# Analysis of the in Vitro Antitumor Activity of Novel Purine-6-sulfenamide, -sulfinamide, and -sulfonamide Nucleosides and Certain Related Compounds Using a Computer-Aided Receptor Modeling Procedure

Vellarkad N. Viswanadhan,[‡] Arup K. Ghose,*[,†] Naeem B. Hanna, Steven S. Matsumoto, Thomas L. Avery, Ganapathi R. Revankar,[§] and Roland K. Robins

*ICN Nucleic Acid Research Institute, 3300 Hyland Avenue, Costa Mesa, California 92626.* *Received January 17, 1990*

The comparative antileukemic activities of 21 novel nucleosides were determined in vitro by using cultured L1210 cells and analyzed for structure-related efficacy by a computer-aided receptor modeling method (REMOTEDISC) as recently described (Ghose, A. K.; et al. *J. Med. Chem.* **1989**, *32*, 746). The algorithm can be classified as a 3D-QSAR method and consists of the following steps: selection of a reference structure from the low-energy conformations of the active compounds; an automated superposition of the low-energy conformations of the other compounds so that there is maximum matching (or overlapping) of the atom-based physicochemical properties; construction of the binding-site cavity from the location of the atoms of the superimposed molecules; and determinations of the relative importance of the various physicochemical properties at different regions of the site cavity using reverse stepwise regression analysis. The model was based on the minimum energy conformation of (*R,S*)-2-amino-9-β-D-ribofuranosylpurine-6-sulfinamide (sulfinosine, **5**), an effective antileukemic agent in vivo, in the data set. The model fit the biological data with a standard deviation of 0.363, a correlation coefficient of 0.933 and a explained variance of 0.815. The method targeted a syn conformation as the probable active form and the 2′-OH, 5′-OH as well as C2-$NH_2$ group of the purine ring as favoring the stability of the syn conformation, thereby establishing the major contributions of these three molecular entities to overall antitumor activity.

The purine derivatives and analogues have played a significant role in cancer chemotherapy since the introduction of Elion and Hitchings[1] in 1952 of purine-6-(1*H*,9*H*)-thione (6-mercaptopurine, 6-MP), which is still widely used to treat lymphoblastic leukemia in children.[2] Medicinal chemists have continued to search for novel purine derivatives with the hope of obtaining more effective anticancer agents with greater specificity.[3] We recently initiated a program to synthesize and evaluate the nucleoside derivatives of certain purine-6-sulfonamides as potential anticancer agents.[4,5] During the course of these studies, it was observed that the introduction of a sulfenamido, sulfinamido or sulfonamido group at the 6-position of certain pure ribonucleosides resulted in highly water soluble compounds with significant antitumor activity.[4,5] Administered qd (once daily) on day one, 2-amino-9-β-D-ribofuranosylpurine-6-sulfenamide (**3**) at 22 mg/kg exhibited a T/C of 170, whereas a diastereomeric 2-amino-9-β-D-ribofuranosylpurine-6-sulfinamide (sulfinosine, **5**) at 173 mg/kg showed a T/C of 167 against L1210 leukemia in mice. T and C are the survival times in the test and the control animals, respectively. The 5′-deoxy analogue of sulfinosine (**8**) at 104 mg/kg also showed a T/C of 172. A single treatment with **3**, **5**, or **8** reduced body burdens of viable L1210 leukemia cells by more than 99.8%.[4] Structural alterations in the aglycon and carbohydrate moieties of this series of nucleosides produced compounds with different solubilities and anticancer activities in mice.[4-6]

In the present study, a number of purine-6-sulfenamide, -sulfinamide, and -sulfonamide nucleosides and certain related derivatives were analyzed for their activity against cultured L1210 cells by using a computer-aided drug design methodology,[7] REMOTEDISC (REceptor MOdeling from the ThreE DImensional Structure and physicoChemical properties of the ligand molecules) in order to develop a complementary model of the receptor binding site. Such a model is useful in analyzing the antitumor data and in rationally designing other structural analogues with increased antitumor activity and selectivity. This method[7] takes into account both the conformational and physical properties of the ligand nucleosides to suggest a quantitative model for the hypothetical binding site cavity.[8-10] The method, in general, is a 3D-QSAR approach for modeling the receptor binding site cavity from activity data produced by the actual anticancer evaluation of a set of structurally related, biologically active compounds. The problem of modeling the binding site of the receptor solely from the biological activities of a set of ligands is well documented, and several approaches to this complex problem have been developed.[11-16]

[†] Present address: Sterling Research Group, 81 Columbia Turnpike, Rensselaer, NY 12124.

[‡] Present address: Theoretical Immunology Section, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

[§] Present address: Triplex Pharmaceutical Corporation, 9391 Grogans Mill Road, The Woodlands, TX 77380.

(1) For a commentary, see: Chabner, B. A. *J. Natl. Cancer Inst.* **1988**, *80*, 1512.
(2) Burchenal, J. H.; Murphy, M. .; Ellison, R. R.; Sykes, M. D.; Tan, C. T. C.; Leone, L. A.; Karnofsky, D. A.; Craver, L. F.; Dargeon, H. W.; Rhoads, C. P. *Blood* **1953**, *8*, 965.
(3) Robins, R. K.; Revankar, G. R. *Med. Res. Reviews* **1985**, *5*, 273.
(4) Revankar, G. R.; Hanna, N. B.; Imamura, N.; Lewis, A. F.; Larson, S. B.; Finch, R. A.; Avery, T. L.; Robins, R. K. *J. Med. Chem.* **1990**, *33*, 121.
(5) Revankar, G. R.; Hanna, N. B.; Ramasamy, K.; Larson, S. B.; Smee, D. F.; Finch, R. A.; Avery, T. L.; Robins, R. K. *J. Heterocycl. Chem.* **1990**, in press.
(6) Ramasamy, K.; Imamura, N.; Hanna, N. B.; Finch, R. A.; Avery, T. L.; Robins, R. K.; Revankar, G. R. *J. Med. Chem.* **1990**, *33*, 1220.
(7) Ghose, A. K.; Crippen, G. M.; Revankar, G. R.; McKernan, P. A.; Smee, D. F.; Robins, R. K. *J. Med. Chem.* **1989**, *32*, 746.
(8) Ghose, A. K.; Crippen, G. M. *J. Med. Chem.* **1984**, *27*, 901.
(9) Ghose, A. K.; Crippen, G. M. *J. Med. Chem.* **1985**, *28*, 333.
(10) Ghose, A. K.; Crippen, G. M. In *Comprehensive Medicinal Chemistry*; Hansch, C., Sammes, P. G., Taylor, J. B., Ramsden, C. A. Eds.; Pergamon Press, Elmsford, NY; Vol. 4, pp 715-733.
(11) Marshall, G. R. *Ann. Rev. Pharmacol. Toxicol.* **1987**, *27*, 193.
(12) Gund, P.; Halgren, T. A.; Smith, G. M. *Ann. Rep. Med. Chem.* **1987**, *22*, 269.
(13) Cohen, N. C. *Adv. Drug Res.* **1985**, *14*, 41.
(14) Hopfinger, A. J. *J. Med. Chem.* **1985**, *28*, 1133.
(15) Sheridan, R. P.; Venkataraghavan, R. *Acc. Chem. Rev.* **1987**, *20*, 322.
(16) Cramer, R. D.; Patterson, D. E. *J. Am. Chem. Soc.* **1988**, *110*, 5959.

0022-2623/91/1834-0526$02.50/0 

**Table I.** Observed and Calculated in Vitro Anti-L1210 Activity ($ID_{50}$) of Certain Sulfur-Containing Purine Nucleosides and Related Compounds

| compd no. | nucleosides | cytotoxicity, log (1/$ID_{50}$)[a] obs[b] | calcd | $\Delta_{c-a}$[c] |
|---|---|---|---|---|
| 1 | 2-amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-purine-6(1*H*)-thione | 7.30 (±0.21) | 7.36 | 0.06 |
| 2 | 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine-6-sulfenamide | 6.66 (±0.27) | 5.66 | −1.00 |
| 3 | 2-amino-9-β-D-ribofuranosyl-purine-6-sulfenamide | 6.52 (±0.35) | 6.26 | −0.26 |
| 4 | 2-amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-purine-6-sulfenamide | 6.06 (±0.03) | 6.14 | 0.08 |
| 5 | (*R,S*)-2-amino-9-β-D-ribo-furanosylpurine-6-sulfin-amide | 6.00 (±0.28) | 5.41 | −0.59 |
| 6 | 2-amino-9-(5-deoxy-β-D-ribo-furanosyl)purine-6-sulfen-amide | 6.00 (±0.35) | 6.28 | 0.28 |
| 7 | 2-amino-9-β-D-ribofuranosyl-purine-6-sulfonamide | 5.70 (±0.24) | 5.54 | −0.16 |
| 8 | (*R,S*)-2-amino-9-(5-deoxy-β-D-ribofuranosyl)purine-6-sulfinamide | 5.55 (±0.32) | 5.41 | −0.14 |
| 9 | 2-amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-purine-6-sulfonamide | 5.52 (±0.28) | 5.38 | −0.14 |
| 10 | 2-amino-9-(5-deoxy-β-D-ribo-furanosyl)purine-6-sulfon-amide | 5.42 (±0.33) | 5.50 | 0.08 |
| 11 | 9-β-D-ribofuranosylpurine-6-sulfenamide | 5.22 (±0.35) | 5.56 | 0.34 |
| 12 | 9-β-D-ribofuranosylpurine-6-sulfonamide | 4.92 (±0.16) | 4.90 | −0.02 |
| 13 | 3-β-D-ribofuranosylpyrazolo-[4,3-*d*]pyrimidine-7-sulfen-amide | 4.69 (±0.29) | 5.09 | 0.40 |
| 14 | (*R,S*)-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine-6-sulfinamide | 4.60 (±0.34) | 4.74 | 0.14 |
| 15 | 9-(2-deoxy-β-D-*erythro*-pento-furanosyl)purine-6-sulfon-amide | 4.56 (±0.26) | 4.83 | 0.27 |
| 16 | 1-β-D-ribofuranosylpyrazolo-[3,4-*d*]pyrimidine-4-sulfen-amide | 4.51 (±0.03) | 4.49 | −0.02 |
| 17 | (*R,S*)-2-amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-purine-6-sulfinamide | 4.45 (±0.15) | 5.30 | 0.85 |
| 18 | 9-β-D-arabinofuranosylpurine-6-sulfonamide | 4.00 (±0.28) | 3.96 | −0.04 |
| 19 | (*R,S*)-9-β-D-arabinofuranosyl-purine-6-sulfinamide | 4.00 (±0.10) | 3.74 | −0.26 |
| 20 | (*R,S*)-6-amino-1-β-D-ribo-furanosylimidazo[4,5-*c*]-pyridine-4-sulfinamide | 3.00 (±0.22) | 2.98 | −0.02 |
| 21 | 1-β-D-ribofuranosylpyrazolo-[3,4-*d*]pyrimidine-4-carbox-amide | 4.38 (±0.29) | 4.55 | 0.17 |

[a] Inhibitory dose 50 ($ID_{50}$) is the concentration (μM) of compound that produced 50% inhibition of tumor cell growth as compared to the untreated controls. According to this scale, the higher the value the more active the compound is. [b] The values within parentheses represent the standard deviation of the measurement. [c] The difference between the calculated and observed values.

## Results and Discussion

The structures of the 21 antitumor purinesulfenamide, -sulfinamide, and -sulfonamide nucleosides and their presently studied analogues are depicted, with atom numbering, in Figure 1. The observed and calculated antitumor activities of these nucleosides in vitro are shown in Table I. We included one carboxamide derivative (**21**) to analyze the effect of planarity of the group structurally equivalent to the sulfinamide moiety on the biological activity.

**Table II.** Description of the Minimum-Energy Conformations of the Nucleosides

| compd no. | torsion angles,[a] deg $\omega_1$ | $\omega_2$ | $\omega_3$ | $\omega_4$ | $\omega_5$ | $\omega_6$ | $\omega_7$ | $\omega_8$ |
|---|---|---|---|---|---|---|---|---|
| 1 | 310 | 170 | 180 | | 30 | 20 | | |
| 2 | 50 | 60 | 180 | | 190 | | 40 | 138 |
| 3 | 290 | 160 | 50 | 300 | 40 | 20 | 40 | 138 |
| 4 | 50 | 60 | 180 | | 190 | 200 | 40 | −97 |
| 5 | 290 | 160 | 50 | 300 | 40 | 20 | −120 | −78 |
| 6 | | 60 | 50 | 300 | 210 | 20 | 40 | −97 |
| 7 | 290 | 160 | 50 | 300 | 40 | 160 | 0 | −69 |
| 8 | | 60 | 50 | 300 | 210 | 200 | −120 | −78 |
| 9 | 50 | 60 | 180 | | 190 | 200 | 0 | −69 |
| 10 | | 60 | 50 | 290 | 210 | 160 | 0 | −69 |
| 11 | 50 | 60 | 50 | 300 | 200 | | 40 | 138 |
| 12 | 50 | 60 | 50 | 300 | 200 | | 0 | −69 |
| 13 | 50 | 60 | 50 | 300 | 210 | | 40 | −97 |
| 14 | 50 | 60 | 180 | | 190 | | −120 | −78 |
| 15 | 50 | 60 | 180 | | 190 | | 0 | −69 |
| 16 | 50 | 60 | 50 | 300 | 210 | | 40 | 138 |
| 17 | 290 | 180 | 180 | | 30 | 20 | −120 | −78 |
| 18 | 310 | 180 | 180 | 180 | 200 | | 0 | −69 |
| 19 | 310 | 180 | 180 | 290 | 200 | | −120 | −78 |
| 20 | 50 | 60 | 50 | 60 | 200 | 20 | −120 | −78 |
| 21 | 50 | 60 | 50 | 300 | 210 | | 0 | 180 |

[a] $\omega_1$ = $\omega(H_{5'}–O_{5'}–C_{5'}–C_{4'})$, $\omega_2$ = $\omega(O_{5'}–C_{5'}–C_{4'}–C_{3'})$, $\omega_3$ = $\omega(C_{2'}–C_{3'}–O_{3'}–H)$, $\omega_4$ = $\omega(C_1–C_{2'}–O_{2'}–H)$, $\omega_5$ = $(C_4–N_9–C_1–C_{2'})$, $\omega_6$ = $\omega(N_3–C_2–N–H)$, $\omega_7$ = $\omega(C_5–C_6–S$ or $C–N)$, $\omega_8$ = $\omega(C_6–S$ or $C–N–H)$.

**Molecule Generation and Conformational Analysis.** The starting geometry for conformational analysis is based on a crystallographic fragment library containing the 2′-endo,3′-exo (type *S*) ribose ring[17] and various heterocyclic rings as obtained from the literature.[7] The furanosyl ring was kept rigid at its crystallographic structure during energy minimization by pattern search and combinatorial search. The use of rigid rings has limitations due to conformational fluctuations that occur in solution. In the present study, however, this aspect was not taken into account nor was it expected to have significant influence on obtained results because, from our analysis, it appears to be the composition and conformation of the fused heterocyclic ring that primarily directs the biological activity of the present set of nucleosides. Although all the substituents were rotated during pattern search energy minimization, only three to four torsion angles were varied during the combinatorial search. These bonds are glycosyl C1′–X bond, where X represents the attached atom of the heterocyclic ring, the backbone C4′–C5′ and C5′–O5′ bonds and any one important dihedral angle involving the substituents of the heterocyclic moiety. Since the torsional energy parameter associated with some of the functional groups attached to the heterocyclic ring were not available, these functional groups were studied through AMPAC (AM1) molecular orbital package[18,19] and kept fixed at the obtained minimum energy conformation.

The conformational stabilities of these nucleosides are related to their chemical structure. Ribofuranosyl nucleosides with an $NH_2$ group in the 2-position of the purine ring favor a syn comformation (see Table II, nucleosides **3**, **5**, and **7**). For the 2′-deoxyribofuranosyl nucleosides with a C2–$NH_2$ group, the C6-substituents have some role in

(17) Low, J. N. *Acta Crystallogr.* **1983**, *C39*, 796.
(18) QCPE Program No. 506, Department of Chemistry, Indiana University, Bloomington, IN.
(19) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.

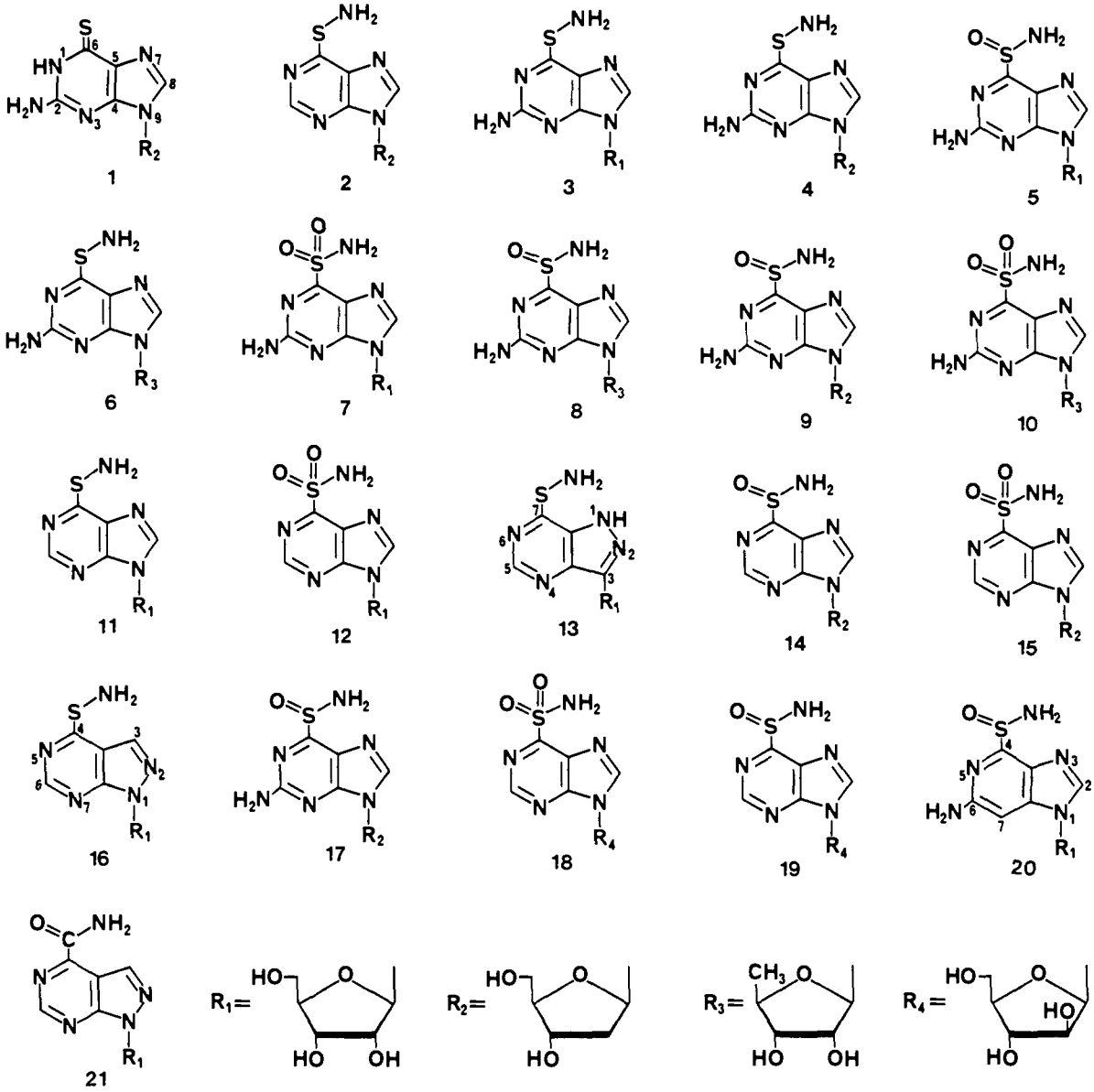

Figure 1. Structures and atom labels for the molecules used in the present study.

deciding the syn/anti stability. There are four such nucleosides, among which two (1 and 17) had syn and the other two (4 and 9) had anti conformation. One major factor which increases the stability of the syn conformation is the interaction between the C2–$NH_2$ and 5′-OH groups, since all nucleosides without one of these groups had anti conformation.

The minimum energy conformation of nucleoside 5, the reference compound in the model building process, is shown in Figure 2.

**Molecular Superpositions.** The receptor-site model presented here has been developed on the basis of the minimum energy conformation of nucleosides 5 as the reference structure. This nucleoside (sulfinosine) is the most active in the animal model and has several biological advantages over the other compounds.[4] The minimum energy conformation of this nucleoside is very similar to that of nucleoside 1, the most active compound in the in vitro cytotoxicity study. The result obtained on the basis of the minimum energy conformation of nucleoside 1 was comparable. The superposition, determined on the basis of physicochemical property matching, suggests that most of the molecules can attain the desired syn superposition in which the sugar ring and the heterocyclic ring match,

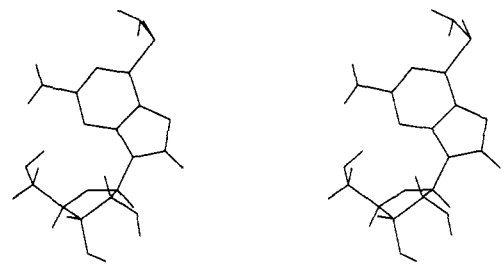

Figure 2. A stereo view of the minimum energy conformation of nucleoside 5 the reference structure in the model building process.

at the energy cost of 1 kcal/mol or less. As exceptions, the arabinofuranosyl nucleosides 18 and 19 at the low-activity end needed nearly 2 kcal/mol energy to attain the desired superposition. The destabilizing factor comes from interaction of the cis hydroxyl group at the 2′-position with the aglycon moiety. The nucleoside with the lowest antitumor activity (20) has an anti minimum energy conformation. It could not attain the desired superposition since its syn conformation was too unstable. As it is a 3-deazapurine derivative, the steric repulsion between the hydrogen attached to C3 and O4′ seems to be responsible

**Table III.** Description of the Active Conformations of the Nucleosides

| compd no. | torsion angles,[a] deg | | | energy, kcal/mol |
|---|---|---|---|---|
| | $\omega_1$ | $\omega_2$ | $\omega_5$ | |
| **1** | 310 | 170 | 30 | 0.00 |
| **2** | 290 | 160 | 40 | 0.91 |
| **3** | 290 | 160 | 40 | 0.00 |
| **4** | 290 | 180 | 30 | 0.20 |
| **5** | 290 | 160 | 40 | 0.00 |
| **6** | | 40 | 50 | 0.74 |
| **7** | 290 | 160 | 40 | 0.00 |
| **8** | | 40 | 50 | 0.78 |
| **9** | 290 | 160 | 40 | 0.31 |
| **10** | | 40 | 40 | 0.83 |
| **11** | 290 | 160 | 40 | 1.02 |
| **12** | 290 | 160 | 40 | 1.03 |
| **13** | 290 | 160 | 50 | 0.95 |
| **14** | 290 | 160 | 50 | 0.92 |
| **15** | 290 | 160 | 40 | 0.99 |
| **16** | 290 | 160 | 50 | 0.89 |
| **17** | 160 | 290 | 30 | 0.15 |
| **18** | 310 | 160 | 40 | 1.71 |
| **19** | 310 | 160 | 40 | 1.84 |
| **20** | 290 | 160 | 220 | 1.97 |
| **21** | 290 | 160 | 50 | 0.89 |

[a] See footnote of Table II for the identification of the dihedral angles.

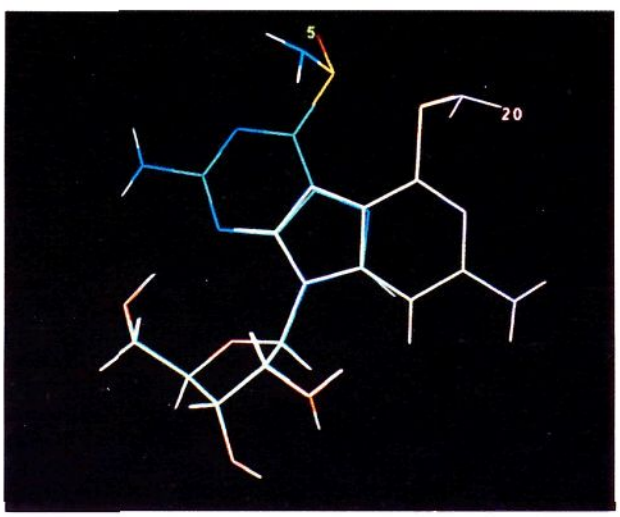


**Figure 3.** Superposition of nucleoside **20** on the reference compound **5**.

for the instability of this molecule's syn conformation. The active conformation (the best superposed structure from the physicochemical property matching) and the corresponding conformational energy of the various nucleosides are given in Table III. The superposition of **20** on the reference structure is shown in Figure 3.

**Binding-Site Cavity.** The hypothetical binding-site cavity[20] was generated from the superposed structure of 21 nucleosides. The site cavity was divided into four different pockets. The description of the site pockets and the relative importance of various physicochemical properties in different pockets are given in Table IV. The structure of the active-site cavity and the center of the four pockets are shown relative to the reference nucleoside **5** and **20** and are presented in Figure 4. The model showed a standard deviation of 0.363 between the calculated and observed biological activities and a correlation coefficient

(20) The model consisted of 59 small spheres of four different types. The coordinates of the center of the spheres and their radii and types may be obtained on request from the authors.

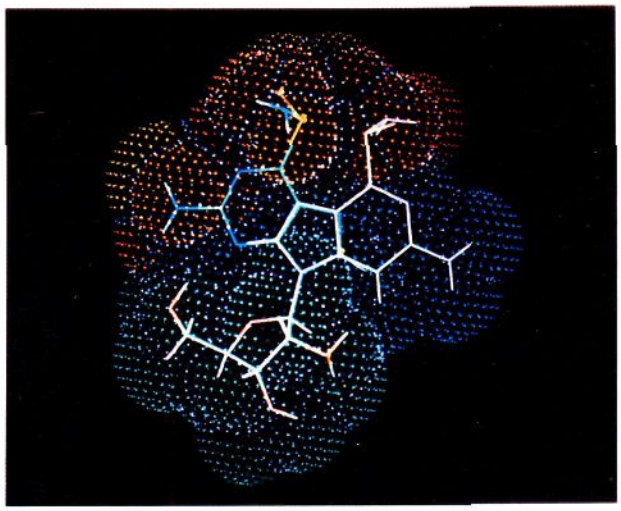

**Figure 4.** The surface of the hypothetical binding-site cavity relative to the reference nucleosides **5** and **20**. The numbers indicate the approximate location of the center of various site pockets.

of 0.933. The overall statistics of fit is given in Table V. The active physicochemical properties of the nucleosides at various site pockets are given in Table VI. The corresponding interaction energy can be obtained by multiplying the physicochemical properties by the corresponding coefficients in Table IV. The calculated biological activity is the sum of all the interaction terms. The evaluation of the local physicochemical properties in the various site pockets using the reference nulcleoside **5**. The sum of any physicochemical properties of the atoms in a particular site pocket will give the local property. Since the nucleoside **20**, bound in the anti conformation, the cavity is extended on the anti region. During superposition only a limited number of low-energy conformations were considered; however, the steric requirement may force this molecule to bind in the syn conformation, and the conformational energy may alone be responsible for the low biological activity of this molecule. For nucleoside **21** the carboxamide group remained in the site pocket 3. Bond angle, bond distances, and the planarity of the group are responsible for such distribution of the group. Since the molecular interaction in this site pocket is negligible (see Table IV), it exhibited poor biological activity.

The present model suggests that there are two aspects for the biological activity of these molecules, viz. conformational and physicochemical. A syn conformation is necessary for the observed antitumor activity of these nucleosides and this conformation is favored by the presence of 5′- and 2′-hydroxy groups and an amino group at 2-position of purine ring. A major attraction comes from the dispersive interaction of the sugar ring with the active site which, in turn, suggests that the presence of the hydroxyl groups helps to attain the active conformation rather than direct interaction with the receptor site. It may be worth trying to freeze the syn conformation and replace oxygen by more dispersive atoms like sulfur. Since the nucleoside with the lowest activity (**20**) was bound in anti conformation, the method assumed that the anti region (site pocket 2) was sterically sensitive. This observation suggests that a proper tailoring of the five-membered heterocyclic moiety of the purine ring may improve the cytotoxicity. The interaction of the atoms at site pocket 3 (see Figure 4) is negligible with the receptor. Only one hydrogen from the C2–$NH_2$ and one oxygen attached to the sulfur interacts with site pocket 4. The interaction

**Table IV.** Description of the Site Pockets

| site pocket | coefficients[a] of phys chem param: hydrophobicity[b] | dispersive[c] | electrostatic[d] | description (geometrical[e] and physicochemical[f]) |
|---|---|---|---|---|
| 1 | | 0.3007 | 15.5943 | Binds the glycon moiety. Large and positive group preferred. |
| 2 | -4.4693 | -0.1262 | | Binds the five-membered heterocyclic ring and $N_3$ atom of the purine ring. Small hydrophilic atoms are preferred. |
| 3 | | | | Binds $N_1$, $C_2$, $C_6$ and part of the 2- and 6-substituents. Interaction negligible. |
| 4 | 0.7351 | | 2.1031 | Binds one H of the $C_2$-$NH_2$ and one O of the 6-substituent. Hydrophobic and positively charged groups favored. |

[a] The coefficient corresponding to the conformational energy is -0.6875. Except for the dispersive interactions the error limit of all other coefficients was very low. [b] Octanol-water partition coefficient was used to model the hydrophobic interaction. [c] Molar refractivity was used to model the dispersive interaction. [d] Atomic charge distribution was used to model the electrostatic interaction. [e] The geometrical description is provided with respect to the reference nucleoside **5**, which is generally applicable for most other nucleosides. [f] The physicochemical properties of the preferred atoms suggested here does not consider its effect on the conformational properties or on the physicochemical properties of the other parts of the molecule. The conclusion regarding the preferred properties can be drawn from the fact that the positive contribution to biological activity is preferred and the contribution is the product of the coefficient (as given in this table) and the property of the ligand. Since the atomic refractivity, which is directly related to the volume of the atom, cannot be negative, a negative coefficient suggests that a small group or atom is preferred.

**Table V.** Statistics of the Study

| no. of compds | no. of site pocket | no. of parameter | standard deviation | correl[a] coeff | explained[b] variance | $F$ test,[c] % |
|---|---|---|---|---|---|---|
| 21 | 3 | 7 | 0.363 | 0.933 | 0.715 | 99.998 |

[a] For definition, see: Snedecor, G. W.; Cochran, W. G. In *Statistical Methods*; Iowa State University Press: Ames, IA 1967; p 172. [b] The definition and necessity of the use of "explained variance" have been described by Porcell et al.: Purcell, W. P.; Bass, G. E.; Clayton, G. E. In *Strategy of Drug Design: A Guide to Biological Activity*; Wiley, New York, 1973; p 29. [c] The regression is significant at this level, which corresponds to a $F$ value of 9.431 with degrees of freedom 8 and 12, see reference above.

**Table VI.** Local Physicochemical Properties in Different Site Pockets for Certain Sulfur-Containing Purine Nucleosides and Related Compounds[a]

| compd no. | site pocket/physicochemical properties[b]: $1/D$ | $1/E$ | $2/H$ | $2/D$ | $4/H$ | $4/E$ | conformation energy, kcal/mol |
|---|---|---|---|---|---|---|---|
| 1 | 26.8585 | 0.0036 | -0.3851 | 19.7596 | -0.3260 | 0.1118 | 0.00 |
| 2 | 26.8585 | -0.0080 | -0.2290 | 21.2080 | -0.3260 | 0.1084 | 0.91 |
| 3 | 27.3055 | -0.0162 | -0.2290 | 21.2080 | -0.6520 | 0.2055 | 0.00 |
| 4 | 26.8585 | -0.0068 | -0.2290 | 21.2080 | -0.6520 | 0.2077 | 0.20 |
| 5 | 27.3055 | -0.0137 | -0.2290 | 21.2080 | -0.6774 | -0.2093 | 0.00 |
| 6 | 26.5160 | 0.1204 | 0.0970 | 20.4079 | -0.6520 | 0.2015 | 0.74 |
| 7 | 27.3055 | -0.0075 | -0.2290 | 21.2080 | -0.6774 | -0.1922 | 0.00 |
| 8 | 26.5160 | 0.1226 | 0.0970 | 20.4079 | -0.6774 | -0.2035 | 0.78 |
| 9 | 26.8585 | 0.0020 | -0.2290 | 21.2080 | -0.3514 | -0.2871 | 0.31 |
| 10 | 26.5160 | 0.1290 | 0.0970 | 20.4079 | -0.6774 | -0.1927 | 0.83 |
| 11 | 27.3055 | -0.0185 | -0.2290 | 21.2080 | -0.3260 | 0.1083 | 1.02 |
| 12 | 27.3055 | -0.0060 | -0.2290 | 21.2080 | -0.3514 | -0.2848 | 1.03 |
| 13 | 27.1986 | -0.1172 | -0.4736 | 21.6422 | -0.3260 | 0.1150 | 0.95 |
| 14 | 26.8585 | -0.0116 | -0.2290 | 21.2080 | -0.3514 | -0.2928 | 0.92 |
| 15 | 26.8585 | -0.0041 | -0.2290 | 21.2080 | -0.3514 | -0.2794 | 0.99 |
| 16 | 27.3489 | -0.0207 | -0.0066 | 22.4339 | -0.3260 | 0.1163 | 0.89 |
| 17 | 26.8585 | -0.0046 | -0.2290 | 21.2080 | -0.6774 | -0.2145 | 0.15 |
| 18 | 27.3055 | -0.0361 | -0.2290 | 21.2080 | -0.3514 | -0.2855 | 1.71 |
| 19 | 27.3055 | -0.0431 | -0.2290 | 21.2080 | -0.3514 | -0.2977 | 1.84 |
| 20 | 27.3055 | -0.0160 | -0.2243 | 36.7089 | 0.0000 | 0.0000 | 1.97 |
| 21 | 27.3489 | -0.0165 | -0.0066 | 22.4339 | 0.0000 | 0.0000 | 0.89 |

[a] The interaction at any site point may be obtained by multiplying the physicochemical properties with the corresponding coefficients as given in Table IV. [b] $D$ is the sum of the atomic refractivities to account for the dispersive interaction; $E$ is the sum of the CNDO/2 atomic charge distribution to account for the electrostatic interaction; $H$ is sum of the atomic contribution to octanol-water partition coefficient to account for the hydrophobic interaction.

with oxygen is, however, repulsive. Since the substituent here was not rotated, due to the unavailability of the torsional parameter, the $NH_2$ group attached to the sulfur was not considered for interaction in this region. Although that will lead to an attractive interaction, such rotation needs conformational energy which may nullify the attractive interaction.

In conclusion, the present computer-aided receptor modeling procedure was very helpful in rationalizing our previously generated biological activity data. It shows the binding conformation as well as the various important physicochemical parameters and conformational factors which are related to the observed antitumor activity. The hypothetical binding-site cavity generated in this method may also be used for designing new compounds.

The present model suggests that a syn conformation is necessary for the antitumor activity of these nucleosides. The 2′-OH and 5′-OH groups, as well as the $NH_2$ group at the 2-position of the purine ring help to attain this conformation. The anti conformation occupies mostly the site pocket 2 whose coefficient for atomic refractivity (see Table IV) is negative. Since there is a direct relationship between the atomic volume and atomic refractivity, these observations suggest that in the anti conformation the nucleosides get steric repulsion.

The major advantage of this molecular modeling approach is that it provides a physical picture which may help medicinal chemists develop the hypotheses needed

Table VII. Illustration of the Evaluation of the Local Physicochemical Properties at the Various Site Pocket Using (*R,S*)-2-Amino-9-β-D-ribofuranosylpurine-6-sulfinamide (**5**)

| atom | atom type[a] | site pocket[b] | hydroph[a] | molar refrac[a] | charge density[c] |
|---|---|---|---|---|---|
| C1′ | 9 | 1 | −0.4042 | 3.1069 | 0.2372 |
| C2′ | 8 | 1 | −0.5210 | 2.9566 | 0.1013 |
| C3′ | 8 | 1 | −0.5210 | 2.9566 | 0.1332 |
| C4′ | 8 | 1 | −0.5210 | 2.9566 | 0.1207 |
| C5′ | 6 | 1 | −0.8370 | 3.3267 | 0.1244 |
| O2′ | 56 | 1 | 0.1402 | 1.4673 | −0.2535 |
| O3′ | 56 | 1 | 0.1402 | 1.4673 | −0.2462 |
| O4′ | 59 | 1 | 0.1720 | 1.2000 | −0.2449 |
| O5′ | 56 | 1 | 0.1402 | 1.4673 | −0.2480 |
| N1 | 75 | 3 | −0.1106 | 4.4916 | −0.2089 |
| C2 | 32 | 3 | 0.2074 | 2.5000 | 0.2848 |
| N3 | 75 | 2 | −0.1106 | 4.4916 | −0.2276 |
| C4 | 43 | 2 | 0.0498 | 2.5001 | 0.1840 |
| C5 | 28 | 2 | 0.1290 | 2.5000 | 0.0397 |
| C6 | 29 | 3 | 0.1652 | 2.5831 | 0.0771 |
| N7 | 75 | 2 | −0.1106 | 4.4916 | −0.2055 |
| C8 | 42 | 2 | −0.1316 | 2.8842 | 0.1780 |
| C9 | 73 | 2 | 0.4198 | 2.7404 | −0.1234 |
| S(6) | 109 | 3 | −0.1708 | 5.6011 | 0.2230 |
| O(S) | 58 | 4 | −0.3514 | 1.4001 | −0.2991 |
| N(2) | 69 | 3 | 0.3132 | 3.6403 | −0.2422 |
| N(S) | 72 | 3 | −0.0528 | 2.9645 | −0.2410 |
| H1′ | 48 | 1 | 0.3161 | 0.8000 | −0.0125 |
| H2′ | 47 | 1 | 0.3343 | 0.8000 | 0.0054 |
| H3′ | 47 | 1 | 0.3343 | 0.8000 | −0.0222 |
| H4′ | 47 | 1 | 0.3343 | 0.8000 | 0.0066 |
| H5′ | 47 | 1 | 0.3343 | 0.8000 | 0.0111 |
| H5′ | 47 | 1 | 0.3343 | 0.8000 | −0.0049 |
| HO2′ | 50 | 1 | −0.3260 | 0.8001 | 0.1447 |
| HO3′ | 50 | 1 | −0.3260 | 0.8001 | 0.1339 |
| HO5′ | 50 | 2 | −0.3260 | 0.8001 | 0.1313 |
| HC8 | 49 | 2 | −0.1488 | 0.8000 | 0.0082 |
| HN(2) | 50 | 4 | −0.3260 | 0.8001 | 0.0898 |
| HN(2) | 50 | 3 | −0.3260 | 0.8001 | 0.0958 |
| HN(S) | 50 | 3 | −0.3260 | 0.8001 | 0.1163 |
| HN(S) | 50 | 3 | −0.3260 | 0.8001 | 0.1332 |

[a] For atom classification and atomic parameters for hydrophobicity and molar refractivity see ref 24. [b] The physicochemical property in any site pocket for nucleoside **5**, as illustrated in Table VI, can be obtained by summing up the properties of the atoms in that site pocket. [c] Charge density is obtained from CNDO/2 calculations.

to guide future synthetic work.

The conclusions reached by this procedure are largely dependent on the precision of the available biological activity data. Accordingly care must be taken in the generation and utilization of such data if erroneous conclusions are to be avoided.

## Experimental Section

**Chemistry.** 2-Amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine-6(1*H*)-thione (**1**) was prepared as reported previously.[21] The synthesis of the ribonucleosides **3**, **5**–**8**, and **10**–**12** has recently been reported from our laboratory.[4] The synthesis of the 2′-deoxyribonucleosides **2**, **4**, **9**, **14**, **15**, and **17**, as well as the arabinofuranosyl nucleosides **18** and **19** were also reported recently from our laboratory.[5] 6-Amino-1-β-D-ribofuranosylimidazo[4,5-c]-pyridine-4-sulfinamide (**20**)[6] and 1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4-carboxamide (**21**)[22] were prepared as reported.

**1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4-sulfenamide (16).** Commercial sodium hypochlorite solution (Clorox, 0.77 M, 5.25%, 8 mL) was cooled to 0 °C in an ice bath. Ammonium hydroxide (0.77 M, 20 mL) was similarly cooled in an ice bath and added with stirring to the above bleach solution. The mixture was stirred at 0 °C for 15 min and then a cold (0 °C) solution of 1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4-(5*H*)-thione[23] (1.42 g, 5 mmol) in 2 N KOH solution (2.5 mL) was added. The flask was stoppered and the contents were stirred for 45 min, during which time the reaction mixture had warmed to room temperature. The reaction mixture was allowed to stand at room temperature for 2 h, and the product that crystallized out was collected by filtration. After washing with cold EtOH (2 × 10 mL), the product was recrystallized from aqueous EtOH to yield 0.61 g (41%) of the title compound: mp 166–169 °C; IR $\nu_{max}$ 3200–3400 ($NH_2$, OH) $cm^{-1}$; UV $\lambda_{max}$ (pH 1) 295 nm (ε 28 000), (pH 7) 293 nm (ε 24 000), (pH 11) 292 nm (ε 21 000); $^1H$ NMR ($Me_2SO$-$d_6$) δ 3.53 (m, 2 H, $C_{5'}CH_2$), 3.83 (m, 1 H, $C_{4'}H$), 4.05 (m, 1 H, $C_{3'}H$), 4.35 (m, 1 H, $C_{2'}H$), 4.70 (s, 2 H, $SNH_2$), 5.05 (t, 1 H, $C_{5'}OH$), 5.10 (d, 1 H, $C_{3'}OH$), 5.25 (d, 1 H, $C_{2'}OH$), 6.24 (d, 1 H, $J_{1',2'}$ = 4.53 Hz, $C_{1'}H$), 8.67 (s, 1 H, $C_3H$) and 8.75 (s, 1 H, $C_6H$). Anal. ($C_{10}H_{13}N_5O_4S$, MW 299.3) C, H, N, S.

**3-β-Ribofuranosylpyrazolo[4,3-*d*]pyrimidine-7-sulfenamide (13).** In a similar manner as described for **16**, amination of 3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine-7-thione[22] (thioformycin B, 0.28 g, 1 mmol, in 0.5 mL 2 N KOH) with chloramine solution (prepared from 1.5 mL of commercial sodium hypochlorite and 0.4 mL of 0.7 M $NH_4OH$ at 0 °C) gave the crude reaction product. The product was dissolved in MeOH (20 mL) and adsorbed onto silica gel (5 g). The excess solvent was evaporated to dryness, and the dried silica gel was loaded onto a flash silica gel column (1.5 × 20 cm) packed in $CH_2Cl_2$. The column was eluted with $CH_2Cl_2$–MeOH (8:2, then 6:4, v/v). The homgeneous fractions were pooled and the solvents evaporated, and the residue was crystallized from aqueous EtOH to yield 0.15 g (50%) of the title compound: mp >100 °C dec; IR $\nu_{max}$ 3150–3300 (NH, $NH_2$ OH) $cm^{-1}$; UV $\lambda_{max}$ (pH 1) 331 nm (ε 21 000), (pH 7) 323 nm (ε 23 000), (pH 11) 332 nm (ε 19 100); $^1H$ NMR ($Me_2SO$-$d_6$) δ 3.63 (m, 2 H, $C_{5'}CH_2$), 3.97 (m, 1 H, $C_{4'}H$), 3.99 (s, 2 H, $SNH_2$), 4.15 (m, 1 H, $C_{3'}H$), 4.61 (m, 1 H, $C_{2'}H$), 5.15 (t, 1 H, $C_{5'}OH$), 5.27 (d, 1 H, $C_{2'}OH$), 5.45 (d, 1 H, $J_{1',2'}$ = 6.1 Hz, $C_{1'}H$), 5.56 (d, 1 H, $C_{2'}OH$), 9.1 (s, 1 H, $C_5H$). Anal. ($C_{10}H_{13}N_5O_4S$, MW 299.3) C, H, N, S.

**Cell Culture Toxicity Studies.** A murine leukemia cell line L1210 was used. Cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 20 mM HEPES (pH 7.4), and 2 mM glutamine. The cytotoxicity determination was carried out in 96-well microliter dishes containing a starting number of (5–10) × $10^3$ cells per well and 0.1–100 μM concentrations of the test nucleosides in triplicate wells. L1210 cells were incubated with the compounds at 37 °C for 3 days. After this time period, 25 μL of 4 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added to each well and incubation was continued for 5 h.[24] The formazan product was dissolved in 2-propanol containing 0.04 M HCl, and the absorbance was determined with microtiter plate reader. The absorbance was proportional to the number of cells. The absorbance values were used to calculate the $ID_{50}$ value for each compound, the concentration that inhibited cell growth to 50% of the value for untreated, control cells. Special care was taken to generate the $ID_{50}$ values by repeating the experiment several times until a consistent result was obtained.

**Computational Methods.** The method followed here is similar to what we have reported previously,[7] and can be summarized as follows:

**(i) Generation of Molecules.** The starting three-dimensional structure of a ligand molecule is generated by attaching the crystallographic fragments at a standard bond length and arbitrary dihedral angle.

**(ii) Sampling of the Conformational Space.** The fixed valence geometry molecular mechanics program CONFOR[8] was used for the conformational analysis. The details of this program have been published elsewhere.[25] Although a complete geometry

(21) Hanna, N. B.; Ramasamy, K.; Robins, R. K.; Revankar, G. R. *J. Heterocycl. Chem.* **1988**, *25*, 1899.
(22) Goebel, R. J.; Adams, A. D.; McKernan, P. A.; Murray, B. K.; Robins, R. K.; Revankar, G. R.; Canonico, P. G. *J. Med. Chem.* **1982**, *25*, 1334.
(23) Montero, J. L. G.; Bhat, G. A.; Panzica, R. P.; Townsend, L. B. *J. Heterocycl. Chem.* **1977**, *14*, 483.
(24) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.
(25) Viswanadhan, V. N.; Ghose, A. K.; Revankar, G. R.; Robins, R. K. *J. Chem. Inf. Comput. Sci.* **1989**, *29*, 163.

relaxed conformational calculation is more preferable, the unavailability of the molecular mechanics parameter is the major obstacle for such calculation. Only a finite number of the lowest energy conformations from a grid search was selected for the next steps, and each conformation was given a priority value using the priority function

$$P = E_{obs} + E_c \quad (1)$$

where $E_{obs}$ is the observed binding free energy (or any biological activity data which is directly proportional to the binding energy) and $E_c$ is the conformational energy compared to the global minimum energy.

**(iii) Building a Quantitative Model for the Binding Site Cavity.** The conformation having highest priority is chosen as the reference structure and the following steps are executed recursively until an acceptable solution is obtained: (a) Determine the geometrically feasible superposition of the low energy conformations of the other molecules on the reference structure best matching of the physicochemical properties. (b) Construct the hypothetical receptor cavity from the locations of the atoms of the superposed molecules. (c) Divide the site cavity into a minimum number of pockets and correlate the important physicochemical properties at the different pockets with the binding energy or biological activity of the ligands. Store the statistics of fit. Select the structure having the next lower priority. Restart step (a). The loop may end after a certain number of structures have been tried as the reference or a solution with acceptable statistics has been detected.

Three physicochemical properties[25] were used in the regressional study. These were: (1) charge density[26] for electrtostatic interaction, (2) molar refractivity for dispersive interactions, and (3) water–octanol partition coefficient for hydrophobic interactions. The calculated binding energy ($E_{calc}$) of a ligand with the receptor or the biological activity proportional to the binding energy is given by

$$E_{calc} = -CE_c + \sum_{i=1}^{n_s}\sum_{j=1}^{n_p}[C_{ij}\sum_{k=1}^{n_o}P_{jk}] \quad (2)$$

where $E_c$ is the conformational energy, $C$'s are the site and physicochemical property dependent coefficients determined by the regressional method or any optimization procedure; $n_s$ is the number of site pockets, $n_p$ is the number of the physicochemical property active in the site pocket, $n_o$ represents the number of atoms of the ligand occupying the site pocket; $P_{jk}$ is the $j$th physicochemical property of the $k$th atom of the ligand.

The various critical aspects of the dissection of the binding-site cavity merits some discussion. The dissection is based on the choice of the primary site pockets, since the rest of the site pockets (secondary) get the type of the nearest primary site pocket. We used the sum of the correlation coefficient of the three physicochemical properties of the ligand atoms with the binding affinity data to select the primary site pocket. However, since the properties of the ligand atoms occupying the nearest secondary site pocket largely change the distribution of the properties, such dissection often fails to give the best fit. One solution of the problem is to use distance-dependent interaction function that we proposed recently.[27] Also cross validatory least squares or partial least squares and prediction on a totally separated test set may be the best idea to avoid any change correlation.[27]

**Registry No.** 1, 789-61-7; **2**, 124416-61-1; **3**, 123002-38-0; **4**, 124416-63-3; **5**, 124508-99-2; **6**, 123002-41-5; **7**, 123002-39-1; **8**, 124509-01-9; **9**, 124416-58-6; **10**, 123026-03-9; **11**, 123002-35-7; **12**, 123002-37-9; **13**, 130933-22-1; **14**, 124416-62-2; **15**, 124417-93-2; **16**, 124416-83-7; **17**, 124416-58-6; **18**, 124416-60-0; **19**, 124578-21-8; **20**, 124416-73-5; **21**, 83200-36-6; thioformycin B, 13263-91-7; 1-β-D-ribofuranosylpyrazolo[3.4-*d*]pyrimidine-4(5*H*)thione, 54524-71-9.

(26) Pople, J. A.; Veveridge, D. L. In *Approximate Molecular Orbital Theory*; McGraw-Hill: New York, 1970; pp 85–162.

(27) Ghose, A. K.; Crippen, G. M.; *Mol. Pharmacol.* **1990**, *37*, 725.