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New Natural Products of Interest under Development at the National Cancer Institute

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Summary. *Fourteen new agents of natural products origin which are under development as antitumor agents at the National Cancer Institute are discussed with reference to their sources, structures, antitumor activity, current status, and future prospects as clinically effective agents.*

Introduction

Since 1956 the Cancer Chemotherapy National Service Center, now called the Developmental Therapeutics Program (DTP), has had a comprehensive drug development program which includes the screening of compounds obtained from natural products. Approximately 150,000 microbial cultures have been fermented and 200,000 plant extracts have been tested for their cell cytotoxicity and in vivo activity vs. various animal tumors (Hartwell, 1976; Douros, 1976). Approximately 5 years ago a concentrated effort to evaluate animal products (primarily marine) was initiated, and to date approximately 27,000 extracts have been screened, about 2% showing in vivo activity.

The following antibiotics had clinical antineoplastic activity and were evaluated by the National Cancer Institute, (NCI): actinomycin D, isolated at Rutgers (USA), mithramycin (USA), and streptozotocin (USA) (Douros, 1976).

The plant products which are clinically active compounds and were also scrutinized by NCI are: vincristine, vinblastine, and podophyllotoxins. No marine animal product has yet been evaluated by NCI in clinical trials, and although many active extracts have been obtained from marine animals there have been no compounds isolated to date which have been of clinical interest.

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Many compounds have been isolated from the above-mentioned programs. In addition to natural products, NCI has obtained through its worldwide surveillance program many other interesting natural products from industrial concerns, research institutes, universities, and scientists. Some of the more interesting compounds in drug development will be discussed (Table 1). None of the drugs being considered in this paper is yet considered clinically active, although some are now undergoing clinical trials.

Methodology

Natural products when purified (> 90%) are assigned an NSC number, which is an identification developed by NCI for all compounds when purified. Normally we prefer materials to be at least 98% pure, but proteins, peptides, polysaccharides, and some other antibiotics do not lend themselves to easy purification. The various protocols for screening these drugs have been established by the Drug Evaluation Branch, NCI (Geran et al., 1972). Normally P388 leukemia in mice is the first in vivo test in which a natural product compound is evaluated; however, rational selection can result in using another in

Table 1. Natural products undergoing drug development at NCI

Compound	NSC No.
Actinomycin Pip 1β	107,660
Anguidin	141,537
T-2 toxin	138,780
Baccharin	269,757
Aclacinomycin A	208,734
7-0-methyl nogarol	269,148
Nogamycin	265,450
AT-125	163,501
Hydroxy AT-125	176,324
Maytansine	153,858
Tripdiolide	163,063
Homoharringtonine	141,633
Taxol	125,973
Bruceantin	165,563

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Table 2. Division of cancer treatment (DCT) panel of antitumor screens

Tumor	Parameter	Activity criteria	Route of innoculation	Tissue and level of innoculation	
Mouse colon 26 (C6)	Survival (median T/C)	T/C ≥ 140%	i.p.	brei	1 : 100
Mouse breast (CD)	Tumor inhibition (median T/C)	T/C ≤ 42%	s.c.	brei	5×10^6
Human colon xenograft	Survival?[a]	?	s.c.	fragment	14 mg
Mouse breast xenograft	Survival?	?	s.c.	fragment	14 mg
Mouse lung xenograft	Survival?	?	s.c.	fragment	14 mg
Mouse B16 melanosarcoma (B16)	Survival	T/C ≥ 140%	i.p.	brei	1 : 10
Mouse Lewis lung carcinoma (LL)	Survival	T/C ≥ 140%	i.p.	cells	10^5
Mouse L1210 leukemia (LE)	Survival	T/C ≥ 140%	i.p.	cells	10^5

[a] = Limits not firmly established

vivo tumor screen as the primary test. In most cases, a material is tested initially vs. P388 leukemia (PS) to determine toxicity data, even if this is not the primary tumor of interest for a compound to be evaluated against. If an increased life span of 30% or more is obtained vs. P388 in at least two different laboratories, the compound is tested vs. L1210 leukemia, B16 melanoma, and Lewis lung. If activity is confirmed in any of the above three tumors, the compound is then tested in the rest of the panel (Table 2).

All of the in vivo screening data referred to in this paper utilized the routes of tumor administration and levels of tumor indicated in Table 2. All administrations of test compounds were by the intraperitoneal route unless otherwise indicated.

Routinely a compound which is isolated in our natural products program and shows cytotoxicity but no PS activity is additionally tested in at least one solid tumor before being considered of no interest. The reason is that a natural product which has been isolated from the microbial fermentation, plant, or animal programs comes from an extract which has shown reproducible activity vs. PS in vivo or marked cytotoxicity prior to being isolated and thus is not similar to a random compound, which has no history of possibly having any biological activity. If the compound has sufficient activity and is a novel structure or an analog deemed of interest to NCI it will now be scheduled for the tumor panel. If sufficient activity is observed and it is deemed of interest by an NCI decision-making group, it is scheduled for formulation. When a formulation which is suitable for man has been obtained and the natural product is still active in animal tests when used in this formulation, the material is scheduled for toxicology (dog and monkey).

Prior to toxicology, the formulated antineoplastic agent is tested for schedule dependency and oral-route activity. Feasibility of large-scale production is determined and cost/benefit determinations are also made. A decision-making group of scientists determines whether the compound should go to toxicology in dogs and monkeys[1].

When toxicology studies are being done, the pharmacology group determines pharmacokinetics, etc., with the drug. Toxicological results are reviewed and if no irreversible dangerous side effects are observed the drug is deemed ready for Phase I clinical trials. The drug is then evaluated vs. the various signal cancers used by NCI in Phase II trials.

Phase I. After approval of the IND application, the Phase I clinical trials can begin and this research is directed to establishing a maximum tolerated dose in humans at the schedule tested. It is of utmost importance during these trials to establish toxicity parameters and determine whether toxicity is predictable, treatable, and reversible.

Table 3. NCI signal tumors

Acute lymphocytic leukemia	Lung
Acute myelocytic leukemia	Lymphoma
Brain	Melanoma
Breast	Ovary
Colon	Pancreas

Phase II. The drug is screened for clinical activity against a range of 'signal tumors'[2] (Table 3). Normally 15–30 patients are tested in each individual tumor group. If clinical responses are obtained in any of these groups and NCI is interested, then Phase III and Phase IV clinical trials are initiated.

Phase III. The drug is used in comparison with existing drugs against a given cancer type. The selection of patients to be evaluated is based on the results of Phase II studies.

Phase IV. The drug is used in combination studies. Often a drug might be of more value in combination, since two or more drugs may have different modes of action and can act synergistically with each other. Also lower doses of each individual drug in the combination can be used, which can decrease toxicity.

Results

The following drugs derived from natural sources are now in some phase of drug development at NCI:

Actinomycin Pip 1β (NSC-107660) (Fig. 1) is a peptide antibiotic with a phenoxazine chromophore and is pro-

[1] Division of Cancer Treatment, NCI. Treatment Linear Array, December 1976

[2] Division of Cancer Treatment, NCI. Treatment Linear Array, December 1976

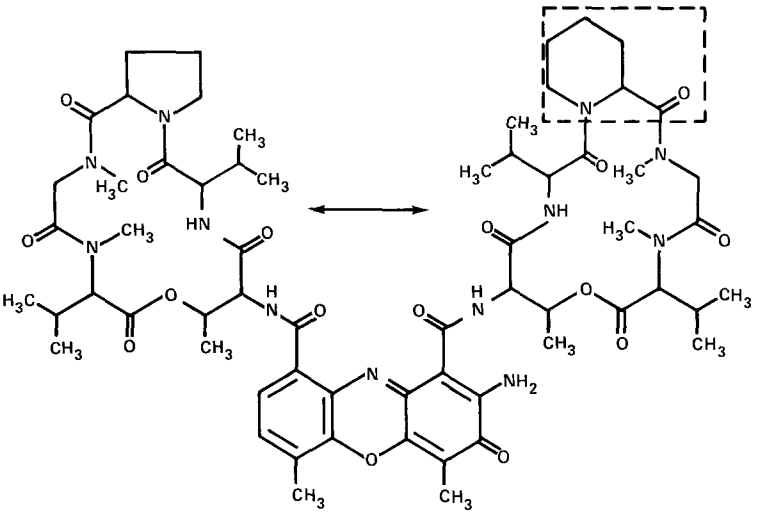

Fig. 1. Structure of actinomycin Pip 1β

duced by *Streptomyces parvulus*. The material was produced under NCI contract by Dr. E. Katz, Georgetown University. This antibiotic differs from actinomycin D in that one of the prolines in the peptide chain is replaced with pipecolic acid. The NCI has shown renewed interest in the actinomycins, as many clinicians have indicated that they would be interested in clinical trials of an actinomycin which had broader activity, less toxicity, or a greater chemotherapeutic index than actinomycin D.

Actinomycin Pip 1β has shown greater activity in B16 melanoma in mice than actinomycin D (Table 4). The superiority is not striking but it is definite. 27 actinomycins (Table 5) have been evaluated at NCI but none has shown a broader spectrum of antitumor activity than actinomycin D, which would be necessary for them to be considered as possible candidates for advanced studies. Limited work continues in fermentation through biotransformation and chemical modifications to obtain novel actinomycins. This work will be phased out if some definite indication of an improvement in activity or toxicity does not occur in the near future.

Anguidin (NSC-141537) (Fig. 2). This trichothene derivative is produced by the fungus *Fusarium* (Brian et al., 1961). We have, or are now evaluating, 36 trichothenes in our program. These agents are produced by fermentation or have been isolated from the Brazilian plant *Baccharis megapotamica*. A comparison of the antitumor activity of anguidin, T-2 toxin (Bamburg et al., 1968) (Fig. 3) and baccharin (Kupchan et al., 1976a) (Fig. 4) appears in Table 6. The various trichothenes under testing at NCI appear in Table 7. The major interest in anguidin has occurred from its activity in the Colon 38 (C8) system, as seen in Table 8.

Table 4. Comparison of actinomycin D and Pip 1β in animal antitumor activity[a]

Tumor	Actinomycin D		Pip 1β	
	Dose range	ILS	Dose range	ILS
P388	125–1000 μg	200	125– 2000 μg	125
L1210	188–1000 μg	53	50– 800 μg	50
B16	25– 100 μg	86	50– 800 μg	119
Lewis lung	65– 500 μg	7	625–10000 μg	11
Colon	108– 500 μg	56	108– 5000 μg	53

[a] Data from Dr. Randall Johnson, NCI

AT-125 (5-isoxazoloacetic acid, α-amino-3-chloro-4,5-dihydro) (NSC-163501). This antineoplastic agent was isolated from a *Streptomyces svicus* fermentation by the Upjohn Co. (Hanka and Dietz, 1973). It was one of the two isoxazoles isolated from the antimetabolite screen used by this pharmaceutical company. Both isoxazoles have P388 and L1210 activity. Due to a greater quantity of NSC-163501 being produced than its analog NSC-176324, 5-isoxazoleacetic acid, amino-3-chloro-4,5-dihydro-4-hydroxy, the NCI has pursued this drug (Fig. 5).

One unusual facet of this fermentation was that crude broths did not show any activity vs. P388 but when the isoxazole was isolated and purified good P388 and L1210 activity was observed.

These drugs are L-glutamine antagonists and inhibit L-asparagine synthetase (Neil et al., 1975; Cooney et al., 1974). Both compounds have been extensively tested in the animal tumor tests (Table 9).

Nogalomycin is an anthracycline antibiotic produced by *Streptomyces nogalates*, but due to toxicity

Table 5. Actinomycins tested at NCI

Actinomycin C	NSC-18,268
Actinomycin C_2	NSC-87,221
Actinomycin C_3	NSC-87,222
Actinomycin D, 3A (or B)-(cis-4-chloro-L-proline)	NSC-244,388
Actinomycin D	NSC-3,053
Actinomycin E	NSC-26,742
Actinomycin F or Z	NSC-58,240
Actinomycin K_1C	NSC-281,262
Actinomycin K_2C	NSC-269,150
Actinomycin K_2t	NSC-271,682
Actinomycin Pip 1α	NSC-244,391
Actinomycin Pip 1β	NSC-107,660–241,535
Actinomycin II (A_2F_8)	NSC-236,660
Actinomycin III (A_3, X_0, F_9)	NSC-236,661
Actinomycin P_2	NSC-48,077–250,428
Actinomycin X-diacetate	NSC-237,669
Actinomycin X-acetate	NSC-237,668
Actinomycin X-didecanoate	NSC-237,670
Actinomycin XO_a	NSC-241,534
Actinomycin XO_b	NSC-241,536
Actinomycin Z_5	NSC-282,745
Actinomycin 3′-azetidine-3′azetidine	NSC-244,393
Actinomycin 3′-azetidine-3′proline	NSC-244,392
Actinomycin C_3-2-chloro-2-desamino	NSC-237,105
Actinomycin 2-hydroxy-2-desaminoactinomycin C_3	NSC-237,106
Actinomycin X-methoxine	NSC-237,670
Actinomycin D, 7-amino-2A-D-alloisoleucine-2B-D-alloisoleucine	NSC-241,490
Actinomycin D, 7-nitro-2A-D-alloisoleucine-2B-D-alloisoleucine	NSC-241,491
Actinomycin D, 3A-(cis-4-chloro-L-proline)-3B-(cis-4-chloro-L-proline)	NSC-246,113

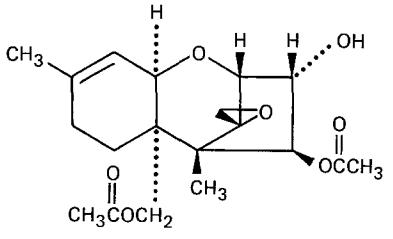

Fig. 2. Structure of anguidine

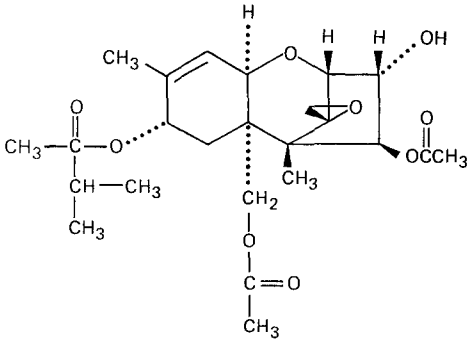

Fig. 3. Structure of T-2 toxin

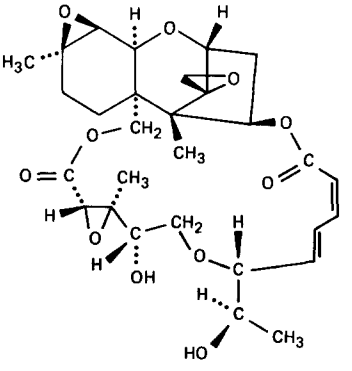

Fig. 4. Structure of baccharin

this compound was dropped from the NCI program. Several interesting nogalomycin analogs were developed through the microbial-chemical biotransformation program (Wiley et al., 1977). Two of these are scheduled for tumor panel evaluation, 7-0-methyl nogarol (Fig. 6) and nogamycin (Fig. 7). Both have shown good activity vs. PS and B16 (Table 10). 7-0-methyl nogarol (NSC-

Table 6. Comparison of antitumor results of anguidin, T-2 toxin and baccharin

Compound	PS		LE		B16		LL	
	Dosage	ILS[b]	Dosage	ILS	Dosage	ILS	Dosage	ILS
Anguidin	2 mg/kg 1 mg/kg	120 35[a]	1 mg/kg	40	2 mg/kg	40	1–4 mg/kg	21–44
T-2 toxin	3 mg/kg 2 mg/kg	31[a] 140	1 mg/kg	40	2 mg/kg	50	2 mg/kg	22
Baccharin	7 mg/kg 7 mg/kg	58[a] 144	40 mg/kg	57	5.4 mg/kg	38	–	–

[a] Adriamycin-resistant PS tumor line
[b] Percentage increase in life span of treated vs control tumored animals

Table 7. Trichothenes undergoing testing at NCI

Compound	NSC No.
T-2 toxin	138,780
Anguidin	141,537
Fusarenon X	197,211
Neosolaniol	197,212
Trichothene derivative	D245,352[a]
Trichothec-9-ene-3,4,15,-triol,12,13-epoxy-15-acetate(3α, 4β)	267,030
Trichothec-9-ene-3,4,15,-triol,12,13-epoxy-triacetate(3α, 4β)	267,031
Trichothecane-3,4,15,-triol,12,13-epoxy-4,15-diacetate(3α, 4β)	267,032
Trichothec-9-en-8-one,4,5-bis(acetyloxy)-12,13-epoxy-3,7-dihydroxy(3α, 4β, 71)	267,034
Trichothec-9-en-8-one,3,4,7,15-tetra-kis(acetyloxy)-12,13-epoxy(3α, 4β, 71)	267,035
Trichothec-9-en-8-one,3(acetyloxy)-12,13-epoxy-7,15-dihydroxy-, 3α, 7D	267,036
Trichothec-9-ene-3,4,15-Triol,12,13-epoxy	269,142
Trichothec-9-en-8-one,12,13-epoxy-3,4,7,15-tetrahydroxy, 13α, 4β, 7α	269,143
Trichothec-9-3n-8-one,12,13-epoxy-3,7,15-trihydroxy(3α, 7α)	269,144
Trichothec-9-en-8-one,3,7,15-tris(acetyloxy)-12,13-epoxy(3α, 7α)	269,145
Trichothene (structure not completely elucidated)	267,693
Trichothene (structure not completely elucidated)	272,704
Trichothen-9-ene-3,4,8,15-tetrol,12,13-epoxy-15-acetate-8-(3-methylbutonoate)3α, 4β, 8α	278,571
Trichothec-9-ene-3,4,15-triol,12,13-epoxy-4-acetate(3α, 4β)	281,805
Trichothec-9-ene-3,4,15-triol,12,13-epoxy,3,4-diacetate(3α, 4β)	283,150
15,16-dinortricotheca-6,8,10-triene,12,13-epoxy,8-methoxy	283,834
15,16′dinortricotheca-6,8,10-triene,12,13-epoxy,8-methoxy(12α)	283,835
Isocrotanic acid, ester with 2a,3,4,4a,4b,7-trimethyl-1H-oxeto[3′,2′:1,5]cyclo-penta [1,2-b)benzofuran-6(5H)-one	92,492
Trichothec-9-en-4-o1,12,13-epoxy, acetate	73,846
Verrucarin A,7′-deoxo-2′deoxy-4′,8-dihydroxy,7′-(1-hydroxyethyl)	269,753
Verrucarin A,7′-deoxo-8-hydroxy,7′-(1-hydroxyethyl)	269,755
Verrucarin A,7′-deoxo-2′-deoxy-2′,3′-epoxy-4′,8-dihydroxy-7-(1-hydroxyethyl) [2′*S*,3′*S*,4′*S*,7′*R*(R)]	269,756
Verrucarin A,7′-deoxo-2′-deoxy-2′,3′:9,10-diepoxy-9,10-dihydro-4′-hydroxy-7′-(1-hydroxyethyl), [2′*S*,3′*S*,4′*S*,7′R(*R*), 9R, 10S]	269,757
Verrucarin A	200,736
Roridin A	200,737
Verrucarin A,2′,3′-didehydro-7′-deoxo-2′-deoxy-7′,5′-(ethylideneoxy)	274,540
Verrucarin A,7′-deoxo-2′-deoxy-2′,3′epoxy-4′,8-dihydroxy-7′-(1-hydroxyethyl)-, [2′*S*,4′*S*,7′*R*(*S*),8*S*]	269,758
Verrucarin A,9,10-epoxy-9,10-dihydro(9*R*,10*S*)	283,445
Verrucarin A,7′-deoxo-8-hydroxy-7′-(1-hydroxyethyl)-2′E,3′E,8*S*)	269,759
Verrucarin A,7′-deoxo-2′-deoxy-2′,3′-epoxy-4′,8-dihydroxy-7′-(1-hydroxyethyl)-, [2′*S*,4′*S*,7′*R*(*S*),8S]	269,758
Verrucarin A,9,10-epoxy-9,10-dihydro(9*R*,10*S*)	283,445

[a] A discreet compound. Data, structure, and supplier cannot be revealed at this time

Table 8. Screening data for anguidin in mouse colon 38

Dose	Activity
8 mg/kg	97% inhibition
4 mg/kg	76% inhibition
2 mg/kg	70% inhibition

Table 9. AT-125 (NSC-163501) and hydroxy AT-125 (NSC-176324) antitumor results

Compound	Tumor	Dose	ILS
163,501	B16	4 mg/kg	27%
	LE	3 mg/kg	96%
	PA[a]	60 mg/kg	47%
	PS	2 mg/kg	98%
176,324	B16	64 mg/kg	38%
	LE	50 mg/kg	67%
	PS	50 mg/kg	128%

[a] PS adriamycin-resistant line

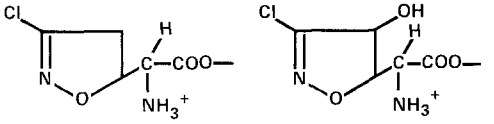

Fig. 5. Structure of AT-125 (left) and hydroxy AT-125

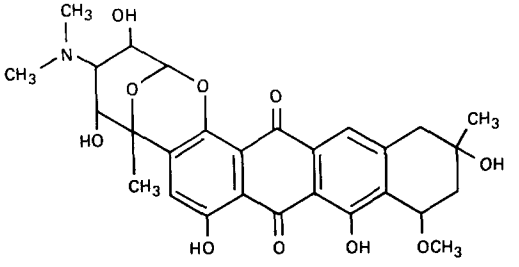

Fig. 6. Structure of 7-0-methyl nogarol

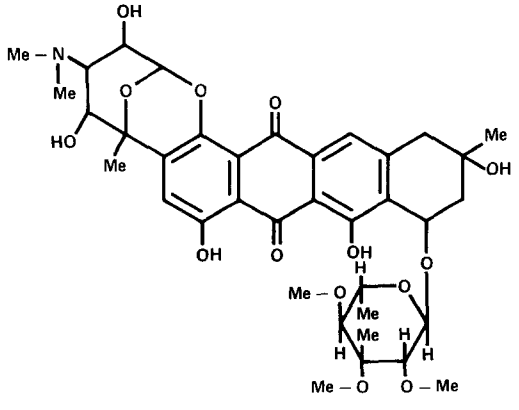

Fig. 7. Structure of nogamycin

269148) seems to be superior vs. the leukemias while nogamycin (NSC-265450) has shown greater activity vs. B16 at the Upjohn Co. NCI would also be quite interested in these compounds if they have less cardiac toxicity than adriamycin. A total of 13 nogalomycins have been evaluated at NCI. Biotransformation of many of these natural products seems to be a worthwhile avenue to pursue in order to determine quickly whether an analog might be more active than the present candidates.

Aclacinomycin A (NSC-208734) (Fig. 8) is a cinerubin-like anthracycline, which was isolated by Dr. Umezawa's group in Japan and has been developed as a possible anticancer agent by Sanraku Ocean (Oki et al., 1975). NCI has evaluated over 250 anthracyclines in its program and unless aclacinomycin shows less cardiac toxicity than other active anthracyclines it will be just one of the 20 best anthracyclines evaluated in the program. The drug is being thoroughly tested in the NCI tumor panel screen (Table 11). This compound has shown complete inhibition of the Danny Martin mammary tumor in mice. In addition, LE, PS, and B16 activity was observed.

Taxol (NSC-125973) (Fig. 9) is a diterpene of the taxane type which is unusual in that it has large and complex ester groupings that have been shown to be related to its activity (Wall and Wani, 1971). As a result of the nitrogenous side chain present this compound is sometimes classed under the alkaloids rather than the terpenes. The active material was isolated by Dr. Monroe Wall's group at Research Triangle Institute from several species of *Taxus* (Wani et al., 1971).

The best source appears to be stem bark of *Taxus brevifolia,* the western yew, a small evergreen native to the Pacific Northwest. There are relatively few species of the genus *Taxus* (family Taxaceae), and compounds having the taxane skeleton occur predominantly in this group. The novel structure of taxol presents a new type of chemical lead for production of antitumor agents, which may be developed further as supplies of the parent compound become available. Taxol has shown antitumor activity in the B16 melanoma, L1210, P388, and P1534 leukemias and the Walker 256 carcinosarcoma tumor systems and was selected for preclinical development on the basis of its activity in the B16 melanoma system.

Tripdiolide (NSC-163063) is a diterpene triepoxide which was isolated by Dr. Kupchan's group at the University of Virginia from *Tripterygium wilfordii* (Celastraceae) (Kupchan et al., 1972). The most active plant part is the root and it is interesting that production of the active substances tripdiolide and triptolide appears

Table 10. Nogalamycin derivatives of interest

Compound	Tumors					
	B16		PS		LE	
	Dose	% ILS	Dose	% ILS	Dose	% ILS
7-0-Methylnogarol	12.5 mg/kg	91	12.5 mg/kg	142	12.5 mg/kg	87
Nogamycin	1.25 mg/kg	172	1.25 mg/kg	72	2.5 mg/kg	25

Table 11. Aclacinomycin antitumor test results

Tumor	Dosage	Activity
B16	3 mg/kg	48% ILS
Mammary	3 mg/kg	100% (inhibition of tumor)
Colon (C6)	3 mg/kg	21% ILS
LE	25 mg/kg	41% ILS
LL	3 mg/kg	25% ILS
PS	5 mg/kg	122% ILS

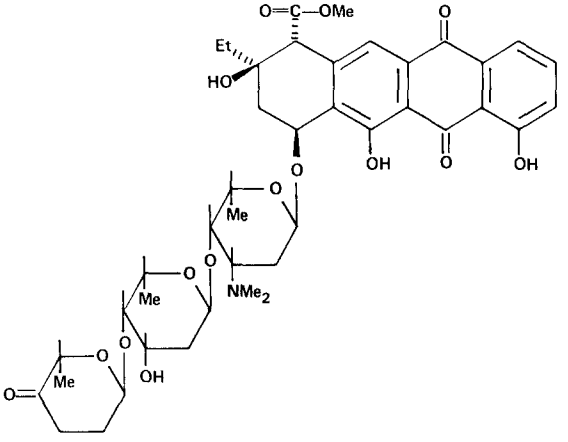

Fig. 8. Structure of aclacinomycin

dependent on location of collection, as root from Taiwan showed excellent activity, while samples procured in Hong Kong were devoid of active substance.

Examination of the structure of tripdiolide (Fig. 10) shows an unusual triepoxide grouping, which appears to be intimately associated with antitumor activity since derivatives without the triepoxide or with altered sterochemistry of the epoxides are inactive (Kupchan, 1976). Triptolide, a closely related compound, differs only in the presence of a ketone instead of the hydroxy group present in the left side of the molecule and is also active.

The selection of tripdiolide as a candidate for preclinical development was on the basis of its activity in the L1210 leukemia system. Tripdiolide is also active against the Lewis lung and P388 leukemia systems.

Homoharringtonine (NSC-141633) is one of a group of several cephalotaxine esters originally isolated by Powell, Smith and coworkers from *Cephalotaxus harringtonia* var. *drupacea* (family Cephalotaxaceae) (Powell et al., 1970). The structure of the cephalotaxine nucleus is found only in *Cephalotaxus* and closely allied genera, and again represents a small group of compounds with unusual biological activity which were detected through random screening. The active materials were originally isolated from wood, bark, and twig samples of the plant, but it has since been determined that seed is a much richer source of the active alkaloids. This evergreen is a native of the China mainland and has presented problems in procurement of sufficient plant material for scale-up isolation work; however, a supply from Japan has been obtained.

There are two closely related active compounds present in extracts of *C. harringtonia,* harringtonine and homoharringtonine, which differ only in the number of methylene groups in the ester side chain as shown in Figure 11. These appear to be equally active, but homoharringtonine was selected for preclinical development at NCI based on its higher yields in the plant. Relevant biological data on the two compounds are given in Table 12.

Bruceantin (NSC-165563) is a member of the group known as quassinoids or simaroubolides and was originally isolated from the stem bark of *Brucea antidysenterica* (Simaroubaceae), a small tree native to Ethiopia. (Kupchan et al., 1973). The isolation was performed by Dr. S. M. Kupchan's group at the University of Virginia, and the structure of bruceantin is presented in Figure 12. Extracts of various plants of the family Simaroubaceae have been used in folk medicine in the treatment of warts, condyloma acuminata, and amebiasis, and studies on other plants of this family in the NCI program have led to isolation of over a dozen related compounds with significant antitumor activity. The quassinoids will be an exciting group of compounds to investigate, since

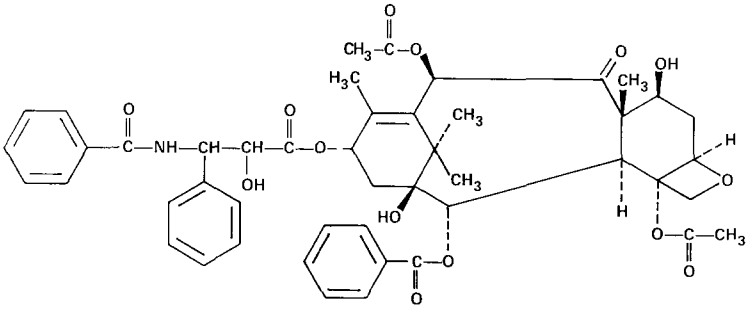

Fig. 9. Structure of taxol

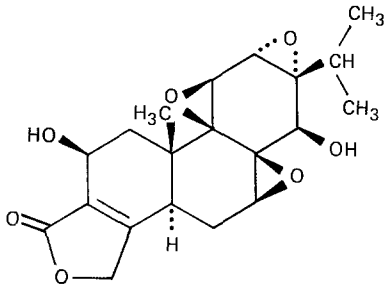

Fig. 10. Structure of tripdiolide

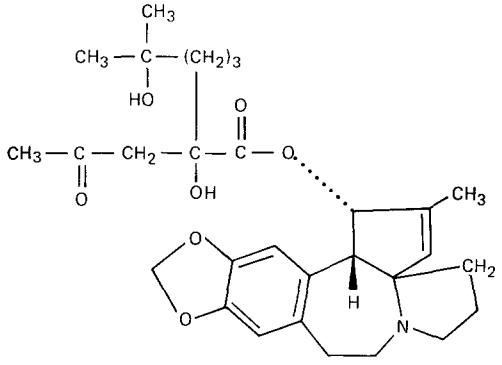

Fig. 11. Structure of homoharringtonine

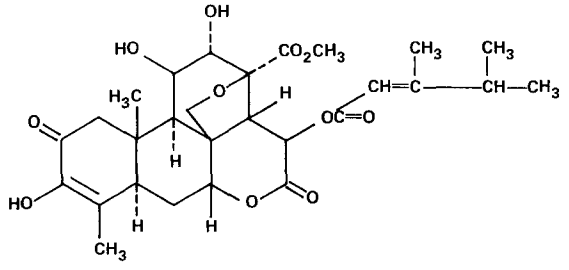

Fig. 12. Structure of bruceantin

Table 12. Comparative antitumor screening data of harringtonine and homoharringtonine

Mouse tumor system	Harringtonine		Homoharringtonine	
	Dose (mg/kg)	% ILS[a]	Dose (mg/kg)	% ILS
B16 melanoma	1.0	50	1.0	11
	0.5	42	0.5	26
L-1210 leukemia	2.0	36	2.0	50
	1.0	86	1.0	30
	0.5	17	0.5	20
	0.25	24	0.25	13
P-388 leukemia	2.0	204	2.0	200
	1.0	140	1.0	145
	0.5	109	0.5	140
				% inhibition[b]
Colon 38	Not tested		4.0	100
			2.0	47
			1.0	36

[a] Percent increase in life span in treated vs. control tumored mice
[b] Percent inhibition of tumor growth in treated vs. control tumored mice

a considerable number of them have now been isolated and it will be possible to do structure-activity correlations to determine which functionalities are needed for antitumor activity. Some correlations in the structures closely related to bruceantin have already been made (Kupchan et al., 1976). Development of other drugs of the quasisinoid type will depend on the results obtained in the clinical studies of bruceantin. Bruceantin was selected for development on the basis of activity in the P388 leukemia system and also shows significant activity in the B16 melanoma, colon 38, and L1210 leukemia systems in mice.

Maytansine (NSC-153858) (Fig. 13) is an ansa macrolide isolated from several plant species in the genera

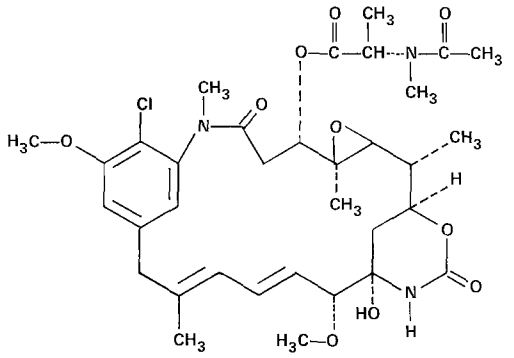

Fig. 13. Structure of maytansine

Table 13. Maytansine: in vivo animal test summary

Tumor	Optimum dose	ILS
B16	16 μg/kg	57%
C6	64 μg/kg	31%
L1210	256 μg/kg	81%
LL	32 μg/kg	32%
PS	256 μg/kg	142%

Maytenus and *Putterlickia* (fam. Celastraceae) (Kupchan et al., 1972a). Structurally maytansine is somewhat related to the rifamycin, tolypomycin, geldanomycin, and streptovaricin series, but none of these has antitumor activity comparable to maytansine. All of the other agents are of microbial origin, which led to the suspicion that maytansine might be produced by a microorganism which was either parasitic on, symbiotic with, or co-occurring with *Maytenus* and *Putterlickia* species. A detailed search failed to find such an organism, and in addition maytansine appears to be present in its source plants regardless of place of collection, so it may truly be a plant constituent. However, the low yield (0.1—10 mg/kg of dried plant) leaves open the possibility that maytansine might be a transformation product in the plant of a substance of microbial origin which is taken up by the plant.

The original isolation work was done in Dr. S. M. Kupchan's laboratories, and was a truly magnificent piece of work in view of the difficult isolation process required by the low yield of material present (0.00002%). Fortunately, maytansine is extremely potent and is active in μg/kg doses, so it has been successfully developed in spite of the poor yield. A summary of the activity of maytansine in murine tumor systems is given in Table 13.

Discussion

The NCI has had a very active natural products program which has constantly supplied novel potential antineoplastic agents for clinical trial. 15 such compounds are now in various stages of testing at NCI (Table 1).

An interest still remains with clinicians to evaluate a new actinomycin if there are indications that it has a broader spectrum of activity, lower toxicity, or a better chemotherapeutic index than actinomycin D. 27 actinomycins have been tested to see whether any were better than actinomycin D. If none of the present candidates and a few new selected derivatives shows a marked superiority to actinomycin D, this work will be terminated.

Anguidin is entering Phase II studies, but at this time it is too early to tell whether it will be a clinically acceptable antineoplastic agent. Some activity has been observed with the trichothenes vs. adriamycin-resistant P 388 leukemias, and this might indicate the possibility of using this drug in combination with adriamycin. Some B16 and L1210 activity has been observed with anguidin and T-2 toxin. Also substantial activity vs. colon cancer in the mouse has been observed with anguidin (Table 8). At the time clinical trials were to be conducted, anguidin was more easily produced than T-2 toxin and more information was available on anguidin, so it was selected for trials. Baccharin is a much newer compound and was identified by Dr. S. M. Kupchan. At present work is being done with fungi found growing on the *Baccharis* plant in order to determine whether the drug is really of microbial origin. The NCI will continue evaluation of this type of compound because indications are that the best candidate from this type of compound may as yet not have been discovered.

The isoxazoles AT-125 and hydroxy AT-125 were two of three antineoplastic drugs to be observed from the 10,000 cultures evaluated in the antimetabolite screen, the other being 5-azacytidine. At present NSC-163501 is scheduled for toxicology studies and additional evaluation in the tumor panel. A great deal of difficulty was encountered in scaling up this fermentation and in the isolation of these compounds. Upjohn has been successful in this work, and this should allow speedy evaluation of NSC-163501. New isoxazoles are of interest to the program and work continues in the area, a total of 14 such compounds having been evaluated at NCI.

7-0-Methyl nogarol and nogamycin are the best of the nogalomycin derivatives which have been isolated and tested in the NCI program. These drugs are now in tumor panel testing and when activity is confirmed at another testing facility these drugs will be eligible to be considered for toxicology studies. 7-0-Methyl nogarol has been scheduled for cardiotoxicity evaluation in rab-

bits. Investigation of transformations of the nogalomycin structure seems to be highly worthwhile and work in this research area will continue.

Aclacinomycin is being evaluated in Phase I clinical trials in Japan. The Japanese groups in their preliminary results have indicated that this compound does not cause alopecia and cardiac toxicity. At the present time the NCI is scheduling cardiac toxicity tests with this compound in rabbits and will postpone toxicology or other testing until the Phase I clinical trials are completed in Japan.

Taxol is currently being isolated on a large enough scale for formulation and toxicology studies, and the prospects for development of this agent toward clinical trials appear good. Tumor panel testing of taxol is currently in progress. This agent appears from preliminary data to function as a mitotic spindle poison.

Tripdiolide has presented a scale-up isolation problem due to the reactivity of the epoxides present and instability of tripdiolide in some common organic solvents, but it is anticipated that this problem will be solved in the near future. Toxicology studies preparatory to clinical trial will commence as soon as sufficient pure compound is available and a suitable formulation can be prepared. The mechanism of action of tripdiolide may be through inhibition of sulfhydryl containing enzymes which can be related chemically to the alkylating ability of the epoxides present.

Homoharringtonine is of high interest to the NCI program as a result of antileukemic activity shown in murine tumor systems and a recent study in China, which indicated that an alkaloidal fraction from *C. harringtonia* which contains harringtonine and homoharringtonine was highly effective in treatment of several types of leukemia in man (*Cephalotaxus* Research Coordinating Group, 1976). Homoharringtonine is currently being isolated on a pilot-plant scale and preclinical toxicology is expected to begin in 1978.

Bruceantin shows good activity in both leukemias and solid tumors in mice and may have potential as an agent with a broadened antitumor spectrum. Toxicology studies have been completed and bruceantin entered Phase I clinical trials in August 1977. It is suggested that bruceantin acts as an inhibitor of protein synthesis and has a minimal effect on DNA and RNA synthesis.

Maytansine has recently entered Phase II clinical trials at several cancer centers under a variety of dosage schedules, and preliminary data on these trials will be available sometime in 1978. The mechanism of action of maytansine is clearly involved with inhibition of mitosis, and there is evidence which suggests that it acts by inhibition of tubulin polymerization. On the basis of the data obtained so far, maytansine appears to be one of the most promising antitumor agents isolated from higher plants which is now under development at the NCI.

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