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RELATION OF STRUCTURE OF PURINE AND PYRIMIDINE NUCLEOSIDES TO ANTITUMOR ACTIVITY[1,2]

Abraham Goldin, Harry B. Wood, Jr., and Robert R. Engle[3,4]

SUMMARY

The structure-activity relations have been reviewed for the purine and pyrimidine nucleoside derivatives tested in the screening program of the Cancer Chemotherapy National Service Center. Data are presented on 648 compounds in 36 structural groupings. The test systems include leukemia L1210, Walker carcinosarcoma 256, Adenocarcinoma 755, Sarcoma 180, and the Lewis lung tumor in mice, and the KB tissue culture screen. Also indicated is the clinical status of the nucleoside derivatives. Twenty-one nucleoside derivatives are in the clinical category; 16 of these have shown clinical activity or are being actively processed for the clinic and were active in the L1210 system. One of the compounds active in the clinic was inactive in the L1210 system but was active against KB in tissue culture. One additional compound active in the clinic was inactive in the screening systems. Three compounds listed as inactive in the clinic or too toxic for clinical use were active only in the KB system. One hundred twenty-nine additional compounds in which to date there has been no clinical interest were active in one or more of the experimental test systems. Included were 35 compounds active against L1210, one compound active against Walker 256, 69 compounds active against Carcinoma 755, one compound active against Lewis lung tumor, and 23 compounds active against KB cells in tissue culture. These compounds appeared in 20 of the 36 structural groupings, indicative of the wide range of activity of purine and pyrimidine nucleoside congeners. The structure-antitumor relations for the compounds in this report emphasize the desirability for the further investigation and possible introduction into the clinic of additional active nucleoside derivatives. The analysis also indicates that simple structural alterations in this class of compounds may lead to wide alteration in biologic and antitumor activity, suggesting the importance of additional stress on the synthesis of new congeners.

Since the inception of the screening program of the Cancer Chemotherapy National Service Center, a relatively large body of screening data has been accumulated for a wide variety of classes of compounds (1). Although in general compounds were introduced into the screening program on an empirical basis without regard to their structure, compounds have been introduced into the program on the basis of the class to which they belonged and their specific structure.

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[2] This material was presented in one of a series of meetings on the analysis of structure-activity relations being conducted by Chemotherapy, National Cancer Institute (NCI). The members present at the nucleoside meeting which was held on May 5, 1967, include the following: F. J. Ansfield, H. H. Baer, B. R. Baker, L. L. Bennett, Jr., R. W. Brockman, G. B. Brown, J. H. Burckhalter, E. E. Campaigne, P. Carbone, C. C. Cheng, L. R. Duvall, R. R. Ellison, R. R. Engle, H. G. Fletcher, Jr., J. J. Fox, E. Frei III, M. E. Friedkin, J. L. Glass, A. Goldin, L. Goodman, T. T. Grossnickle, C. Heidelberger, J. F. Henderson, R. W. Ihndris, R. B. Ing, L. V. Kedda, I. Kline, G. A. LePage, E. L. May, J. A. Montgomery, M. B. Naff, J. W. Newman, C. A. Nichol, G. R. Pettit, W. Prusoff, D. Rall, E. Reich, R. K. Robins, L. J. Sargent, S. A. Schepartz, A. W. Schrecker, S. M. Schwartz, E. M. Sloane, S. Takahashi, D. W. Visser, V. S. Waravdekar, F. R. White, M. L. Wolfrom, H. B. Wood, and C. G. Zubrod. The authors wish to acknowledge the interest of the members of this conference and the contributions that they have made in the synthesis and investigation of compounds listed in this report. The authors are also indebted to all who synthesized and submitted compounds for testing in this program.

[3] Cancer Chemotherapy National Service Center (CCNSC), Chemotherapy, NCI, Public Health Service, Bethesda, Md.

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Over a period of years, with the progress in cancer chemotherapy, there have emerged classes of compounds for which representatives have shown activity not only in experimental animals but also in the clinic (2-17). These have included the folic acid antagonists, alkylating agents, purines and pyrimidines, and antibiotics. Another important category which has shown potential for treating patients with cancer is the nucleosides, both purine and pyrimidine nucleosides, including antibiotic nucleosides. In view of the interest in the nucleosides it was decided to summarize the extant CCNSC data (as of May 1967) for these compounds and to examine the structure-activity relations.

After the retrospective analysis by Goldin, Serpick, and Mantel (18) of the relation of CCNSC screening data to clinical activity, emphasis was placed on leukemia L1210 and Walker carcinosarcoma 256 as primary screens. The available data for the nucleosides in the L1210 and Walker systems were therefore summarized. In addition the data were collected for other systems, including Adenocarcinoma 755 (Ca755), Sarcoma 180 (S180), Lewis lung carcinoma, and the KB tissue culture system.

Prior to the shift in emphasis to the L1210 and Walker screens, new compounds were screened in one or more of a wide variety of tumor systems (1,19-22). The gaps in the data stem from either a lack of compound or a loss of interest in the compound as the result of the compound failing to pass the initial primary screen.

The structure-activity studies, of which this report is one of a series, may be important to (1) the screening program in determining what additional testing ought to be done—it may identify the gaps in the data and indicate which of these gaps ought to be filled; (2) the chemist in helping him to decide what new structures might best be synthesized; (3) the biochemist in determining which structures are worthy of detailed studies of mechanism of action; (4) the pharmacologist in deciding which structures are worthy of additional pharmacologic investigation; and (5) the clinician in helping to decide which compounds are of greatest potential interest.

Structure-activity analyses are important in order to uncover new drugs and the structural characteristics which may lead to the improvement of drug effectiveness. The important progress in recent years in the treatment of acute leukemia and allied disorders (23-28) provides additional stimulus for the search for new types of agents and for structure-activity analyses in active groups.

METHODS

The methods are those used in the CCNSC screening program (19-21). The tumor systems are outlined in table 1. The L1210 system uses survival time of the animals as the index of drug effectiveness. A 30% increase in the survival time of the treated animals relative to the controls is indicative of a significant and reproducible chemotherapeutic effect against L1210. For the tumors Walker carcinosarcoma 256, Ca755, S180, and Lewis lung carcinoma, a 75% inhibition of tumor growth relative to the untreated controls was taken as the level of inhibition indicative of drug activity. (In the CCNSC protocols the level of activity required is more extensive, but for this analysis, as for the retrospective study by Goldin, Serpick, and Mantel [18] 75% inhibition was considered to be satisfactory.) The KB system is in essence a cytotoxicity test which is indicative of biologic activity for a compound. In this system 50% inhibition of growth, as reflected by inhibition of protein production, at 1 μg/ml or less was taken as the level of activity. However, 50% inhibition in the KB system in the range of 1 to 10 μg/ml suggests that the compound may be of biologic interest.

EXPERIMENTAL RESULTS

The experimental results are summarized in table 2, in which the compounds are listed in 36 groups.

1. Uracil Ribosides

In addition to uracil there were 21 compounds in this group (entry Nos. 88551-88572), involving substitutions in both the ribose and the uracil moieties. Only one compound met the criterion for activity in the L1210 system, 5-diazouridine (NSC-70390, entry No. 88566). Two additional compounds, though not definitely active in the L1210 system, did show sufficient activity to warrant interest: 1-β-D-ribofuranosyl isobarbituric acid (NSC-73376, entry No. 88560), which has an oxygen at position 5, and uridine 5'-pyrophosphate, glucopyranosyl ester (NSC-20269, entry No. 88570). The other compounds showed no activity in the in vivo systems in which they were tested, nor were they active against KB cells in tissue culture. The uracil ribosides as a class were relatively nontoxic.

2. Uracil 2'-Deoxyribosides

In addition to the free base 5-fluorouracil there were 23 uracil 2'-deoxyribosides (entry Nos. 88573-88596). Most of them lacked test data in 2 or more of the systems. 5-Fluorouracil (NSC-19893, entry No. 88573) was active against L1210, Ca755, S180, and KB cells in tissue culture. Although 2'-deoxyuridine (NSC-23615, entry No. 88574) appeared to be inactive, halogenated derivatives of this compound were generally effective in the various test systems.

2'-Deoxy-5-fluorouridine (5-FUDR, NSC-27640, entry No. 88576) was a notably active compound in this series. It definitely increased the survival time of mice with L1210, and resulted in a marked inhibition of growth of the Ca755, S180, and Lewis lung tumors. It elicited borderline inhibition of growth of Walker 256, and was also active in the KB system.

In contrast to 5-FUDR (the 2'-deoxy-5-β-D-ribofuranoside), the 2'-deoxy-5-fluoro-α-D-ribofuranoside (NSC-66259, entry No. 88575) was inactive against L1210 and S180 in vivo, as well as against KB in tissue culture. No other data were available on the alpha riboside.

2'-Deoxy-5-trifluoromethyluridine (F_3TDR, NSC-75520, entry No. 88595) appeared to be even more active than 5-FUDR in the L1210 system. As with 5-FUDR, F_3TDR exerted a partial inhibitory effect against Walker 256. It was almost as effective as 5-FUDR against Ca755 but less effective against S180; however, F_3TDR was tested at only one dose level in the S180 system. No test data were available in the Lewis lung or KB systems.

A compound of possible interest was the 3'-acetate derivative of 5-FUDR (3'-acetate 5-FUDR, NSC-407335, entry No. 88577). There were no test data in the animal tumor systems, but the compound was active in KB tissue culture. Similarly, the 5-bromo-5,6-dihydro-6-methoxy derivative of 5-FUDR (5-bromo-5,6-dihydro-6-methoxy-5-FUDR; NSC-80870; entry No. 88584) was active in KB tissue culture; in addition it was active against the Lewis lung tumor. Both of these compounds warrant additional testing. Other fluorinated derivatives (entry Nos. 88578-88582) were less active against KB in tissue culture; in vivo data were not available. The loss of activity in the L1210 system when the primary hydroxyl group is converted to a carboxyl group at the 5' position of 5-FUDR (NSC-103704, entry No. 88583) is of interest and should be substantiated.

Substitution of bromine instead of fluorine in the 5 position as in 5-bromo-2'-deoxyuridine (5-BUDR, NSC-38297, entry No. 88585) resulted in a loss of activity against L1210 and the other tumors. However, the iodo derivative, 2'-deoxy-5-iodouridine (5-IUDR, NSC-39661, entry No. 88587) did retain effectiveness in the L1210 system. It was less active than 5-FUDR in Ca755 and S180. Both 5-BUDR and 5-IUDR were considerably less toxic than 5-FUDR, as indicated by the marked increase in their optimal doses. They were also less toxic than 5-FUDR in the KB tissue culture system.

In view of the significant activity and the clinical interest in 5-FUDR and 5-IUDR, it would be desirable to test more structural analogs of these compounds.

3. Uracil Arabinosides and Uracil Lyxosides

None of the 10 uracil arabinosides and uracil lyxosides (entry Nos. 88597-88606) showed definite evidence of activity, when tested in the in vivo tumor systems, or against KB in tissue culture. The lack of activity of uracil 1-β-D-arabinofuranoside (NSC-68928, entry No. 88597) was in marked contrast to the activity observed with cytosine 1-β-D-arabinofuranoside (NSC-63878, entry No. 88635). 5-Fluorouracil 1-β-D-arabinofuranoside (NSC-406444, entry No. 88599) given as a single injection was inactive against L1210; in other studies (7,29), however, it was effective in the treatment of leukemia B82. 5-Bromouracil 1-β-D-arabinofuranoside (NSC-82222, entry No. 88600) and 5-iodouracil 1-β-D-arabinofuranoside (NSC-82221, entry No. 88601) were tested only in the KB system and were found to be nontoxic. Because of the general clinical interest in halogenated derivatives, it is suggested that additional testing be done with the fluoro, bromo, and iodo derivatives.

The two lyxofuranosyl derivatives (NSC-88790, entry No. 88605; and NSC-88791, entry No. 88606) were inactive in the L1210, S180, and Lewis lung systems and against KB. They were not tested against Walker 256 and Ca755.

4. Uracil Hexopyranosides

Of the 23 uracil hexopyranosides (entry Nos. 88607-88629), only the complex (2-β-D-glucopyranosyl)oxy derivative (NSC-100050, entry No. 88624) was tested in the L1210 system. It was inactive at the one dose level tested. No compounds were tested against the other in vivo screening systems, and none of them had

definite activity in the in vitro KB tissue culture system. The 2',3',4',6'-tetraacetate, 4-methoxy derivative of a 1-β-D-glucopyranoside (NSC-401826, entry No. 88609) had a KB rating of 11 which was suggestive of biologic activity.

In vivo test data would have to be obtained in order to evaluate this class of compounds.

5. Uracil Miscellaneous

The 4 compounds in this category (entry Nos. 88630-88633) were inactive in the KB tissue culture system. All 4 were pyranyl derivatives of uracil. Two compounds (NSC-92707, entry No. 88631; and NSC-92709, entry No. 88632) were inactive against L1210 and against other tumor systems where tested. All compounds, however, were tested at only one dose level.

6. Cytosine Furanosides

In addition to cytosine itself, 17 cytosine furanosides were listed in this series (entry Nos. 88634-88651). The free base cytosine (NSC-27787, entry No. 88634) was tested against S180 and was inactive. Cytosine 1-β-D-arabinofuranoside (Ara-C, NSC-63878, entry No. 88635), a drug of clinical interest, was highly active against L1210. It was inactive in the screen against Walker 256 and Lewis lung carcinoma, but was active against Ca755 and S180. It was also active in vitro against KB cells.

The 2',3',5'-triacetate derivative of cytosine 1-β-D-arabinofuranoside (2',3',5'-triacetate Ara-C, NSC-93150, entry No. 88636) given orally was active against L1210. In other studies against advanced leukemia L1210[5] the 2',3',5'-triacetate derivative was less effective than cytosine 1-β-D-arabinofuranoside when administered subcutaneously, but the 2 agents had similar antileukemic activity when given orally. The triacetate derivative was active in the KB system but showed reduced activity against S180. The 2',3',5'-triacetate derivative of *N*-acetylcytosine 1-β-D-arabinofuranoside (2',3',5'-triacetate *N*-acetyl-Ara-C, NSC-92717, entry No. 88637) was also quite active in the L1210 system and active against KB cells. The increased optimal dose for leukemia L1210 (1350 mg/kg) was in decided contrast to the 20-mg/kg optimal dose for cytosine 1-β-D-arabinofuranoside.

The 5'-phosphate derivative of cytosine 1-β-D-arabinofuranoside (5'-phosphate Ara-C, NSC-99445, entry No. 88638) (30) produced a 250% increase in the survival time of mice with advanced L1210;[6] however, in the advanced L1210 study the phosphorylated derivative was approximately as effective as cytosine 1-β-D-arabinofuranoside.[6] No data were available for this compound in the primary screen against early L1210.

One additional arabinofuranoside was active in the L1210 system: the 5-fluoro derivative of cytosine 1-β-D-arabinofuranoside (5-fluoro-Ara-C, NSC-529180, entry No. 88639). Data were not available for this compound in the other screening systems.

It should be noted that the 5-methyl derivative of cytosine 1-β-D-arabinofuranoside (NSC-96372, entry No. 88640) was inactive in the L1210 system and in the in vitro KB tissue culture system. However, the compound was tested at only one dose level in the L1210 system; it is suggested that a series of dose levels of this drug be studied.

The 5-fluoro derivative of 2'-deoxycytidine (NSC-48006, entry No. 88649) was active in the L1210 system and against S180. 5-Bromo-2'-deoxycytidine (NSC-61765, entry No. 88650) was active against Ca755 but inactive in L1210 and in other in vivo systems.

The other cytosine furanosides (entry Nos. 88641-88648 and 88651) were inactive in the systems in which they were tested; however, there were wide gaps in the data.

7. Cytosine 2'-Deoxypyranosides

There were only 2 compounds in this series (entry Nos. 88652-88653). Both were inactive in the KB system, and no data on in vivo studies were available.

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[5] Kline, I., Tyrer, D. D., Gang, M., Venditti, J. M., and Goldin, A. To be published.
[6] Schrecker, A. W., and Goldin, A. To be published.

8. Cytosine Glucopyranosides

All 7 cytosine glucopyranosides (entry Nos. 88654-88660) were inactive in the KB system. It will be necessary to obtain data on in vivo tests in order to make any further structure-activity analysis for this group of compounds.

9. Cytosine 2'-Aminopyranosides

There were 13 compounds in this series (entry Nos. 88661-88673). Twelve compounds were tested only in the KB system, and none met the requirement for activity. Of these, 2'-acetamido-2'-deoxycytosine 1-β-D-glucopyranoside (NSC-401829, entry No. 88664) had a KB rating of 13, suggesting possible biologic activity. The other compound in this series (NSC-24832, entry No. 88667) was not tested against KB but was inactive in the Ca755 and S180 systems.

10. Cytosine Miscellaneous

None of these 5 compounds (entry Nos. 88674-88678) showed activity in the systems in which they were tested. However, NSC-402189 (entry No. 88677) had a rating in the KB system (1.3) approaching the level of activity acceptance; there were no data for this compound in any of the in vivo tumor systems. Another compound (NSC-401861, entry No. 88676) had a rating of 9.5 in the KB system, suggesting possible biologic activity; however, it was not active against L1210 and the other systems tested.

11. Hypoxanthine Ribosides

Twelve hypoxanthine ribosides and the free base were tested (entry Nos. 88679-88691). The free base, hypoxanthine (NSC-14665, entry No. 88679), was inactive against L1210, Ca755, S180, and Lewis lung tumors, as well as against KB in tissue culture.

Inosine (NSC-20262, entry No. 88680) was inactive against L1210, Ca755, and S180. One of the compounds in this series, a complex codehydrogenase I derivative (NSC-20271, entry No. 88687), was active in the L1210 system. The 10 additional hypoxanthine ribosides in this series were inactive in the systems in which they were tested.

No data were available for the hypoxanthine ribosides in the Walker 256 system, and only 5 of the 13 compounds were tested in the KB system. Where the substituents in this series were in the 6-hydroxy group (NSC-30606, entry No. 88688; NSC-88727, entry No. 88689; and NSC-31144, entry No. 88690) the available data were quite limited.

12. Hypoxanthine Arabinosides

There were 5 compounds in this series (entry Nos. 88692-88696). Four of them, including hypoxanthine 9-β-D-arabinofuranoside (NSC-405122, entry No. 88695) were tested at one dose level in the L1210 system and were inactive. The compounds were also inactive in the other systems in which they were tested. They were also relatively nontoxic as shown by the high dosage which could be used. Hypoxanthine 9-β-D-arabinofuranoside has been reported to be almost as lethal to purine-requiring *Escherichia coli* as adenine 9-β-D-arabinofuranoside (Ara-A, NSC-404241, entry No. 88930) (7) and should therefore undergo further testing.

13. Hypoxanthine Miscellaneous

The 15 compounds in this group (entry Nos. 88697-88711) have a wide variety of substituents in the sugar moiety, and in some cases substitution was made in the hypoxanthine base. Data were available for only 5 of the compounds in the L1210 system, and no compounds were tested in the Walker system. The compounds were ineffective in the systems in which they were tested, but there were wide gaps in the available data.

14. 6-Mercaptopurine Ribosides

There were 59 compounds in this group (entry Nos. 88712-88770). The parent compound, 6-mercaptopurine (6-MP, NSC-755, entry No. 88712), was active against L1210, Walker 256, and Ca755. In addition, it was active in vitro in the KB system. 6-Mercaptopurine 9-β-D-ribofuranoside (6-MPR, NSC-4911, entry No. 88713) also was active in the L1210, Walker 256, and Ca755 systems. In addition it had definite antitumor activity in the S180 system, and like the free base it inhibited KB in tissue culture.

A series of compounds is listed (entry Nos. 88714-88744) in which substitutions were made in the sugar moiety. None of the compounds appeared to be more effective than 6-MP or 6-MPR. Compounds which were approximately as effective as 6-MP and 6-MPR against L1210 included the 2',3',5'-triacetate derivative of 6-mercaptopurine 9-β-D-ribofuranoside (2',3',5'-triacetate 6-MPR, NSC-66385, entry No. 88715), the 2',3',5'-trivalerate derivative (2',3',5'-trivalerate 6-MPR, NSC-77495, entry No. 88717), the 2',3',5'-tributyrate derivative (2',3',5'-tributyrate 6-MPR, NSC-76952, entry No. 88718), the 2',3',5'-tribenzoate derivative (2',3',5'-tribenzoate 6-MPR, NSC-28416, entry No. 88722), the 2',3',5'-tris(*p*-nitrobenzoate) derivative [2',3',5'-tris(*p*-nitrobenzoate) 6-MPR, NSC-76126, entry No. 88725], the 5'-phosphate butyl ester (5'-butyl phosphate 6-MPR, NSC-45635, entry No. 88733), and the 5'-phosphate diethyl ester (5'-diethyl phosphate 6-MPR, NSC-40634, entry No. 88734). The 5'-phosphate diphenyl ester (5'-diphenyl phosphate 6-MPR, NSC-40632, entry No. 88739) had somewhat reduced activity. These compounds which were active in L1210 usually showed activity in one or more of the Walker 256, Ca755, S180, and KB systems in which they were tested.

A number of compounds not tested or not active against L1210 were active against Ca755. The 2',3'-diacetate derivative of 6-mercaptopurine 9-β-D-ribofuranoside (2',3'-diacetate 6-MPR, NSC-408371, entry No. 88714) was active against Ca755 and KB, but was not tested in the other systems. The activity of the 2',3',5'-triisobutyrate derivative (2',3',5'-triisobutyrate 6-MPR, NSC-78308, entry No. 88716) in the Ca755, S180, and KB systems (despite the lack of activity against L1210) suggests that this compound is worthy of additional testing. The 2',3',5'-tri-*p*-anisate derivative (2',3',5'-tri-*p*-anisate 6-MPR, NSC-76519, entry No. 88723) was also active in the Ca755 system, but was inactive at the one dose level tested against L1210. The 2',3',5'-tris(*p*-chlorobenzoate) derivative (2',3',5'-tris(*p*-chlorobenzoate) 6-MPR, NSC-76520, entry No. 88724) inhibited Ca755, but was ineffective at the one dose level tested against L1210. The 5'-phosphate derivative (5'-phosphate 6-MPR, NSC-46024, entry No. 88731), and the 5'-phosphate ethyl ester (5'-ethyl phosphate 6-MPR, NSC-45636, entry No. 88732) were active against Ca755 and also against KB; neither compound was tested in the other systems. The 5'-phosphate dibutyl ester (5'-dibutyl phosphate 6-MPR, NSC-40633, entry No. 88735), and the 5'-phosphate phenyl ester (5'-phenyl phosphate 6-MPR, NSC-45637, entry No. 88737), also active against Ca755, were not tested against L1210.

A number of other substitutions in the sugar moiety led to loss of antitumor effectiveness, as reflected in a failure to increase the survival time of mice with L1210 or failure to produce appreciable inhibition of solid tumor growth in the other tumor systems. Also with the exception of NSC-85389 (5'-(*p*-nitrophenyl) phosphate 6-MPR, entry No. 88738) and NSC-405278 (2'-phosphate 6-MPR, entry No. 88744), no activity was noted in the KB system.

Apparently, if the substitution in the sugar moiety is too cumbersome it may lead to a loss of activity: 3 such compounds were the 2',3',5'-trilaurate derivative (NSC-76123, entry No. 88719), the 2',3',5'-trioleate derivative (NSC-76124, entry No. 88720), and the 2',3',5'-tristearate derivative (NSC-76949, entry No. 88721). With these compounds the loss of activity may be related to failure of penetration of the drugs into the tumor cells.

The one 9-β-L-ribofuranosyl-6-mercaptopurine derivative listed (NSC-92428, entry No. 88745) was inactive against KB in tissue culture and was not tested in the other systems.

Compounds NSC-76465 through NSC-91745 (entry Nos. 88746-88760) have alterations involving the 6-sulfhydryl (SH) group. Substitution of a methyl group into the SH group of 6-MPR (6-methyl-MPR, NSC-40774, entry No. 88747) permitted retention of activity against L1210. The methyl derivative was also active in the KB system. However, there did appear to be a loss in activity against Walker 256, Ca755, and S180. A second compound involving the substitution of a methyl group into SH also involved the introduction of a 5'-phosphate group (5'-phosphate 6-methyl-MPR, NSC-407746, entry No. 88748); with this compound there was activity in the KB system, but no other data were available. Substitution of an ethyl group for the hydrogen in the SH moiety (6-ethyl-MPR, NSC-39368, entry No. 88749) resulted in an apparent loss in activity against L1210, but only one dose level of the drug was tested. The compound was active against Ca755 and had borderline activity against the S180 tumor. The 6-propylthiopurine derivative (6-propyl-MPR, NSC-39044, entry No. 88750) was not tested against L1210, but it too was active against Ca755. 6-Allylthiopurine 9-β-D-ribofuranoside (6-allyl-MPR, NSC-39367, entry No. 88751) was possibly more active in the L1210 system than either 6-MP or 6-MPR; this compound was also active against the Ca755 and Walker 256 tumors. At the one dose level tested the 6-acetonylthio derivative (6-acetonyl-MPR, NSC-39045, entry No. 88757) was active against L1210 and against Ca755. The nitroimidazolylthio derivative [6-(1-methyl-4-nitroimidazol-5-yl)-MPR, NSC-91745, entry No. 88760] was not tested in vivo; it was active against KB cells.

Other complex substitutions in SH may result in loss of L1210 activity. This was noted with NSC-27307 (entry No. 88754), NSC-39043 (6-cinnamyl-MPR, entry No. 88756), and NSC-39848 (6-cyanomethyl-MPR, entry No. 88759).

Most of the analogs of 6-MPR that have substitutions for the hydrogen in the SH group alone or accompanied by additional substituents in the sugar moiety were active in the Ca755 system. Among those listed in table 3 are NSC-40630 (6-cyclopentyl-MPR, entry No. 88752), NSC-26273 (6-benzyl-MPR, entry No. 88753), NSC-55467 [6-(2-methyl-1-naphthyl)methyl-MPR, entry No. 88755] and NSC-41847 [6-(2-thenyl)-MPR, entry No. 88758].

Of the remaining compounds in the 6-MP riboside series, NSC-46786 through NSC-76932 (entry Nos. 88761-88770), only 2 were tested in any of the tumor systems, and they were negative. Five compounds, NSC-407427 [6-MPR phosphate (5' → 5') 6-MPR, entry No. 88762], NSC 409841 [5-FUDR phosphate (3' → 5') 6-MPR, entry No. 88763], NSC-409289 [5-FUDR phosphate (5' → 5') 6-MPR, entry No. 88764], NSC-407338 [thymidine phosphate (5' → 5') 6-MPR, entry No. 88765], and NSC-45404 (1-[2-(6-MP)ethyl]-6-MPR, entry No. 88769) were active in the KB system, and it is suggested that further testing in the tumor systems be done. The possible role of the phosphate in entry Nos. 88762-88765 in relation to KB activity awaits clarification.

15. 6-Mercaptopurine (Nonribose) Nucleosides

The 19 compounds (entry Nos. 88771-88789) in this category included deoxy sugars, arabinofuranosides, lyxofuranosides, xylofuranosides, glucofuranosides, glucopyranosides, galactofuranosides, allofuranosides, and one 4'-thioribofuranoside.

2'-Deoxy-6-mercaptopurine 9-β-D-ribofuranoside (2'-deoxy-6-MPR, NSC-409366, entry No. 88771) was active against L1210, Walker 256, and KB cells in tissue culture. 6-Mercaptopurine 9-β-D-arabinofuranoside (6-MPAra, NSC-406021, entry No. 88773) was possibly more active than 6-MP or 6-MPR against L1210. This compound was also very active against Ca755 but was inactive in the KB system. 6-Mercaptopurine 9-α-D-arabinofuranoside (NSC-99193, entry No. 88774) appeared to be inactive against L1210; however it was tested at only one dose level. The other alpha derivative, the 2',3',5'-triacetate derivative of 6-mercaptopurine 9-α-D-arabinofuranoside (NSC-98668, entry No. 88775) was also inactive in the L1210 system and failed to inhibit tumor growth in the Walker 256 system.

The 2',3'-diacetate derivative of 5'-deoxy-6-mercaptopurine 9-β-D-xylofuranoside (NSC-101586, entry No. 88782) was inactive against L1210, as was 6-mercaptopurine 9-β-D-galactofuranoside (NSC-84636, entry No. 88784).

The tetraacetate derivative of 6-propylthiopurine 9-β-D-glucopyranoside (NSC-80919, entry No. 88789) was active against L1210 but inactive in both the S180 and KB systems. No other data on animal tests were available for the compounds in the 6-MP (nonribose) nucleoside series.

One of the compounds, 6'-deoxy-6-mercaptopurine 9-β-D-allofuranoside (6'-deoxy-6-MPAllo, NSC-409352, entry No. 88787), was active in the KB system and should be tested in vivo against the animal tumors. The 9-β-D-lyxofuranosyl derivative (NSC-92429, entry No. 88777) had a rating of 3.0 in the KB system, suggesting that the compound is biologically active.

16. 6-Mercaptopurine: Nonsugar Analogs

There were 15 compounds listed in this category (entry Nos. 88790-88804) and all were 9-tetrahydropyranyl or 9-tetrahydrofuryl derivatives. One of these, a 9-tetrahydrofuryl derivative of 6-mercaptopurine [9-(tetrahydro-2-furyl)-6-MP, NSC-45153, entry No. 88790], was quite active in the L1210 system and was active against Ca755. Two tetrahydropyranyl derivatives [9-(tetrahydro-2-pyranyl)-6-MP, NSC-33186, entry No. 88794; and 9-(tetrahydro-2-pyranyl)-6-butyl-MP, NSC-38305, entry No. 88798] showed borderline activity against L1210 and were also active against Ca755. Nine other compounds were active in the Ca755 system. This type of structure appears to be worthy of additional testing.

17. 6-Halopurine Nucleosides

Including the free base 6-chloropurine, there were 23 compounds in this group (entry Nos. 88805-88827). The free base, 6-chloropurine (6-ClP, NSC-744, entry No. 88805), was active against both L1210 and Ca755 but was inactive in the Walker 256, S180 and KB systems.

6-Chloropurine 9-β-D-ribofuranoside (6-ClPR, NSC-4910, entry No. 88806) and its 2',3',5'-tribenzoate derivative (NSC-88182, entry No. 88807) were inactive against L1210. 6-ClPR, however, retained its effectiveness against Ca755 and was active against KB cells. 6-Bromopurine 9-β-D-ribofuranoside (NSC-62630, entry No. 88808) and 6-iodopurine 9-β-D-ribofuranoside (6-IPR, NSC-66384, entry No. 88809) were also inactive against

L1210 and did not meet the requirement for activity against Ca755. 6-Chloro-2'-deoxypurine 9-β-D-ribofuranoside (NSC-409824, entry No. 88810) was inactive in the KB system but was not tested in any other system. 6-Chloro-2'-deoxypurine 9-α-D-ribofuranoside (NSC-90251, entry No. 88811) was inactive not only in the KB system but also against L1210, Walker 256, and S180.

In a series of 6-halogen-2-substituted purine nucleosides, 16 compounds (NSC-99440 through NSC-100280; entry Nos. 88812-88827) were essentially inactive in the systems in which they were tested. 2-Amino-6-iodopurine 9-β-D-ribofuranoside (NSC-66383, entry No. 88821), however, was active against Ca755. Although none of these compounds was definitely active against KB, one of them received a rating bordering on activity: the 2',3',5'-triacetate derivative of 2,6-dichloropurine 9-β-D-ribofuranoside (NSC-76763, entry No. 88814). Additional testing with this type of structure is required to permit further comparisons.

18. 6-Halopurine: Nonsugar Analogs

The nonsugar substituents for the 11 compounds in this group (entry Nos. 88828-88838) included tetrahydrofurans and tetrahydropyrans. The 9-tetrahydro-2-pyranyl derivative of 6-chloropurine [9-(tetrahydro-2-pyranyl)-6-ClP, NSC-33187, entry No. 88830] showed borderline activity against L1210 and was also active against Ca755. Three other compounds [9-(tetrahydro-2-furyl)-6-IP, NSC-45152, entry No. 88835; 9-(tetrahydro-2-pyranyl)-6-IP, NSC-33188, entry No. 88836; and NSC-70386, entry No. 88838] were tested against L1210 but failed to meet the criterion for effectiveness. The first two of these, NSC-45152 and NSC-33188, were active against Ca755. Four other compounds were active in the Ca755 system, and 2 had borderline activity. One compound [9-(tetrahydro-6-ethoxy-2-pyranyl)-6-ClP, NSC-42372, entry No. 88832] was active against the Walker 256 tumor.

No additional activity was found in the other systems in which the drugs were tested; however, the testing was incomplete.

19. Adenine Ribosides

In addition to the free base adenine, there were 64 adenine ribosides in this series (entry Nos. 88839-88903). The free base adenine (NSC-14666, entry No. 88839) was not tested against L1210 or Ca755. It was inactive against S180, Walker 256, and Lewis lung carcinoma, and was also inactive in the KB system. Adenosine (AdR, NSC-7652, entry No. 88840) was inactive in all the tumor systems and was ineffective against KB. Only 2 of 30 adenine ribosides that were tested in the L1210 system were active. 6-Hydrazinopurine 9-β-D-ribofuranoside (6-hydrazino-PR, NSC-29408, entry No. 88866), had an L1210 rating of 37 but was inactive in the Walker 256, Ca755 and S180 systems. 3'-Amino-3'-deoxy-*N*,*N*-dimethyladenosine (3'-amino-3'-deoxy-*N*,*N*-dimethyl-Adr, NSC-3056, entry No. 88875) had an L1210 rating of 32 and was also active against Ca755 and KB.

Two additional compounds, 3'-amino-3'-deoxyadenosine (NSC-18192, entry No. 88874) and 2-fluoroadenosine (2-fluoro-AdR, NSC-30605, entry No. 88888), were inactive against Walker 256.

Three compounds were active against Ca755: *N*-(2-hydroxyethyl)-adenosine (*N*-(2-hydroxyethyl)-AdR, NSC-54251, entry No. 88868); the 6-trimethylammonium chloride derivative of adenosine (6-trimethylammonium chloride-PR, NSC-66382, entry No. 88872) which also had an activity rating of 1.4 in the KB system; and as indicated above, the L1210-active 3'-amino-3'-deoxy-*N*,*N*-dimethyladenosine (NSC-3056, entry No. 88875). Puromycin (NSC-3055, entry No. 88876) partly inhibited the growth of Ca755 and was active against KB. It is interesting to note that all 4 of these compounds have alkylated amino groups in position 6. Two other such compounds, *N*-methyladenosine (NSC-29409, entry No. 88867) and the 6-(1-aziridinyl) derivative of purine 9-β-D-ribofuranoside (NSC-39091, entry No. 88870), had ratings of 66 and 68 respectively against Ca755.

A number of additional compounds were active in the KB system. *N*-Allyladenosine (*N*-allyl-AdR, NSC-98777, entry No. 88869) had a rating of 1 in the KB system but was not tested in vivo. NSC-400643 [N^{α}-(3-methoxy-4-ethoxybenzylidene) puromycin, entry No. 88880] and NSC-400641 (N^{α}-(piperonylidene) puromycin, entry No. 88881), were active in the KB system, as was 2-fluoroadenosine (NSC-30605, entry No. 88888). Another compound active in KB was 8-bromoadenosine (8-bromo-AdR, NSC-79213, entry No. 88892). Adenine 3-β-D-ribofuranoside (NSC-82825, entry No. 88901) had a KB rating of 1.7 which suggested possible biologic activity, but the compound was inactive against L1210 at the one dose level tested.

The adenine ribosides do offer interesting leads but much additional data is required in order to make precise structure-activity analyses.

20. Adenine Deoxypentofuranosides

There were 25 adenine deoxypentofuranosides (entry Nos. 88904-88928). Thirteen of them were tested against L1210 and had no activity. Only one of the compounds was tested in the Walker system and it too was inactive. Where tested, the compounds were inactive against Ca755 and S180 and there was no definite evidence of activity in the KB system. No data were available for the Lewis lung tumor. Further analysis of the adenine deoxypentofuranosides would require additional data.

21. Adenine Arabinofuranosides

The data on adenine arabinofuranosides (entry Nos. 88929-88958) were scanty. Of the 30 compounds in this group only 5 were tested against L1210, and they were inactive. The 3 compounds tested against Walker 256 also were inactive. Where tested, there was no evidence of activity in the other tumor systems. No compound tested in the KB system met the criterion for effectiveness. Several compounds, however, had sufficient activity in the KB system to suggest that they might be biologically active. These included adenine 9-β-D-arabinofuranoside (NSC-404241, entry No. 88930) (7,31-33), its alpha derivative (NSC-70422, entry No. 88929), and its 3'-azido-3'-deoxy derivative (NSC-98670, entry No. 88936). The 2-chloro derivative of adenine 9-β-D-arabinofuranoside (NSC-76356, entry No. 88955) had a KB rating of 2.5, which approached the level of acceptance for tissue culture activity; however, given as a single injection it was inactive against L1210.

Most of these compounds had substitutions in the sugar moiety. There were wide gaps in the data and additional testing would be required for further structure-activity analysis.

22. Other Adenine Pentofuranosides

There were 31 compounds (entry Nos. 88959-88989) listed under this heading; 26 of them were xylofuranosides and the rest were lyxofuranosides. These compounds have not been examined in any detail. Only 4 were tested against L1210 and they were negative: 5'-deoxyadenine 9-α-D-xylofuranoside (NSC-102642, entry No. 88961); adenine 9-β-D-xylofuranoside (NSC-7359, entry No. 88962); adenine 9-β-D-xylofuranoside-1-oxide (NSC-97110, entry No. 88979); and the 3',5'-*O*-isopropylidene derivative of 2-chloroadenine 9-β-D-xylofuranoside, 2'-methanesulfonate (NSC-90841, entry No. 88983). Adenine 9-β-D-xylofuranoside (NSC-7359, entry No. 88962) was also inactive against the Walker 256 tumor, as was 5'-deoxyadenine 9-β-D-xylofuranoside (NSC-86102, entry No. 88970). The methanesulfonate derivative (NSC-90841, entry No. 88983), in addition to being inactive against L1210, was inactive against the S180 and Lewis lung tumors. No additional in vivo test data were available.

None of the compounds tested in the KB system had an important degree of activity. The drug most active against KB was the 3',5'-*O*-isopropylidene-4'-thio derivative of adenine 9-β-D-xylofuranoside (NSC-98675, entry No. 88968) which had a rating of 7.9; no in vivo data were available for this compound. Structure-activity studies in this group of compounds were severely hampered by the wide gaps in the in vivo data.

23. Adenine Hexonucleosides

The 23 compounds in this group (entry Nos. 88990-89012) included a variety of hexopyranosides and hexofuranosides. Where tested, none showed activity in vivo against the animal tumor systems, or in vitro in the KB system. One of the compounds, 6'-deoxyadenine 9-β-D-allofuranoside (NSC-18193, entry No. 88996) had a KB rating of 8.1, suggesting biologic activity. There were wide gaps in the data and additional testing should be done. The substituents in all but one of the adenine hexonucleosides were limited to the sugar moiety. NSC-91743 (entry No. 88999) which had a 2-chloro substitution in the adenine moiety was inactive in the KB system, but was not tested in vivo.

24. Adenine: Nonsugar Analogs

There were 15 compounds in this group. Six were tested against L1210 and all were inactive. Only one compound, a 9-(tetrahydro-2-furyl)-6-trimethylammonium chloride derivative (NSC-53347, entry No. 89016) was tested in the Walker 256 system, and it was inactive; it was also inactive against the L1210, S180, and Lewis lung tumors but was quite active against Ca755. The 9-(5-methyl-tetrahydro-2-furyl)-6-trimethylammonium chloride derivative (NSC-67617, entry No. 89018) was active against Ca755 and inactive against L1210 and S180. The 9-(tetrahydro-2-pyranyl)-6-trimethylammonium chloride derivative (NSC-53346, entry No. 89022) was also active against Ca755 but was not tested in the other systems. None of the other derivatives showed definite activity in the in vivo test systems for which data were available, and no activity was observed in the KB system.

25. Adenine Miscellaneous

None of the 5 miscellaneous adenine compounds (entry Nos. 89028-89032) showed activity in the L1210 system. Four of the compounds were adenine pyranosides; one of these, 2-chloroadenine 9-β-D-ribopyranoside (NSC-101087, entry No. 89030) was also inactive in the Walker 256 system. Adenine 9-β-D-xylopyranoside (NSC-90560, entry No. 89029) was also inactive against the Lewis lung tumor. No other in vivo test data were available. Two of the compounds were tested in vitro against KB, and one of them, a cyclopentanemethanol derivative (NSC-103526, entry No. 89032) was active.

26. 2,6-Diaminopurine Nucleosides

The free base 2,6-diaminopurine (NSC-743, entry No. 89033) was active against Walker 256 but was ineffective against L1210, Ca755, and S180. No data were available for this compound in the Lewis lung system. Unlike the 2,6-diaminopurine nucleosides tested, the free base was active in the KB system.

Besides the free base, there were 14 2,6-diaminopurine nucleosides in this group (entry Nos. 89033-89047), only 2 of which had been tested against L1210. 2,6-Diaminopurine 9-β-D-ribofuranoside (2,6-diamino-PR, NSC-7363, entry No. 89034) was inactive at the only dose level tested (500 mg/kg). It was also inactive against Ca755 and S180, and had a rating of 12 in the KB system. 2'-Deoxy-2,6-diaminopurine 9-β-D-ribofuranoside (NSC-104303, entry No. 89038), given in a single injection, was inactive in the L1210 system. It was also inactive against Walker 256 at the only dose level tested, 400 mg/kg. No data were available for any other compounds in the Walker 256 system.

The 6-trimethylammonium chloride derivative of 2-aminopurine 9-β-D-ribofuranoside (NSC-66380, entry No. 89035) had borderline activity in the Ca755 system and had a rating in the KB system suggestive of biologic activity. The 2-(dimethylamino) derivative of 6-aminopurine 9-β-D-ribofuranoside (NSC-36901, entry No. 89036) showed definite activity in the Ca755 system.

More data are needed for this group of compounds, particularly in the L1210 and Walker systems, to permit additional structure-activity comparisons.

27. Xanthine Nucleosides

There were eight compounds in this group (entry Nos. 89048-89055), seven of which were tested in one or more in vivo tumor systems. None elicited antitumor activity. Five were inactive against L1210: xanthine (NSC-14664, entry No. 89048); xanthosine (NSC-18930, entry No. 89049); xanthosine, 2',3',5'-triacetate (NSC-70898, entry No. 89050); xanthosine, 2',3',5'-tribenzoate (NSC-76769, entry No. 89051), and 8-(benzyloxy)xanthosine (NSC-101165, entry No. 89054).

The 2',3'-dideoxy-2'-enyl derivative of theophylline-7-D *glycero* pentopyranoside (NSC-91780, entry No. 89055) was active against KB in tissue culture but received no in vivo testing.

28. Guanine Nucleosides

The free base and 18 guanine nucleosides were tested (entry Nos. 89056-89074). The free base, guanine (NSC-3702, entry No. 89056) was inactive against the Walker 256 and Lewis lung tumors; it was not tested in the other systems. Guanosine (GR, NSC-19994, entry No. 89057), tested only at 500 mg/kg, was inactive against L1210. It was also ineffective at the dose level used against the Ca755 and S180 tumors and inactive in vitro against KB. The 2',3',5'-triacetate derivative of guanosine (NSC-66387, entry No. 89058) was also inactive in the L1210 system and failed to meet the requirements for activity in Ca755, S180, and KB.

None of the other guanine nucleosides were active in the systems in which they were tested. In general the 19 compounds in this group were relatively nontoxic. Although 9 of the compounds were inactive in the L1210 system, all but one were tested at only one dose level. It is indicated that additional data are needed on L1210 and Walker 256 in order to make further comparisons.

29. Guanine Miscellaneous

There were 14 compounds listed in this category (entry Nos. 89075-89088), of which only 2 showed antitumor activity. 8-Methoxyguanosine (8-methoxy-GR, NSC-90392, entry No. 89085) inhibited Ca755 by 80% at the one dose level tested. This compound was not tested in the other in vivo tumor systems and was inactive in the KB tissue culture system. 8-Iodoguanosine (8-iodo-GR, NSC-79218, entry No. 89080) inhibited growth of the Ca755 tumor by 74%. It too was inactive in the KB system but was not tested in the other systems.

Four compounds were tested and found to be negative in the L1210 system. None were tested against the Walker 256 or Lewis lung tumors. No activity was observed against KB.

It is suggested that more extensive testing be done with guanosine derivatives that have substituents in the 8 position.

30. 6-Thioguanine Ribosides

Forty-one 6-thioguanine ribosides plus the free base were listed (entry Nos. 89089-89130). The free base 6-thioguanine (NSC-752, entry No. 89089) was active against L1210, Walker 256, Ca755, S180 and KB. 6-Thioguanine-9-β-D-ribofuranoside (6-TGR, NSC-29422, entry No. 89090) produced a 64% increase in survival time of mice with L1210 and thus is at least as effective as 6-thioguanine and 6-mercaptopurine 9-β-D-ribofuranoside (NSC-4911, entry No. 88713). 6-Thioguanine 9-β-D-ribofuranoside was also active against Walker 256 and Ca755. It was moderately effective against S180 and was active against KB cells in tissue culture.

Some of the other 40 6-thioguanine ribosides were approximately as effective as or more effective than 6-thioguanine 9-β-D-ribofuranoside against L1210. The compounds that were active in the L1210 system included the 2',3',5'-triacetate derivative of 6-TGR (2',3',5'-triacetate 6-TGR, NSC-70389, entry No. 89092); the 2',3',5'-tripropionate derivative (2',3',5'-tripropionate 6-TGR, NSC-77556, entry No. 89093); the 2',3',5'-tributyrate derivative (2',3',5'-tributyrate 6-TGR, NSC-76955, entry No. 89094); the 2',3',5'-triisobutyrate derivative (2',3',5'-triisobutyrate 6-TGR, NSC-78307, entry No. 89095); the 2',3',5'-tribenzoate derivative (2',3',5'-tribenzoate 6-TGR, NSC-30688, entry No. 89097), which was somewhat less effective; the 2',3',5'-tris(*p*-chlorobenzoate) derivative (2',3',5'-tris(*p*-chlorobenzoate) 6-TGR, NSC-76954, entry No. 89099); the 2',3',5'-tris(*p*-nitrobenzoate) derivative [2',3',5'-tris(*p*-nitrobenzoate) 6-TGR, NSC-77424, entry No. 89101]; the 6-[(6-methyl-2-pyridyl)methyl]thio derivative (6-[(6-methyl-2-pyridyl)methyl]-TGR, NSC-46386, entry No. 89125); and the 6-(1-methyl-4-nitroimidazol-5-yl)thio derivative [6-(1-methyl-4-nitroimidazol-5-yl)-TGR, NSC-40665, entry No. 89129]. The 2 highest ratings were achieved by 2',3',5'-triacetate 6-TGR (NSC-70389, rating of 76) and 6-[(6-methyl-2-pyridyl)methyl]-TGR (NSC-46386, rating of 92), compared with a rating of 64 for 6-thioguanine 9-β-D-ribofuranoside (NSC-29422, entry No. 89090).

Introduction of a phosphate group in the 5' position of 6-thioguanine 9-β-D-ribofuranoside (5'-phosphate 6-TGR, NSC-408090, entry No. 89091) resulted in the retention of activity in KB tissue culture, but no data were available in the animal systems.

The compounds active against L1210 were, in general, active in one or more of 3 in vivo systems (Walker 256, Ca755, and S180) and with some exceptions in the in vitro KB system.

Apparently loss of activity against L1210 and other systems can occur with the introduction of some of the complex substituents. This occurred with the 2',3',5'-tripalmitate derivative of 6-TGR (NSC-78312, entry No. 89096); the 2',3',5'-tri-*p*-toluate derivative (2',3',5'-tri-*p*-toluate 6-TGR, NSC-78317, entry No. 89098); and the 2',3',5'-tri-*p*-anisate derivative (2',3',5'-tri-*p*-anisate 6-TGR, NSC-77423, entry No. 89100). The last 2 compounds, however, retained their effectiveness against Ca755.

Despite the fact that one of the compounds with a substitution for hydrogen in the SH group of the purine moiety (6-[(6-methyl-2-pyridyl)methyl]-TGR, NSC-46386, entry No. 89125) had markedly increased activity against L1210, a number of similarly substituted analogs of 6-TGR (entry Nos. 89104-89115, 89117-89123, 89126-89128) were not tested in the L1210 system. That such testing should be done is emphasized by the observation that almost all of these analogs were active in the Ca755 system. Those substituents which conferred activity in the Ca755 system were: a 6-methylthio (6-methyl-TGR, NSC-38727, entry No. 89105); a 6-ethylthio (6-ethyl-TGR, NSC-55469, entry No. 89106); a 6-isopropylthio (6-isopropyl-TGR, NSC-47785, entry No. 89108); a 6-butylthio (6-butyl-TGR, NSC-46391, entry No. 89109); a 6-*sec*-butylthio (6-*sec*-butyl-TGR, NSC-55468, entry No. 89110); a 6-isobutylthio (6-isobutyl-TGR, NSC-49815, entry No. 89111); a 6-benzylthio (6-benzyl-TGR, NSC-31730, entry No. 89112); a 6-(*o*-fluorobenzyl)thio [6-(*o*-fluorobenzyl)-TGR, NSC-54265, entry No. 89114]; a 6-(*p*-fluorobenzyl)thio [6-(*p*-fluorobenzyl)-TGR, NSC-54266, entry No. 89115]; a 6-(*o*-chlorobenzyl)-thio [6-(*o*-chlorobenzyl)-TGR, NSC-40666, entry No. 89116]; a 6-(*p*-chlorobenzyl)thio [6-(*p*-chlorobenzyl)-TGR, NSC-42376, entry No. 89117]; a 6-(2,4-dichlorobenzyl)thio [6-(2,4-dichlorobenzyl)-TGR, NSC-42377, entry No. 89118]; a 6-(*m*-bromobenzyl)thio [6-(*m*-bromobenzyl)-TGR, NSC-54264, entry No. 89119]; a 6-(*p*-bromobenzyl)thio [6-(*p*-bromobenzyl)-TGR, NSC-54263, entry No. 89120]; a 6-phenethylthio (6-phenethyl-TGR, NSC-46392, entry No. 89121); a 6-(*o*-nitrobenzyl)thio [6-(*o*-nitrobenzyl)-TGR, NSC-49814, entry No. 89122]; a 6-(*p*-nitrobenzyl)thio [6-(*p*-nitrobenzyl)-TGR, NSC-49813, entry No. 89123]; a 6-(2-pyridylmethyl)thio [6-(2-pyridylmethyl)-TGR, NSC-46385, entry No. 89124]; a 6-[(6-methyl-2-pyridyl)methyl]thio (6-[(6-methyl-2-pyridyl)methyl]-TGR, NSC-46386, entry No. 89125); a 6-(3-pyridylmethyl)thio [6-(3-pyridylmethyl)-TGR,

NSC-53349, entry No. 89126]; a 6-phenacylthio [(6-phenacyl)-TGR, NSC-46390, entry No. 89127]; a 6-(*p*-chlorophenacyl)thio [6-(*p*-chlorophenacyl)-TGR, NSC-46389, entry No. 89128]; and a 6-(1-methyl-4-nitroimidazol-5-yl)thio [6-(1-methyl-4-nitroimidazol-5-yl)-TGR, NSC-40665, entry No. 89129].

31. 6-Thioguanine (Nonribose) Nucleosides

There were only 7 compounds in this series (entry Nos. 89131-89137). 2'-Deoxy-6-thioguanine 9-β-D-ribofuranoside (2'-deoxy-6-TGR, NSC-71261, entry No. 89131) was definitely active in the L1210 and Walker 256 systems and also inhibited KB cells in tissue culture. This compound had a higher rating than 6-thioguanine 9-β-D-ribofuranoside (6-TGR, NSC-29422, entry No. 89090) in the L1210 system. 2'-Deoxy-6-thioguanine 9-α-D-ribofuranoside (NSC-71851, entry No. 89132) was about as effective as 2'-deoxy-6-TGR in the L1210, Walker, and KB systems.

2'-Deoxy-6-benzylthioguanine 9-β-D-ribofuranoside (2'-deoxy-6-benzyl-TGR, NSC-71262, entry No. 89133) was inactive against KB and Walker 256, but it too had an activity rating against L1210 that was somewhat higher than the rating for 6-TGR.

3'-Deoxy-6-thioguanine 9-β-D-ribofuranoside (3'-deoxy-6-TGR, NSC-102635, entry No. 89136), though tested only as a single injection, also was quite active in the L1210 system. The 6-benzylthio derivative of this compound (3'-deoxy-6-benzyl-TGR, NSC-103063, entry No. 89137) had somewhat diminished activity against L1210 but was nevertheless still positive. The anti-L1210 activity of these nonribose sugar analogs of 6-thioguanine warrant additional study.

32. 6-Thioguanine: Nonsugar Analogs

There were only 2 compounds (entry Nos. 89138 and 89139) in this group, both of which were 9-(tetrahydro-2-furyl) derivatives of 6-thioguanine [9-(tetrahydro-2-furyl)-6-TG and 9-(tetrahydro-2-furyl)-6-benzyl-TG]. Both were active in the Ca755 system and inactive in the L1210 system; however, the testing against L1210 was probably inadequate. No data were available for the Walker 256 or the Lewis lung tumor. In the KB system there was no definite evidence of activity.

33. 6-Unsubstituted Purine Nucleosides

The free base, purine (NSC-753, entry No. 89140), was inactive against L1210, Walker 256, Ca755, and S180. Of the 12 purine nucleosides in this group (entry Nos. 89140-89152), only 2 were tested against L1210, and both were inactive. Where tested in the other systems, none of the compounds showed evidence of activity. There are wide gaps in the test data.

34. Pyrrolopyrimidine Ribofuranosides (7-Deazapurine Ribofuranosides)

There were 19 compounds in this group (entry Nos. 89153-89171), and all were tested against L1210. Only 2 were active in the L1210 system: sangivamycin (NSC-65346, entry No. 89167) and thiosangivamycin (NSC-105827, entry No. 89168). Sangivamycin was active in the KB system as well, but was inactive against the Ca755, S180, and Lewis lung tumors. Thiosangivamycin was inactive against Walker 256.

4-Aminopyrrolo[2,3-*d*]pyrimidine 7-β-D-ribofuranoside (tubercidin, NSC-56408, entry No. 89153) and 4-amino-5-carbonitrilepyrrolo[2,3-*d*]pyrimidine 7-β-D-ribofuranoside (toyocamycin, NSC-63701, entry No. 89166) were the only other active compounds in this series. Both compounds were active against KB but inactive against the Ca755, S180, and Lewis lung tumors.

Twelve compounds, including thiosangivamycin (NSC-105827, entry No. 89168) were tested against Walker 256, and no definite activity was noted.

In view of the activity of sangivamycin and its thio derivative and the general interest in 7-deaza compounds, additional studies are warranted.

35. Imidazole Ribofuranosides

None of the 15 compounds in this series (entry Nos. 89172-89186) were rated as active in the systems in which they were tested. There were, however, large gaps in the data and much of the testing was done at only one dose level.

36. Miscellaneous Nucleosides

There were 12 miscellaneous nucleosides (entry Nos. 89187-89198). Eleven were tested in the L1210 system and 3 were found to be active. 4-Amino-*s*-triazin-2-one 1-β-D-ribofuranoside (5-azacytidine, NSC-102816, entry No. 89189) was highly active—about 3 times as effective against L1210 as 6-mercaptopurine 9-β-D-ribofuranoside (NSC-4911, entry No. 88713), and about twice as effective as 2'-deoxy-6-thioguanine 9-β-D-ribofuranoside (NSC-71261, entry No. 89131). 5-Azacytidine received a rating of 68 against the Walker 256 tumor which was below the level of acceptance; it was not tested in the other systems.

as-Triazine-3,5-dione 2-β-D-ribofuranoside (6-azauridine, NSC-32074, entry No. 89187) was moderately active against L1210, but showed no definite evidence of activity in the other in vivo animal tumor systems. It was, however, active against KB in tissue culture. The 2', 3', 5'-triacetate derivative of 6-azauridine (2', 3', 5'-triacetate 6-azauridine, NSC-67239, entry No. 89188) was possibly more active against L1210 than the nonacetylated compound. In contrast to the parent compound the acetylated derivative was relatively inactive against KB.

The only other active compound in this category was 6-methylpurine 9-β-D-ribofuranoside (6-methyl-PR, NSC-101619, entry No. 89194). This compound was active against KB, but was not tested in vivo.

In view of the extensive activity of 5-azacytidine (NSC-102816, entry No. 89189) against L1210 it is suggested that comprehensive studies be done with this compound and with other *s*-triazine or *s*-triazinone derivatives.

DISCUSSION

Structure-Activity Relations with Respect to Chemotherapeutic Activity Against Leukemia L1210

In view of the interest in compounds active against leukemia L1210 in relation to potential clinical activity (1, 18) it is of value to review the structure-activity relations in this system.

Pyrimidines.—The only base-sugar combination that had definite activity against L1210 without further substitution in the base or sugar moieties was cytosine 1-β-D-arabinofuranoside (Ara-C, NSC-63878, entry No. 88635). The other natural base evaluated, uracil, failed to show activity when combined with arabinose (uracil 1-β-D-arabinofuranoside, NSC-68928, entry No. 88597).

Alteration of the structure has a pronounced influence on activity. The importance of selective substitution in the 5 position of the ring is a prime example. Substitution of fluorine, creating an electron-rich center, caused significant activity against L1210 for the following compounds: 5-fluorouridine (34), 2'-deoxy-5-fluorouridine (NSC-27640, entry No. 88576), 5-fluorocytosine 1-β-D-arabinofuranoside (5-fluoro-Ara-C, NSC-529180, entry No. 88639), and 2'-deoxy-5-fluorocytidine (NSC-48006, entry No. 88649).

In similar fashion the 5-trifluoromethyl substituent in 2'-deoxy-5-trifluoromethyluridine (F_3TDR, NSC-75520, entry No. 88595) brought about definite activity against L1210.

The introduction of fluorine at the 5 position per se did not necessarily lead to activity. It did not result in antileukemic activity in the case of 5-fluorouracil 1-β-D-arabinofuranoside (NSC-406444, entry No. 88599) but this compound was tested only as a single injection.

Substitution in the sugar moiety may lead to inactivation as seen in a block of the 5' position despite the presence of a 5-fluoro substituent. This apparently occurred when a carboxyl group was substituted for the alcohol moiety as in the 2'-deoxy-ribofuranuronic acid derivative of 2'-deoxy-5-fluorouridine (NSC-103704, entry No. 88583). In this connection it would be of interest to substitute a methyl group at the 5' position to determine whether it too would give an inactive compound, since in both cases there would be a block of phosphorylation.

Halogenation at the 5 position with bromine (5-bromo-2'-deoxyuridine, NSC-38297, entry No. 88585) or iodine (2'-deoxy-5-iodouridine, NSC-39661, entry No. 88587), resulted in reduced activity relative to the fluorinated derivatives.

5-Diazo substitution resulted in definite activity in the case of 5-diazouridine (NSC-70390, entry No. 88566) and suggestive activity for 2'-deoxy-5-diazouridine (NSC-70900, entry No. 88591). Other substituents at the 5 position which gave partial activity were oxygen, as in isobarbituric acid 1-β-D-ribofuranoside (NSC-73376, entry No. 88560) and an allyl group, as in 5-allyl-2'-deoxyuridine (NSC-80805, entry No. 88593).

Those substituents at position 5 which did not elicit activity were: an amino group (5-amino-2'-deoxyuridine, NSC-97433, entry No. 88590); a morpholino group (5-morpholinouridine, NSC-79073, entry No. 88563); and a dimethylamino group [5-(dimethylamino)uridine, NSC-75792, entry No. 88564].

For base-sugar combinations that were inactive per se, acetylation of the sugar failed to impart activity, eg, the 2',3',5'-triacetate derivative of uracil 1-β-D-arabinofuranoside (NSC 79269, entry No. 88598). On the other hand, acetylation of active derivatives resulted in retention of activity, eg, the 2',3',5'-triacetate derivative of cytosine 1-β-D-arabinofuranoside (NSC-93150, entry No. 88636). In fact, additional acetylation on the nitrogen as in *N*-acetyl-2',3',5'-triacetylcytosine 1-β-D-arabinofuranoside (NSC-92717, entry No. 88637) may have resulted in an actual enhancement of activity.

Although the introduction of 5'-phosphate per se into the sugar moiety failed to impart activity to inactive pyrimidine nucleosides, in one instance, the substitution of a more complex 5'-pyrophosphate moiety into an inactive base-sugar combination did result in a compound with moderate activity, ie, the 5'-pyrophosphate glucosyl ester of uridine (NSC-20269, entry No. 88570). For active compounds the 5'-phosphate moiety did not interfere with activity and may have even increased the activity, as with the 5'-phosphate derivative of cytosine 1-β-D-arabinofuranoside (NSC-99445, entry No. 88638).

The activity appears to reside only in the beta configuration of the 2'-deoxypyrimidine nucleosides. Despite the substitution of a fluorine in the 5 position of an alpha nucleoside (2'-deoxy-5-fluorouracil 1-α-D-ribofuranoside, NSC-66259, entry No. 88575) the compound was inactive.

Although cytidine (NSC-20258, entry No. 88641) was ineffective against L1210, the introduction of an additional nitrogen into the ring in the 5 position, forming 5-azacytidine (NSC-102816, entry No. 89189), resulted in a compound with very extensive L1210 activity. Similarly the introduction into the ring of an additional nitrogen in the 6 position of uridine, forming 6-azauridine (NSC-32074, entry No. 89187), resulted in a compound with moderate activity against L1210. The 2',3',5'-triacetate derivative of 6-azauridine (NSC-67239, entry No. 89188) also was active.

Purine nucleosides.—Purine (NSC-753, entry No. 89140) and purine 9-β-D-ribofuranoside (NSC-65423, entry No. 89141) were inactive against L1210.

The importance of appropriate substitution in the 6 position of the base is well illustrated with SH substitution at the 6 position as in 6-mercaptopurine (6-MP, NSC-755, entry No. 88712) and the 6-mercaptopurine nucleosides. Not only was activity noted with 6-MP alone, but it also was noted for the following 6-MP derivatives: 6-mercaptopurine 9-β-D-ribofuranoside (6-MP, NSC-4911, entry No. 88713); 2'-deoxy-6-MPR (NSC-409366, entry No. 88771); 6-MP 9-β-D-arabinofuranoside (NSC-406021, entry No. 88773); 9-(tetrahydro-2-furyl)-6-MP (NSC-45153, entry No. 88790); and 9-(tetrahydro-2-pyranyl)-6-MP (NSC-33186, entry No. 88794).

The 6-sulfhydryl substitution accompanied by a 2-amino substituent also results in antileukemic activity. This was noted with 6-thioguanine itself (NSC-752, entry No. 89089), 6-thioguanine 9-β-D-ribofuranoside (6-TGR, NSC-29422, entry No. 89090) and 2'-deoxy-6-TGR (NSC-71261, entry No. 89131). However, substitution of an amino group in the 2 position without the presence of an appropriate substituent such as SH at the 6 position failed to impart activity, eg, 2-aminopurine 9-β-D-ribofuranoside (NSC-36906, entry No. 89147), guanosine (NSC-19994, entry No. 89057), and 2'-deoxyguanosine (NSC-22837, entry No. 89068).

Substitution of a hydroxyl group at the 6 position on the purine, eg, hypoxanthine alone (NSC-14665, entry No. 88679), inosine (NSC-20262, entry No. 88680), hypoxanthine 9-β-D-arabinofuranoside (NSC-405122, entry No. 88695), or a D-glucitol derivative of hypoxanthine (NSC-78412, entry No. 88709) failed to impart activity in all but one instance, ie, a complex 5'-diphosphate codehydrogenase derivative (NSC-20271, entry No. 88687).

The phosphorylation of hypoxanthine nucleosides appears to impart some activity, as noted for the complex codehydrogenase derivative and also for the 5'-phosphate 9-β-D-ribofuranoside (NSC-20263, entry No. 88685) and the 5'-triphosphate 9-β-D-ribofuranoside (NSC-20266, entry No. 88686).

Substitution of a hydroxyl group in both the 2 and 6 positions as in xanthine (NSC-14664, entry No. 89048), and xanthosine (NSC-18930, entry No. 89049) failed to impart activity. As already mentioned 2-amino plus 6-hydroxy substitution, as in guanosine and 2'-deoxyguanosine, also failed to impart activity.

Substitution of chlorine at the 6 position of purine (6-chloropurine, NSC-744, entry No. 88805) did result in antileukemic activity. But there was no activity noted for 6-chloropurine 9-β-D-ribofuranoside (NSC-4910, entry No. 88806). Interestingly, however, 9-(tetrahydro-2-pyranyl)-6-chloropurine (NSC-33187, entry No. 88830) did have an active rating.

The 6-bromo and 6-iodo substituents failed to impart activity for 6-bromopurine 9-β-D-ribofuranoside (NSC-62630, entry No. 88808) and for 6-iodopurine 9-β-D-ribofuranoside (NSC-66384, entry No. 88809). However, with 9-(tetrahydro-2-pyranyl)-6-iodopurine (NSC-33188, entry No. 88836) a rating was obtained that was suggestive of activity. This had also been observed with 9-(tetrahydro-2-pyranyl)-6-mercaptopurine (NSC-33186, entry No. 88794). Thus other sugars or cyclic compounds besides ribofuranose, deoxyribofuranose, and arabinofuranose may yield compounds which are active, eg, glucopyranose, tetrahydrofuran, and tetrahydropyran.

In one instance a nucleoside with the alpha configuration was particularly active: 2'-deoxy-6-thioguanine 9-α-D-ribofuranoside (NSC-71851, entry No. 89132). This is unusual, since the alpha derivatives as a rule have been quite ineffective.

In two instances 3'-deoxyribofuranosides were active: 3'-deoxy-6-thioguanine 9-β-D-ribofuranoside (NSC-102635, entry No. 89136) and 3'-deoxy-6-benzylthioguanine 9-β-D-ribofuranoside (NSC-103063, entry No. 89137). The first compound was more effective than either 6-thioguanine alone or 6-thioguanine 9-β-D-ribofuranoside. Both of the 3'-deoxy derivatives were administered as single injections; more activity might have been elicited had the treatment been extended.

Substitution of an amino group at the 6 position of purine did not cause activity in the case of adenosine (NSC-7652, entry No. 88840), 2'-deoxyadenosine (NSC-83258, entry No. 88905), adenine 9-β-D-arabinofuranoside (NSC-404241, entry No. 88930), adenine 9-β-D-xylofuranoside (NSC-7359, entry No. 88962), or in several substituted tetrahydrofuryl and tetrahydropyranyl derivatives. However, definite activity was noted with a hydrazino group (6-hydrazinopurine 9-β-D-ribofuranoside, NSC-29408, entry No. 88866), or a dimethylamino group (3'-amino-3'-deoxy-*N*,*N*-dimethyladenosine, NSC-3056, entry No. 88875) at the 6 position.

Also, several derivatives with substitution at the 6 position of purine appeared to have partial activity, eg, the 6-(aziridinyl) (NSC-39091, entry No. 88870), and 6-(trimethylammonium) chloride (NSC-66382, entry No. 88872) derivatives of purine 9-β-D-ribofuranoside.

The hydrazino substituent may have to be at the 6 position; when it was substituted at the 8 position as in 8-hydrazinoadenosine (NSC-95945, entry No. 88897) no activity was observed. Also, despite the activity of a 6-(dimethylamino) derivative (3'-amino-3'-deoxy-*N*,*N*-dimethyladenosine), an 8-(dimethylamino) derivative [8-(dimethylamino)adenosine, NSC-101164, entry No. 88896] was inactive.

In the two active series of derivatives of 6-MP 9-β-D-ribofuranoside (6-MPR) and 6-thioguanine 9-β-D-ribofuranoside (6-TGR), activity was noted on substitution for the hydrogen in the sulfhydryl group and for substitution in the ribose sugar. Activity was noted with 6-(methylthio)purine 9-β-D-ribofuranoside (6-methyl-MPR, NSC-40774, entry No. 88747), 6-allyl-MPR (NSC-39367, entry No. 88751), and 6-acetonyl-MPR (NSC-39045, entry No. 88757). The 6-allylthio derivative of 6-MPR was possibly more effective than 6-MPR itself, but further introduction of a phenyl group yielding the 6-(cinnamylthio) derivative (NSC-39043, entry No. 88756) led to a decrease in activity.

The introduction of a 6-[(6-methyl-2-pyridyl)methyl]thio group in the case of 6-[(6-methyl-2-pyridyl)-methyl]thioguanine 9-β-D-ribofuranoside (NSC-46386, entry No. 89125) resulted in marked activity. Also, the introduction of a (1-methyl-4-nitroimidazol-5-yl)thio group at the 6 position (NSC-40665, entry No. 89129) resulted in definite antileukemic activity.

However, some of the substitutions for H in the 6-sulfhydryl group of 6-MPR and 6-TGR caused reduced activity, eg, 6-cyanomethyl-MPR (NSC-39848, entry No. 88759) and 6-(2-pyridylmethyl)-TGR (NSC-46385, entry No. 89124).

Where the substituents were on the sugar moiety of 6-MPR, the 2', 3', 5'-triacetate (NSC-66385, entry No. 88715), 2', 3', 5'-tributyrate (NSC-76952, entry No. 88718), 2', 3', 5'-trivalerate (NSC-77495, entry No. 88717), 2', 3', 5'-tribenzoate (NSC-28416, entry No. 88722), and 2', 3', 5'-tris(*p*-nitrobenzoate) (NSC-76126, entry No. 88725) derivatives were all active. In the 6-TGR series the 2', 3', 5'-triacetate (NSC-70389, entry No. 89092), 2', 3', 5'-tributyrate (NSC-76955, entry No. 89094), 2', 3', 5'-triisobutyrate (NSC-78307, entry No. 89095), 2', 3', 5'-tribenzoate (NSC-30688, entry No. 89097), 2', 3', 5'-tris(*p*-chlorobenzoate) (NSC-76954,

entry No. 89099), and 2', 3', 5'-tris(*p*-nitrobenzoate) (NSC-77424, entry No. 89101) derivatives were all active. Some of the more complex substituents on the ribofuranose ring resulted in a loss of activity: the 2', 3', 5'-tris(*p*-chlorobenzoate) (NSC-76520, entry No. 88724), 2', 3', 5'-trilaurate (NSC-76123, entry No. 88719), 2', 3', 5'-trioleate (NSC-76124, entry No. 88720), and 2', 3', 5'-tristearate (NSC-76949, entry No. 88721) derivatives of 6-MPR; and the 2', 3', 5'-tripalmitate (NSC-78312, entry No. 89096), 2', 3', 5'-tri-*p*-toluate (NSC-78317, entry No. 89098) and 2', 3', 5'-tri-*p*-anisate (NSC-77423, entry No. 89100) derivatives of 6-TGR.

The data stress the potential of 7-deazapurine ribofuranosides (purine numbering being retained). Although adenosine was ineffective against L1210, 7-deazaadenosine (tubercidin, NSC-56408, entry No. 89153) had a rating suggestive of activity. The introduction of a carbamoyl group at the 7 position of 7-deazaadenosine as in sangivamycin (NSC-65346, entry No. 89167) or of a thiocarbamoyl group at that position, as in thiosangivamycin (NSC-105827, entry No. 89168), imparted activity. Also, the introduction of a carbonitrile group at the 7 position (toyocamycin, NSC-63701, entry No. 89166) yielded moderate activity.

6-Thio-7-deazapurine 9-β-D-ribofuranoside (NSC-100278, entry No. 89160) had reduced activity relative to 6-MPR. Also, whereas 3'-amino-3'-deoxy-*N*,*N*-dimethyladenosine was active, *N*,*N*-(dimethylamino)-7-deazaadenosine (NSC-100279, entry No. 89155) was inactive. Thus, neither the 6-sulfhydryl nor the 6-(dimethylamino) substituents enhanced the activity of those 7-deazapurine nucleosides studied.

A 6-aminopurine derivative, formycin (NSC-102811, entry No. 89196), in which the 9-nitrogen has been moved to the 8 position (purine numbering being retained), had suggestive activity in the L1210 system. It should also be noted that the sugar, ribofuranose, is still attached at the 9 position, but by a carbon-to-carbon linkage instead of by the usual nitrogen-to-carbon linkage. Additional examples of "C-nucleosides" should be prepared and evaluated.

Thus, compounds active against L1210 were found in the groupings: uracil nucleosides, cytosine nucleosides, azacytosine nucleosides, hypoxanthine nucleosides, 6-mercaptopurine nucleosides, 6-chloropurine nucleosides, adenine nucleosides, thioguanine nucleosides, and 7-deazapurine nucleosides. Active compounds were obtained with ribofuranose, deoxyribofuranose, arabinofuranose, glucopyranose, tetrahydrofuran, and tetrahydropyran, as the sugar moiety in the nucleoside.

Many base-sugar groupings had few or no representatives tested.

Structure-Activity Relations with Respect to Clinical Interest

Purines and pyrimidines and their nucleoside derivatives are of interest for biologic investigation and for treating patients with cancer. Important clinical studies have been done with drugs such as 5-fluorouracil and 2'-deoxy-5-fluorouridine (35-40, 44, 45), 6-mercaptopurine and 6-mercaptopurine 9-β-D-ribofuranoside (46-48), and 6-thioguanine and 6-thioguanine 9-β-D-ribofuranoside (49-51).

This compilation of CCNSC data emphasizes that the number of purine and pyrimidine nucleosides that qualify as active biologically in the tumor screens far exceeds the number of compounds in which there has been clinical interest; table 3 summarizes the structure-activity relations of nucleoside derivatives of biologic and clinical interest. The table includes a compilation of all of the purine and pyrimidine nucleosides that were active in one or more of the screening systems and indicates the clinical activity. Corresponding free bases and inactive nucleosides have been included for purposes of comparison. Also included are clinically active nucleosides that were negative in the experimental screens.

Group 1.—The uracil ribosides have no clinically active compounds listed, whereas one compound is active biologically, namely, 5-diazouridine (NSC-70390, entry No. 88566), which was active in the L1210 system. The corresponding free base 5-diazouracil had no significant antitumor effects in patients (52-53).

Group 2.—In addition to the clinically established free base 5-fluorouracil (5-FU, NSC-19893, entry No. 88573) (35-39) the uracil 2'-deoxyribosides have 3 clinically active compounds: 2'-deoxy-5-fluorouridine (5-FUDR, NSC-27640, entry No. 88576) (40-45,54-62); 2'-deoxy-5-iodouridine (5-IUDR, NSC-39661, entry No. 88587) (63-69); 2'-deoxy-5-bromouridine (5-BUDR, NSC-38297, entry No. 88585) (70-74). 2'-Deoxy-5-trifluoromethyluridine (F_3TDR, NSC-75520, entry No. 88595 (75-79) has recently been entered in a preliminary clinical trial. 5-BUDR was not active in any of the tumor screens presented here. One of the compounds in this group, the 5-bromo-5, 6-dihydro-6-methoxy derivative of 5-FUDR (5-bromo-5, 6-dihydro-6-methoxy-5-FUDR, NSC-80870, entry No. 88584) was active in both the Lewis lung and KB tissue culture systems, but

biologic data were not available for the other in vivo tumor systems. Its activity in the Lewis lung system compares favorably with that observed for 5-FUDR. There are a number of important review articles on 5-FU and its congeners (5, 6, 17, 80–95).

Group 6. —The cytosine furanosides have 3 drugs of clinical interest, namely, cytosine 1-β-D-arabinofuranoside (Ara-C, NSC-63878, entry No. 88635) (7, 96–107), its 2', 3', 5'-triacetate derivative (2',3',5'-triacetate Ara-C, NSC-93150, entry No. 88636) (108), and 5-fluorocytosine 1-β-D-arabinofuranoside (5-fluoro-Ara-C, NSC-529180, entry No. 88639) (109–111). Additional compounds in this category that were active in the biologic systems include the 2', 3', 5'-triacetate derivative of *N*-acetyl-Ara-C (NSC-92717, entry No. 88637) (108), 5'-phosphate Ara-C (NSC-99445, entry No. 88638) (30), and 2'-deoxy-5-fluorocytidine (NSC-48006, entry No. 88649) (112–114), all of which were active in the L1210 system. In addition there was one compound, 5-bromo-2'-deoxycytidine (NSC-61765, entry No. 88650) (115, 116) that was active in the Ca755 system. These and related 2'-deoxycytidine analogs such as the 5-iodo derivative (117) would be worthy of additional study.

Group 11. —The hypoxanthine ribosides have no clinical representatives. One compound, a complex codehydrogenase I derivative (NSC-20271, entry No. 88687), did show marginal activity against L1210.

Group 14. —In addition to the free base 6-mercaptopurine (6-MP, NSC-755, entry No. 88712 (46, 118–123), the 6-mercaptopurine ribosides include 2 compounds of clinical importance, 6-mercaptopurine 9-β-D-ribofuranoside (6-MPR, NSC-4911, entry No. 88713) (124–130) and 6-methyl-MPR (NSC-40774, entry No. 88747) (131–133). Ten additional compounds were active against L1210, 16 were active against the Ca755, and 9 others were active against KB. 6-Mercaptopurine ribosides active in the L1210 system include compounds with substitutions in the ribose moiety and compounds with substitutions for hydrogen in the 6-sulfhydryl moiety. Thus the 2', 3', 5'-triacetate (NSC-66385, entry No. 88715), 2', 3', 5'-tributyrate (NSC-76952, entry No. 88718), 2', 3', 5'-trivalerate (NSC-77495, entry No. 88717), 2', 3', 5'-tribenzoate (NSC-28416, entry No. 88722), and 2', 3', 5'-tris(*p*-nitrobenzoate) (NSC-76126, entry No. 88725) derivatives, as well as the 5'-phosphate butyl ester (NSC-45635, entry No. 88733), 5'-phosphate diethyl ester (NSC-40634, entry No. 88734), and 5'-phosphate diphenyl ester (NSC-40632, entry No. 88739) of 6-MPR all were about as active in the L1210 system as 6-MPR alone. Similarly, in addition to the 6-methylthiopurine riboside (6-methyl-MPR), the 6-allylthio (NSC-39367, entry No. 88751) and 6-acetonylthio (NSC-39045, entry No. 88757) derivatives were active in the L1210 system. The 6-allylthio derivative did have a higher rating than 6-methyl-MPR in the L1210 system. Since a number of the compounds active against Ca755 and KB were not tested in the L1210 system, and in view of the activity of this general category of compounds, more testing of 6-mercaptopurine ribosides against L1210 is suggested. Several important articles have been published pertaining to 6-MP and its congeners (2, 80, 82, 85–87, 91, 92, 94, 134–139).

Group 15. —The one compound in the group of 6-mercaptopurine nonribose sugar analogs that is being prepared for the clinic, 6-MP 9-β-D-arabinofuranoside (6-MPAra, NSC-406021, entry No. 88773) (41,43), was also active in the L1210 system. A second compound in this group, 2'-deoxy-6-MPR (NSC-409366, entry No. 88771) was also active against L1210. In addition, one compound (6'-deoxy-6-MPAllo, NSC-409352, entry No. 88787) was active in the KB system but was not tested in vivo.

Group 16. —The 6-mercaptopurine nonsugar analogs have no representatives in the clinical listing, though 3 were active against L1210 and 9 against Ca755. The 3 compounds active against L1210 include a 9-tetrahydrofuryl derivative of 6-MP [9-(tetrahydro-2-furyl)-6-MP, NSC-45153, entry No. 88790]; a 9-tetrahydropyranyl derivative of 6-MP [9-(tetrahydro-2-pyranyl)-6-MP, NSC-33186, entry No. 88794]; and a 9-tetrahydropyranyl derivative of 6-butylmercaptopurine [9-(tetrahydro-2-pyranyl)-6-butyl-MP, NSC-38305, entry No. 88798]. These agents are currently under development. The compounds active against Ca755 would also be worth additional biologic testing, particularly since most of them have not been tested in L1210 or other systems.

Group 17. —The free base 6-chloropurine (6-ClP, NSC-744, entry No. 88805) has shown clinical activity (49, 143–146) but there are no clinically active compounds listed among the 6-halopurine nucleosides. 6-Chloropurine is the only compound that was active in the L1210 system. However, there were 2 compounds that were active against Ca755, namely, 6-chloropurine 9-β-D-ribofuranoside (6-ClPR, NSC-4910, entry No. 88806) (145) and 2-amino-6-iodopurine 9-β-D-ribofuranoside (6-IPR, NSC-66384, entry No. 88809) and these compounds may be worthy of additional investigation.

Group 18. —The nonsugar analogs of 6-halopurine have no clinical representatives. In this group there was 1 compound active against L1210, 1 compound active against Walker 256, and 7 additional compounds active against Ca755. The compound active against L1210 was NSC-33187 [9-(tetrahydro-2-pyranyl)-6-ClP, entry No. 88830].

Group 19. —The adenine ribosides include 2 compounds in the clinical category, which as yet have not demonstrated definite therapeutic value. One of these, 2-fluoroadenosine (2-fluoro-AdR, NSC-30605, entry No. 88888) (126, 147) was active only in the KB system. The second, puromycin (NSC-3055, entry No. 88876) (143, 148), was partially active against Ca755 and inhibited KB in tissue culture.

Two compounds which have not been evaluated clinically were active in the L1210 system: 6-hydrazinopurine 9-β-D-ribofuranoside (6-hydrazino-PR, NSC-29408, entry No. 88866) and 3'-amino-3'-deoxy-*N*,*N*-dimethyladenosine (3'-amino-3'-deoxy-*N*,*N*-dimethyl-AdR, NSC-3056, entry No. 88875). Two other compounds were active in the Ca755 system and 6 others were active in the KB system.

Group 24. —The nonsugar analogs of adenine include no clinically active compounds, but 3 compounds were active in the Ca755 system. These were all 9-tetrahydrofuryl or 9-tetrahydropyranyl derivatives of 6-trimethylammonium chloride. Their marked activity in the Ca755 system suggests that additional attention be focused on this type of compound.

Group 25. —Among the miscellaneous adenines there were no clinical representatives. One compound, a cyclopentanemethanol derivative of adenine (NSC-103526, entry No. 89032), was active in the KB system, but was inactive in L1210 and was not tested in the other systems.

Group 26. —Although 2,6-diaminopurine (NSC-743, entry No. 89033) has shown clinical as well as biologic activity (22, 49, 80, 149-151), the 2,6-diaminopurine nucleoside group has no compounds in the clinical category. Two compounds were active against Ca755: a 6-trimethylammonium chloride derivative of 2-aminopurine 9-β-D-ribofuranoside (NSC-66380, entry No. 89035), and a 2-(dimethylamino) derivative of 6-aminopurine 9-β-D-ribofuranoside [2-(dimethylamino)-6-amino-PR, NSC-36901, entry No. 89036]. It would be of interest to determine the activity of these compounds against L1210 and Walker 256. 2,6-Diaminopurine 9-β-D-ribofuranoside itself (2,6-diamino-PR, NSC-7363, entry No. 89034) though inactive against L1210, was not tested in the Walker 256 system.

Group 27. —In the xanthine nucleoside series there were no clinically active compounds but there was one compound, a theophylline derivative (NSC-91780, entry No. 89055), which was active in the KB system. This compound was not tested in the in vivo systems.

Group 29. —The group of miscellaneous guanine compounds has no clinically active agents but does have 2 compounds that were active in the Ca755 system: 8-iodoguanosine (8-iodo-GR, NSC-79218, entry No. 89080) and 8-methoxyguanosine (8-methoxy-GR, NSC-90392, entry No. 89085).

Group 30. —The free base 6-thioguanine (6-TG, NSC-752, entry No. 89089) is active clinically (49) and in the L1210, Walker 256, Ca755, and KB systems. The group of 6-thioguanine ribosides includes the clinically active drug 6-thioguanine 9-β-D-ribofuranoside (6-TGR, NSC-29422, entry No. 89090) (18, 50, 51, 152). This group has 10 compounds that were active against L1210, 22 others that were active against Ca755, and 1 (NSC-408090, entry No. 89091) that was active against KB. The compounds active against L1210 included the 2', 3', 5'-triacetate (NSC-70389, entry No. 89092); 2', 3', 5'-tripropionate (NSC-77556, entry No. 89093); 2', 3', 5'-tributyrate (NSC-76955, entry No. 89094); 2', 3', 5'-triisobutyrate (NSC-78307, entry No. 89095); 2', 3', 5'-tribenzoate (NSC-30688, entry No. 89097); 2', 3', 5'-tris(*p*-chlorobenzoate) (NSC-76954, entry No. 89099); and 2', 3',5'-tris(*p*-nitrobenzoate) (NSC-77424, entry No. 89101) derivatives of 6-thioguanine 9-β-D-ribofuranoside. There were also 2 compounds involving substitutions for hydrogen in the sulfhydryl group. One of these, 6-[(6-methyl-2-pyridyl)methyl]thioguanine 9-β-D-ribofuranoside (NSC-46386, entry No. 89125) was particularly active in the L1210 system. The other compound, 6-(1-methyl-4-nitroimidazol-5-yl)-thioguanine 9-β-D-ribofuranoside (NSC-40665, entry No. 89129) had moderate activity. The large number of compounds active in the L1210 and Ca755 systems indicates that this category of compounds is worthy of more intensive investigation. Review articles may be consulted for detailed studies on 6-thioguanine and related compounds (49, 80, 82, 87, 88, 92, 135, 136, 153).

Group 31. —The group of 6-thioguanine nonribose nucleosides has 2 compounds, 2'-deoxy-6-thioguanine 9-β-D-ribofuranoside (2'-deoxy-6-TGR, NSC-71261, entry No. 89131), and the 2'-deoxy-9-α-D-ribofuranoside (NSC-71851, entry No. 89132) (154-156), which are undergoing preclinical evaluation. These compounds and a 2'-deoxy-6-benzylthio derivative of guanine 9-β-D-ribofuranoside (2'-deoxy-6-benzyl-TGR, NSC-71262, entry No. 89133) were active in the L1210 system. 3'-Deoxy-6-thioguanine 9-β-D-ribofuranoside (3'-deoxy-6-TGR, NSC-102635, entry No. 89136) and a 3'-deoxy-6-benzylthio derivative (3'-deoxy-6-benzyl-TGR, NSC-103063, entry No. 89137) also showed definite L1210 activity. These compounds deserve additional attention.

Group 32. —There were no nonsugar analogs of 6-thioguanine in the clinical category. Both of the compounds in this group were quite active against Ca755; both were 9-(tetrahydro-2-furyl) derivatives of 6-thioguanine.

Group 34. —Three compounds in the 7-deazapurine ribofuranoside group are listed in the clinical category. Tubercidin (NSC-56408, entry No. 89153) (8, 9, 157-162) has shown moderate clinical activity. Toyocamycin (NSC-63701, entry No. 89166) (9) has shown no clinical activity but its evaluation has been limited by host toxicity. Sangivamycin (NSC-65346, entry No. 89167) (9) which is active against L1210 has recently entered clinical trial (163). It too may have serious toxic limitations. Thiosangivamycin (NSC-105827, entry No. 89168) was also active against L1210 but has not been tested clinically. Another deaza derivative, 7-deazainosine, has been reported to inhibit the growth of S180 in vitro (164).

Group 36. —The miscellaneous nucleosides group includes 3 azapyrimidines (165), namely, 6-azauridine (NSC-32074, entry No. 89187) (69, 80, 82, 84, 87, 88, 91, 92, 95, 138, 166-174); 2',3',5'-triacetate 6-azauridine (NSC-67239, entry No. 89188) (175), and 5-azacytidine (NSC-102816, entry No. 89189) (176-178), which are in the clinical category. All 3 compounds were active in the L1210 system. An additional compound, 6-methylpurine 9-β-D-ribofuranoside (6-methyl-PR, NSC-101619, entry No. 89194), was active in the KB system. Additional azapyrimidines of interest reported elsewhere include 6-azacytosine (179), 6-azacytidine (180), 6-azathymine (181, 182), and 5-azaorotic acid (183).

It is evident that there are a considerable number of nucleoside derivatives of both purine and pyrimidine that are of potential clinical interest, and many of them are undoubtedly worthy of considerable additional attention for purposes of development for the clinic.

The 21 nucleoside derivatives of clinical interest are listed in table 4 along with their ratings in the 5 in vivo screens and the KB tissue culture screen. Sixteen of these compounds were active in the L1210 screening system. These 16 compounds have the clinical designation, +, ±, or P indicating that they are active or moderately active in the clinic or are being processed for clinical use. Four of the 21 compounds were active only in the KB system. One of these is listed as ± with respect to clinical activity and 3 are listed as negative or too toxic for clinical use. One of the compounds was not active in any screening system and is listed as ± for the clinic. The 21 compounds of clinical interest appeared in 9 of the 36 structural groups listed in this report. Group 2, the uracil 2'-deoxyribosides, had 4 clinically interesting compounds. Group 6, the cytosine furanosides; group 34, the 7-deazapurine nucleosides; and group 36, the miscellaneous nucleosides, each had 3 compounds listed. These data are in agreement with the findings in the previous retrospective analysis (18) pertaining to the effectiveness of the L1210 system in the identification of potentially useful compounds for the clinic.

Table 5 summarizes and compares (A) the number of compounds that are in the clinically active category and (B) the number of additional compounds which are active in one or more of the various screens but which have received no clinical evaluation and are not being actively processed for clinical use. As already indicated, there are 21 nucleoside derivatives in the clinical category. But there are an additional 129 nucleoside derivatives which were rated as active and which await further evaluation for the clinic. Of the 129 clinically untested compounds, 35 were active in the L1210 system and 1 additional compound was active against Walker 256. A total of 69 compounds that were either inactive or untested against L1210 and Walker 256 were active against Ca755. The one compound listed as active against the Lewis lung tumor was inactive against S180 and was not tested in the other 3 in vivo tumor systems. A total of 23 compounds that were inactive or untested in the in vivo screening systems were active in the KB tissue culture system. Many of these were not tested in the in vivo systems because of a lack of sufficient compound.

The current study indicates that careful consideration should be given to the possibility of preclinical and clinical evaluation of additional compounds from this wide array of active nucleoside derivatives. Structural similarity per se should not preclude the clinical trial of congeners of nucleosides already in use. Structurally similar compounds may show a wide divergence in physiologic disposition, biochemical characteristics, and therapeutic effectiveness. Schedule dependency, recently shown to be so important in influencing drug specificity (184-187), undoubtedly may vary considerably for closely related compounds. Differences may occur with respect to the degree of tumor cell kill relative to limiting toxicity for the host and with respect to the origin and treatment of resistant tumor cell variants. Recent studies with 6-MP and 6-methyl-MPR provide a notable example of differences in chemotherapeutic behavior of structurally related congeners. 6-Mercaptopurine is activated by IMP pyrophosphorylase (188) whereas 6-methyl mercaptopurine 9-β-D-ribofuranoside is

activated by adenosine kinase (189-191). It was also demonstrated that 6-methylmercaptopurine 9-β-D-ribofuranoside is capable of inhibiting 6-mercaptopurine resistant tumors that are lacking in IMP pyrophosphorylase (133). 6-MP and 6-methyl-MPR were demonstrated to exert synergistic antitumor action, also indicative of different modes of action for the two drugs (131, 132).

In the current analysis it is demonstrated that closely related nucleoside derivatives show a wide range in their spectrum of activity in the various biological screening systems. In addition the purine and pyrimidine nucleosides show broad differences in their activity in the antitumor test systems as compared with the activity of the corresponding free bases.

The marked activity of a wide variety of nucleoside derivatives of purine and pyrimidines in the antitumor screening systems, the demonstrated differences in biological behavior and the flexibility with respect to the synthesis of new congeners (192) make the nucleoside category a vital area in the experimental and clinical chemotherapeutic effort.

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Table 4.—Nucleoside derivatives of clinical interest

NSC No.	Name	Group No.	Group name	Biologic activity* L1210 ILS	Walker TWI	Ca755 TWI	S180 TWI	Lewis lung TWI	KB	Clinical activity†
27640	5-FUDR (2'-Deoxy-5-fluorouridine)	2	Uracil 2'-deoxyribosides	55	71	85	95	85	<1.0	+
38297	5-BUDR (2'-Deoxy-5-bromouridine)	2	Uracil 2'-deoxyribosides	22	55	62	33	59	30	±
39661	5-IUDR (2'-Deoxy-5-iodouridine)	2	Uracil 2'-deoxyribosides	40	72	60	50		14	±
75520	F_3TDR (2'-Deoxy-5-trifluoromethyluridine)	2	Uracil 2'-deoxyribosides	75	72	73	48			P
63878	Ara-C (Cytosine 1-β-D-arabinofuranoside)	6	Cytosine furanosides	133	32	86	85	45	<1.0	+
93150	2',3',5'-Triacetate Ara-C	6	Cytosine furanosides	90	14		68		<1.0	P
529180	5-Fluoro-Ara-C	6	Cytosine furanosides	156						+
4911	6-MPR (6-Mercaptopurine 9-β-D-ribofuranoside)	14	6-MP riboside analogs	55	87	96	81		<1.0	+
40774	6-Methyl-MPR	14	6-MP riboside analogs	60	23	68	28		<1.0	+
406021	6-MPAra (6-Mercaptopurine 9-β-D-arabinofuranoside)	15	6-MP (nonribose) sugar analogs	63		100		28	>100	P
30605	2-Fluoro-AdR (Adenosine)	19	Adenine ribosides	12	55	13			<1:0	-
3055	Puromycin	19	Adenine ribosides	17	69	34			<1.0	-
29422	6-TGR (6-thioguanine 9-β-D-ribofuranoside)	30	6-Thioguanine ribosides	64	98	95	70		<1.0	+
71261	2'-Deoxy-6-TGR	31	6-Thioguanine (nonribose) nucleosides	99	98				<1.0	P
71851	2'-Deoxy-6-thioguanine 9-α-D-ribofuranoside	31	6-Thioguanine (nonribose) nucleosides	95	91				<1.0	P
56408	Tubercidin	34	7-Deazapurine ribofuranosides	16	25	30	10	40	<1.0	±
63701	Toyocamycin	34	7-Deazapurine ribofuranosides	25		28	21	17	<1.0	- (toxic)
65346	Sangivamycin	34	7-Deazapurine ribofuranosides	50		50	13	20	<1.0	P
32074	6-Azauridine	36	Miscellaneous nucleosides	35	35	0	43	37	<1.0	±
67239	2',3',5'-Triacetate 6-Azauridine	36	Miscellaneous nucleosides	44		57	26		11	±
102816	5-Azacytidine	36	Miscellaneous nucleosides	156	68					P

* Underlining denotes active ratings.
ILS: increased lifespan.
TWI: tumor weight inhibition.
+: clinically active.
±: clinical activity moderate.
-: clinical activity not demonstrated. Either inactive or too toxic.
P: being actively processed for the clinic.
NT: not tested.