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New antitumor substances of natural origin

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Introduction

Since 1956 the Cancer Chemotherapy National Service Center (CCNSC), now incorporated into the Developmental Therapeutics Program (DTP), has had a comprehensive drug development program which includes the screening of compounds obtained from natural products. The fermentation program during the last four years has isolated and evaluated an average of 7500 organisms per year for their ability to produce new antineoplastic agents, but in 1979 approximately 15,000 organisms were screened. Since the inception of the program, approximately 180,000 culture filtrates have been tested *in vivo* against murine tumors and 8.5% of these have been found active (10). A marked improvement in *in vivo* activity in recent years has been due to using *in vitro* pre-screen tests (only those broths active in the *in vitro* screen are then tested *in vivo*) and isolating unusual organisms rather than just Streptomyces. Prior to 1975, no *in vitro* pre-screens were used by the CCNSC and primarily the isolation and fermentation of Streptomyces dominated the program. While Streptomyces were the most prolific organisms for the production of novel antimicrobial antibiotics, this did not necessarily mean they would be the best producers of antineoplastic agents.

Some of the *in vitro* prescreens used in the NCI program and some contemplated tests are shown in Table 1.

There have been 1743 crystalline microbial metabolites screened in the program, with more than 1000 coming into the program since 1975 (Table 2). Japanese companies, research institutes, and academia have supplied NCI with approximately 400 of these compounds during this time.

Pure compounds which entered the NCI Natural Products Program for evaluation in the last year include 233 antibiotics, 180 plant products and 20 animal products (Table 3). A total of 269 compounds have been assigned for tumor panel testing since 1977 (Table 4) (8). Also, 432 compounds have been selected for special testing based on some non-cancer biological activity or data regarding concentration of the agent in

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Table 1. Pre-screens—*in vitro*

In use	Considered for future use
P388 cytoxicity	Differential cell cytotoxicity
L1210 cytotoxicity	Cell differentiation
KB cytotoxicity (liquid, agar)	Cell surface changes
Antimetabolites of purines, pyrimidines, amino acids and sugars	(a) Agglutination of plant lectins
Phage induction (lysogenic, beta galactosidase)	(b) Binding of plant lectins
Aminopeptidase B inhibition	Immunogen stimulation
cAMP phosphodiesterase inhibition	Ornithine decarboxylase inhibition
Protease inhibition	Hypoxic cell
Esterase inhibition	
Antimicrobial activity (including yeasts and fungi)	
Differential sensitivity of normal *vs.* mutant bacterial strains	

Table 2. 1979 natural products program update

Fermentation

Approximately 15,000 microbes isolated
(a) Since inception of program 179,918 culture broths tested *in vivo*
(b) 15,257 confirmed actives (*in vivo*)
(c) 1743 crystalline metabolites tested
(d) 127 new compounds in tumor panel
(e) 11 new compounds in special testing

Plant

1500 to 3000 plant samples/year
(a) 500–900 plant species
(b) Since inception of program about 35,000 plant species, 108,830 extracts
(c) Plant extracts active 4712
(i) genera active 1510
(ii) species active 3286
(d) 64 plant compounds in tumor panel
4 plant compounds in special testing
(e) 2192 crystalline plant compounds tested

Animal

(a) Since inception of program 15,063 extracts screened
(b) Animal extracts active 651
(i) genera active 405
(ii) species active 552
(c) 555 crystalline animal products tested
3 animal compounds in tumor panel
10 animal compounds in special testing

Table 3. 1979 NCI—natural product acquisitions

	Contract	Non-contract	Total
Antibiotics	46	187	233
Plant products	40	140	180
Animal products	2	18	20
		Grand total	433

Table 4. 1979 natural products tumor panel status

	Fermentation	Plant	Animal	Total
Compounds assigned	177	89	3	269
Testing incomplete	127	64	3	194
Testing complete	32	16	0	48
Dropped status	18	9	0	27

a specific organ, e.g. a compound concentrating in the kidney would be tested against renal carcinoma in the mouse (Table 5).

The plant program evaluates approximately 1500–5000 plant extracts/year, which is equivalent to 500–900 plants/year. Since the program's initiation in 1957, about 35,000 plant species and 108,830 extracts have been screened against murine tumors *in vivo* or for cell cytotoxicity against the KB (human nasopharynx) cell line. Approximately 7192 crystalline plant materials have been tested in the program. At present, 80 plant compounds are in special testing and 89 are in the tumor panel (Table 4).

The smallest program has been the animal program and as yet no animal product has been evaluated by NCI in clinical trials. NCI has tested approximately 555 crystalline animal products and at present has 3 animal compounds in the tumor panel and 43 animal-derived materials in special testing (Tables 4, 5).

Many of the pure compounds screened have been isolated from the above-mentioned areas through NCI's extramural research program. NCI also has obtained many compounds through its worldwide surveillance program that includes contacting industrial concerns, research institutes, universities, and individual scientists to acquire compounds of potential interest due to their novel structures or biological activities.

Some of the more interesting drugs from this program will be reviewed. Most of the drugs discussed in the paper are in preliminary stages of evaluation although several agents are in early clinical trials.

Methodology

Compounds are obtained by the Natural Products Branch through contracts, grants, and through an extensive worldwide surveillance program.

Pure compounds are tested initially against the P388 leukemia (*in vivo* pre-screen) at NCI unless there is biochemical data or antitumor data which indicates that other testing would be more desirable. Analogs of known active compounds are tested in direct comparison with the parent compound. Compounds which show preliminary activity are retested for confirmation. Confirmed active compounds are reviewed for suitability for further testing in the NCI tumor panel (Table 6). Generally all compounds which show confirmed activity and are structurally novel are tested in the panel as are analogs which show superior activity to their parent compounds.

Table 5. 1979 natural products special testing status

	Fermentation	Plant	Animal	Total
Compounds assigned	309	80	43	432
Dropped status	149	38	33	220

Table 6. Systems tested in tumor panel

Tumor*	Confirmed T/C %,	
	Initial	DN2
PS	120†	175
B1	125	150
CD	≤42	≤10
C8	≤42	≤10
LE	125	150
LL	140	150
C2H2	≤20	≤10
LKH2	≤20	≤10
MBH2	≤20	≤10
C2G5	≤20	≤10
LKG5	≤20	≤10
MBG5	≤20	≤10

* Tumor abbreviations: PS—P388 lymphocytic leukemia; PA—P388 adriamycin resistant; PV—P388 vincristine resistant; P6—P388 L-alanosine resistant; B1—B16 melanocarcinoma; C6—colon 26; C8—colon 38; CD—CD8F1 mammary tumor; CY—colon 36; CZ—colon 51; EM—ependymoblastoma; LE—L1210 lymphoid leukemia; LL—Lewis lung carcinoma; M5—M5076 ovarian carcinoma; NH—Novikoff hepatoma; C2G2—CX-1 colon renal capsule; C2G5—CX-1 colon renal capsule; C4G5—CX-2 colon renal capsule; C9G5—CX-5 colon renal capsule; C2H2—CX-1 colon xenograft; C9H2—CX-5 colon xenograft; LKG5—LX-1 lung renal capsule; LKH2—LX-1 lung xenograft; MBG5—MX-2 breast renal capsule; MBH2—MX-1 breast xenograft.

† 130% if a crude natural product extract or broth

Compounds demonstrating DN2 level activity in one or more tumor panel systems (Table 6) are reviewed for consideration for preclinical development (bulk production, formulation and toxicology). Decisions for selection for DN2A are made by the Decision Network (DN) Committee which is made up of scientists expert in chemistry, natural products, screening, pharmacology, toxicology, pharmaceutics, biochemistry and clinical treatment. Selection is based on factors which include the spectrum and degree of anti-tumor activity, the novelty of the structure or superiority to the parent compound, cost of procurement, possibility of formulation, and any biochemical and pharmacological data available. Compounds selected for further development at DN2A are returned to the DN committee when procurement and formulation are complete (DN2B) and, if passed, proceed to toxicology studies. When toxicology studies are complete the compound is again reviewed by the DN committee (DN3) and, if satisfactory, materials are assembled for an Investigational New Drug Application (IND). Upon approval of the IND by the Food and Drug Administration, Phase I clinical trials are initiated.

Fermentation program

In the fermentation contract program the first objective is to obtain a broad spectrum of different types of microorganisms, to ferment them under various conditions, and to

Table 7. Microorganisms isolated on unusual carbon sources

Source of carbon	Isolates	*In vitro* actives
Uncommon carbohydrates	189	22 (11.6%)
Substituted Sugars	23	6 (26%)
Carboxylic acids	50	0
Amino acids	200	10 (5%)
Fatty acids	77	3 (3.9%)
Lipids	58	16 (27.6%)
Hydrocarbons	64	13 (20.3%)
Terpenes	10	0
	671	70 (10.4%)

test the broths against various pre-screens (Tables 1 and 7). In addition, cometabolism and biotransformation techniques are used on various substrates of interest to NCI to see if, by subtle microbial chemical modification, one can increase activity, lower toxicity, increase bioavailability, or lower carcinogenicity (39). This work has just been initiated and has started to yield some metabolites that are now being isolated and appear to be novel.

The techniques used to isolate organisms are pollen baiting, enrichment, percolation, and sprinkle plate. In addition, substrates such as unusual sugars, purines, pyrimidines, terpenes, amino acids and hydrocarbons have been used as sole sources of carbon for these organisms. The fermentation broths are then evaluated against various pre-screens. Active broths are refermented and, if active again in the pre-screen, are tested *in vivo* against the P388 leukemia. An expansion of new pre-screens is being contemplated for use in the entire natural products program with the hope of increasing productivity.

If *in vivo* activity is demonstrated in a broth and if presumptive chromatography indicates the presence of a novel compound, the active broth is then assigned to a chemist. The compound is isolated, identified, and is then tested in at least four additional murine tumors if sufficient material is available (7).

Results obtained in 1979 indicate that the present approach to obtaining new cultures that produce novel antineoplastic agents is bearing fruit (Tables 8, 9, 10). The final benefit of using these various methods to obtain new drugs will be determined by how many of these materials have clinical efficacy. In order to obtain more novel fermentation-derived compounds, the NCI has sponsored contracts at the 9 contractors listed in Table 11.

The 147 fermentations currently undergoing chemical isolation studies indicate that the NCI program is obtaining more presumptive leads than ever. Prior to 1975 the program averaged about 18–22 fermentations undergoing isolation studies in any one year.

Table 8. 1979 fermentation statistics

Company	Cultures fermented	Active *in vitro*	Tested *in vivo*	Active *in vivo*	Submitted to chemists	New active compounds
A	894	156 (17.4%)	180	44 (24.4%)	70	6
B	4289	2501 (58.3%)	172	75 (43.6%)	37	2
C	10,134	938 (9.2%)	809	61 (7.5%)	40	2
	15,317	3595 (23.1%)	1161	180 (15.5%)	147	10

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