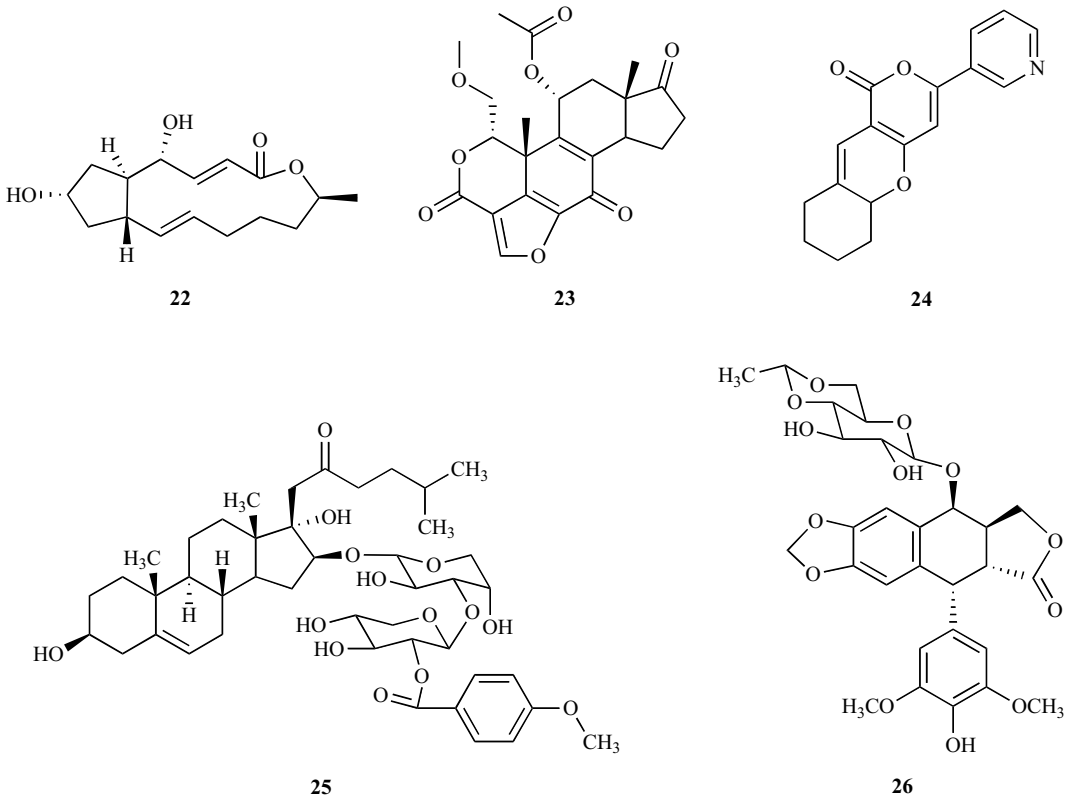

Fig. (8). Compounds effective in reducing leukemia cell growth.

leukemia in young children. It also affects adults. Acute myeloid leukemia *(acute myelogenous leukemia, AML)* accounts for about 10,600 new cases of leukemia each year. It occurs in both adults and children. Plant triterpenes, such as ursolic acid (compound **20**, Fig. **7**) and oleanolic acid, (compound **21**, Fig. **7**) exhibit *in vitro* activity in reducing leukemia cell growth and inhibit the proliferation of several transplantable tumors in animals [72].

Other compounds effective in reducing leukemia cell growth are: brefeldin A (compound **22**, Fig. **8**), wortmannin (compound **23**, Fig. **8**), H10 (compound **24**, Fig. **8**), gossypol, saponin OSW-1 (compound **25**, Fig. **8**), duryne, etoposide (Vepeside) (compound **26**, Fig. **8**).

Brefeldin A is a macrolide antibiotic that was first isolated from *Penicillium decumbens.* It has a wide variety of biological properties, including antiviral, antifungal, antimitotic and anticancer effects [73, 74]. However the use of brefeldin A faces major constraints because of its poor solubility in physiological conditions and undesirable pharmacokinetic properties. To overcome these impediments, Cushman *et al*. [75], synthesized analogues of brefeldin A, by esterification of the two OH groups. The most effective derivatives are the methyl esters, which exhibited higher cytotoxicity in cancer cells.

Wortmannin and its hydroxyl analogue (17-hydroxywortmannin) are natural products which are potent, nonselective inhibitors of phosphoinositidine 3-kinase. However, their toxicity and insolubility, as well as aqueous instability have hampered their development into useful anticancer agents [76]. Conjugates of wortmannin with improved properties were recently reported by the use of pegylation technology [77, 78] and by furan ring opening of wortmannin with diallylamine [79]. Zask and coworkers [80] very recently reported the syntheses of libraries of ring-opened 17-hydroxywortmannin analogues with structurally diverse amines, with superior anticancer properties [80].

Khosla and Ridley reported [81] the preparation of nine novel pyranopyrones through condensation reactions of four engineered polyketides (tetralone mutacin, the hemiacetal SEK4, the diphenyl ketone SEK15 and the quinine SEK15b) with either α,β unsaturated aldehyde citral, 1-cyclohexenecarboxaldehyde, and *S*-perillaldehyde. Aromatic polyketides are natural products that include the clinically used tetracycline and doxorubicin. The obtained pyranopyrones were biologically evaluated toward the three cancer lines: K-562 (human leukemia cell line), L-1210 (murine leukemia cell) and HCT-116 (human colon tumor cell), respectively. The results were not very satisfactory, the products exhibited lower activity than the most investigated member of the tricyclic pyranopyranes H10 (compound **26**, Fig. **8**). OSW-1 (compound **25**, Fig. **8**), and other four natural analogues have been isolated from the bulbs of *Ornithogalum saundersiae*, a perennial grown in southern Africa where it is cultivated as a cut flower and garden plant [82]. The synthesis of OSW-1 has been successfully synthesized in only 10 linear operations, by Jin and Yu [83], from the commercially available starting material 5-androsten-3β-ol-17-one in 28% overall yield. OSW-1 and their five analogues exhibited extremely potent cytostatic activity against human leukemia HL-60

cells, IC_{50} values ranging between 0.1 and 0.3 nM [83]. Etoposide is the most notable analogue of podophyllotoxin, a lignan derivative of the May apple. Etoposide is an important drug for the treatment of leukemia, as well as testicular cancer, and small-cell lung cancer [84, 85].

6. NATURAL PRODUCTS AND THEIR ANALOGUES AGAINST COLON CANCER

Colon cancer is formed in the tissues of the colon (the longest part of the large intestine). Most colon cancers are adenocarcinomas (cancers that begin in cells that make and release mucus and other fluids). According to the National Cancer Institute of USA, more than 100,000 people are diagnosed every year with colon cancer and almost a half die because of colon and rectal cancer. To fight against colon cancer, a new class of anticancer principles, that is, sphingolipids was recently proposed. For instance, D-*erythro*-(2*S*, 3*R*)-sphingosine, (compound **27**, Fig. **9**), has been shown to affect various signaling pathways, including potent inhibition of protein kinase C dependent pathways for cell proliferation [86], and activation of caspase for apoptosis [87]. The anticancer activity of sphingosine and other sphingolipids has been proved to be the most effective against colon cancer cell lines [88]. McDonald *et al.* [89] reported efficient formation of the 1-deoxy-5-hydroxysphingosine analogue (compound **28**, Fig. **10**), with an excellent biological activity against colon cancer.

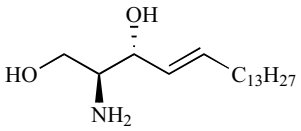

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Fig. (9). Chemical structure of sphingosine.

Gemcitabine is one of the most common drugs used in the treatment of colon cancer [90]. However, gemcitabine has a short plasma half-life (1.5 h) and drug resistance caused by its deamination, which occurs intra and extracellularly by deoxycytidine deaminase, which transform it into the chemotherapeutically inactive uracil derivative [91-93]. To increase the therapeutic index of gemcitabine and other similar molecules, such as cytarabine, Couvreur and coworkers [94] envisaged a new strategy to transform them into more effective drugs by coupling with the acyclic isoprenoid chain of squalene, Fig. (**11**). The resulting squalenoyl nanoassemblies seem to be more effective both *in vitro* and *in vivo* in experimental colon cancer but also in lung, pancreatic, breast, bladder and ovarian cancers [94].

Epothilones A (**32**) and B (**33**), Fig. (**12**) are naturally occurring cytotoxic macrolides. They were first isolated by Hofle and coworkers from the mycrobacterium *Sorangium cellulosum* [95].

Pioneering work carried out by Samuel J. Danishefsky and his coworkers, into the area of total synthesis of both epothilones A and B, suggested that the 12, 13 oxido linkage of the macrolactone is a "*locus of nontumor selective toxicity*" [96, 97]. Another interesting finding was that incorporation of *E*-9, 10 unsaturation in the macrolide framework of epothilone B has the effect of increasing potency and also metabolic stability [98]. The most powerful representative of this class is 26-trifluoro-(*E*)-9,10-dehydroepothilone (Fludelone), the full synthesis and an complex structure-activity relationship was recently reported [99, 100]. The introduction of the three fluorine atoms at C26 attenuated cyctotoxicity of Fludelone as comparative with the $–CH_3$ analogue in leukemic cells. The activity of Fludelone in the treatment of human colon carcinoma (HCT-116) was studied in parallel with paclitaxel, at 20 mg/kg and both resulted in tumor disappearance [99]. It is worth noting that epothilone B (**33**) is in phase III clinical trials, and the lactam analogue of epothilone B (ixabepilone) has just been introduced for clinical use for treatment of certain forms of breast cancer [101].

CONCLUDING REMARKS

Even though synthetic combinatorial chemistry has become a powerful tool for the preparation of a large variety of compounds in many areas, Mother Nature continues to readily offer much more than any "best-equipped" lab. Certainly, there are still many active principles which are neither known nor have been isolated, especially those from exotic plants and marine organisms. Microscopic organisms are

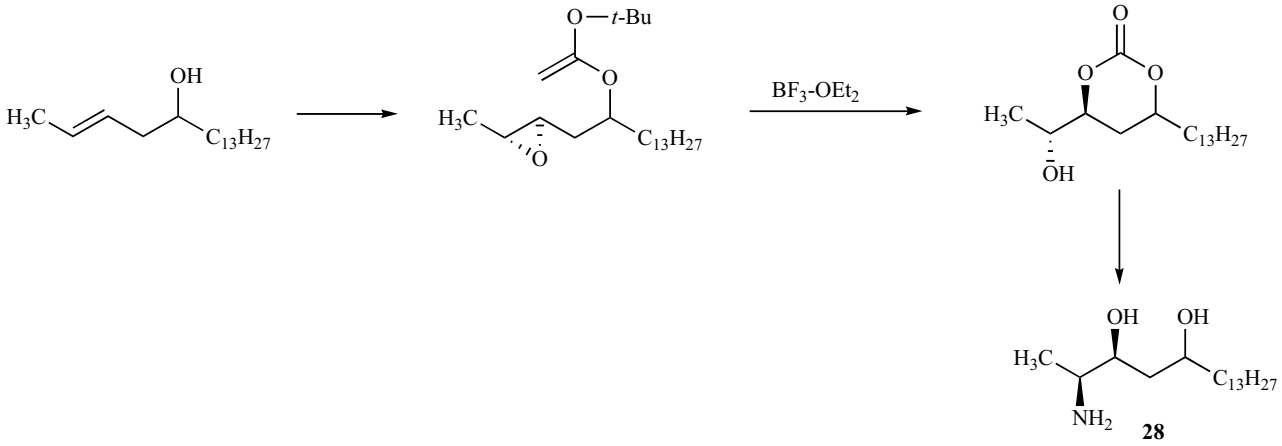

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Fig. (10). Preparation of 1-deoxy-5-hydroxysphingosine analogue.

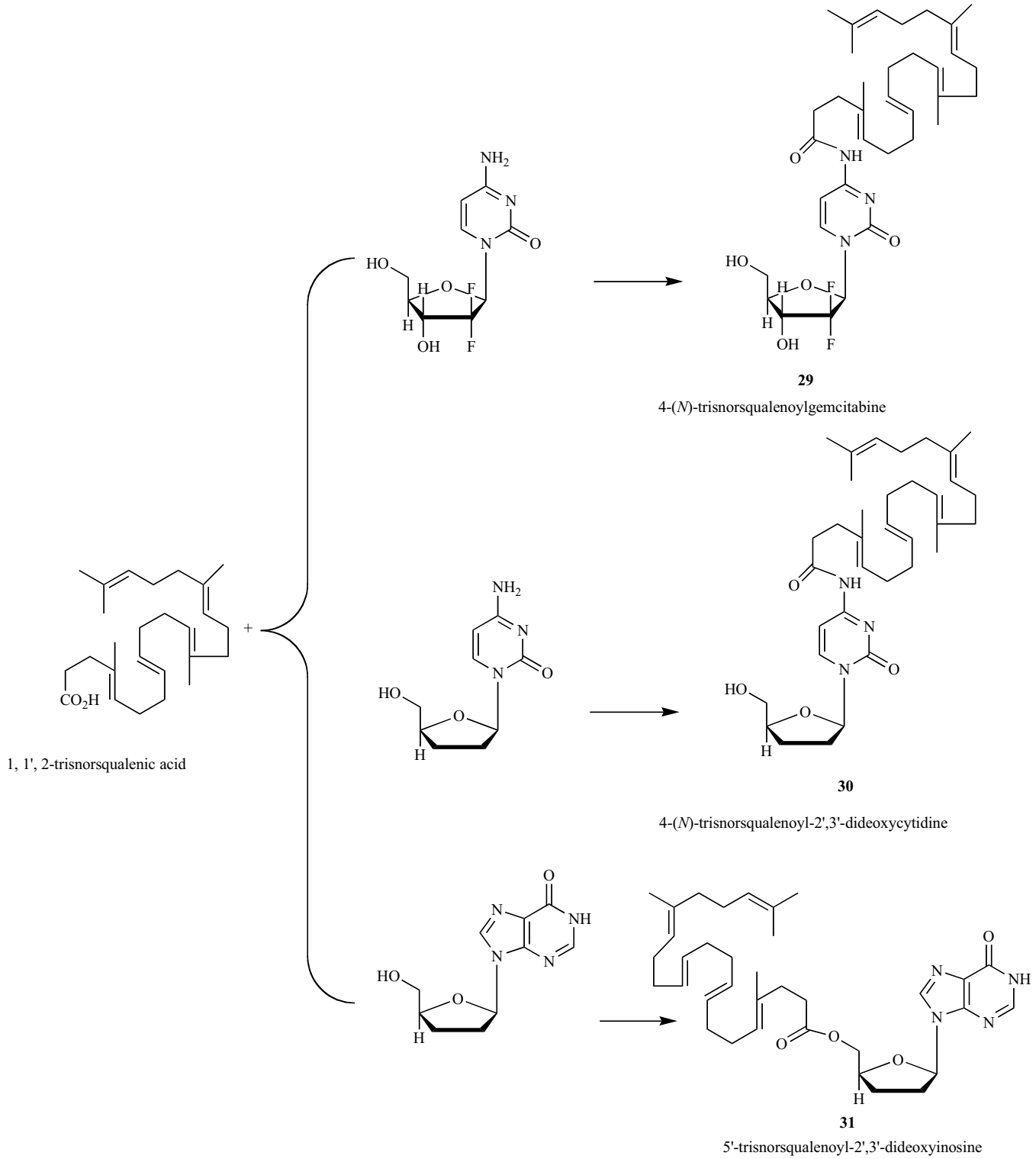
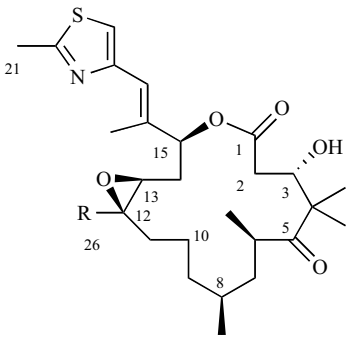


Fig. (11). Synthesis of 4-(*N*)-trisnorsqualenoylgemcitabine, 4-(*N*)-trisnorsqualenoyl-2',3'-dideoxycytidine, and 5'-trisnorsqualenoyl-2',3'-dideoxyinosine.

almost inexhaustible sources in the marine ecosystem, the isolated compounds exerting a high selectivity against certain cancer cell lines. Moreover, unlike the large scale efforts to screen natural products for anticancer purposes that took over the past few decades, modern techniques such as the microbial synthetic route, computational studies, and engineering plant cells, offer the hope of shortening the time until this relentless disease will be totally curable. Without minimizing the work of the individual scientist, we have to admit that the tremendous progress made in cancer treatment were



32: Epothilone A, R = H
33: Epothilone B, R = CH_3

Fig. (12). Structures of epothilones.

largely due to the extensive and concerted multidisciplinary research in areas such as medicine, chemistry, pharmacology, biology, toxicology, and biosynthesis, to name but a few.

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