Quantitative Structure–Activity Relationships in Cytokinin Agonistic and Antagonistic Pyrido[2,3-*d*]pyrimidine Derivatives: Insights into Receptor Topology

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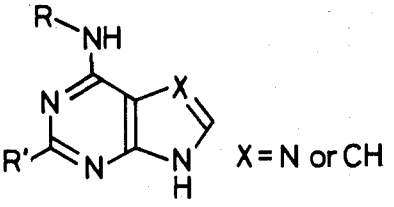
2-(Methylthio)pyrido[2,3-*d*]pyrimidines having various alkylamino and anilino substituents at the 4-position were prepared and tested for their cytokinin agonistic and antagonistic activities by the tobacco callus bioassay. The alkyl series of compounds showed anticytokinin activity, whereas the anilino derivatives exhibited both cytokinin and anticytokinin activities depending on the structure and position of the benzene substituents. Quantitative structure–activity analyses were carried out for each class and for the combined set of compounds with use of physicochemical parameters and regression analysis, indicating that the quality of activity, agonistic or antagonistic, as well as the extent of activity, is significantly affected by the steric features of the molecule. On the basis of the present results and previous quantitative analyses on cytokinins and other classes of anticytokinins, a dimensional map for the cytokinin receptor site can be drawn, which can serve as the basis for the design of novel agonists and antagonists.

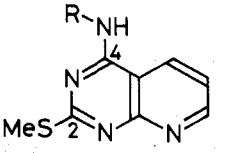
The quantitative structure–activity relationship technique has been proved to be a useful tool in understanding the mechanisms of action of biologically active compounds and in providing information concerning the topology of their receptor sites. In the field of the plant hormones called cytokinins, its application has revealed the structural correspondence between N^6-substituted adenine and *N*,*N*′-diphenylurea derivatives, the classes of compounds having seemingly different structures but the same biological activity.[1] Our earlier studies on cytokinins[1] and anticytkinins[2-4] fitting the generic formula below have shown that steric dimensions play an important role in the binding to the receptor as well as in determining intrinsic activity, agonistic or antagonistic.



In the present study, 2-(methylthio)pyrido[2,3-*d*]pyrimidines having a variety of 4-alkylamino and 4-anilino side chains were prepared and their cytokinin agonistic and antagonistic activities were examined. Quantitative structure–activity analysis indicated that both series of compounds have a common mode of interaction with the receptor, and the maximum width of the N^4 side chains, W_{max}, is an important factor in determining their binding to the receptor and thus the extent of their activity as well as their intrinsic activity. The results coincide with those obtained in earlier studies,[1-4] which adds information providing a basis for developing a model for cytokinin receptor topology.

Test Substances. The compounds **3**, **4**, **8–12**, **14**, **16**, and **20** in Tables I and II have been reported previously.[4] Other compounds were prepared by refluxing 4-chloro-2-(methylthio)pyrido[2,3-*d*]pyridimine[4] with the appropriate amine in 1-butanol. In cases where crystallization was difficult, the compounds were purified as picrate salts.

Biological Parameters. The activity was measured in terms of the fresh weight yield of tobacco callus derived from *Nicotiana tabacum* var. Wisconsin No. 38. For the measurement of anticytokinin activity, the callus was grown on a medium containing 0.05 μM kinetin [6-(furfurylamino)purine] and the test substance. Cytokinin activity was tested similarly in the absence of kinetin. The anticytokinin activity was expressed by the I_{50} (μM) value, which is the concentration at which is obtained 50% of the callus growth on the medium with kinetin but without anticytokinin. The E_{50} (μM) value for cytokinin activity is the concentration at which 50% of the maximum response of callus yield is obtained. The results of the bioassay are summarized in Tables I–III, together with the physicochemical parameters used for the regression analysis.



Substituent Parameters. To best express the steric features of N^4 substituents, we defined the steric parameters *L*, W_{max}, and W_u as shown by Figure 1. *L* is the length of the N^4 substituents along the bond axis that connects them to the N^4 atom. The W_{max} parameter is the maximum width measured from the N^4 bond axis in the direction in which the longest chain of the substituent extends in the fully extended (staggered) conformation. For benzyl substituents, the benzene ring was twisted 30° from the plane in which the skeletal N^4–C–C_1^{Ph} chain lies, minimizing steric constraints. The conformation adopted and the steric parameters defined are essentially the same as those used in the previous cytokinin and anticytokinin studies.[1,3] The W_{max} of the phenyl substituents is the width in the direction to which ortho or meta substituents extend. The W_u parameter is the thickness upward from the plane in which the zigzag alkyl chain lies. It was adopted to express the steric features of those substituents having a branch, i.e., the bulkiness of the region around the C-1–C-2 atoms. For phenyl derivatives, this parameter corresponds to the thickness of the benzene plane. These steric parameters were calculated by the STERIMOL program developed by Verloop et al.,[5] which gives the coordinate

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Table I. Anticytokinin Activity and Physicochemical Properties of 4-(Alkylamino)-2-(methylthio)pyrido[2,3-*d*]pyrimidines

		activity				physicochemical parameters				
			log $1/I_{50}$							
no.	N^4 substit	I_{50}, μM	obsd	calcd[a]	Δ log $1/I_{50}$	W_{max}	W_u	L	π	σ_1
1	CH_3	2.92	−0.47	−0.67	0.20	2.04	1.90	3.00	0.50	−0.01
2	$CH_2CH_2CH_3$	0.27	0.56	0.47	0.09	3.49	1.90	4.92	1.50	−0.01
3	$CH_2(CH_2)_2CH_3$	0.27	0.57	0.42	0.15	4.54	1.92	6.17	2.00	−0.01
4	$CH_2(CH_2)_3CH_3$	0.65	0.19	0.37	−0.18	4.94	1.92	6.97	2.50	−0.03
5	$CH_2(CH_2)_4CH_3$	3.33	−0.52	−0.51	−0.02	5.96	1.92	8.22	3.00	−0.03
6	$CH_2(CH_2)_5CH_3$	4.07	−0.61	−0.94	0.33	6.39	1.92	9.16	3.50	−0.04
7	c-C_3H_5	4.90	−0.69	−0.46	−0.24	3.24	3.08	4.14	1.14	0.01
8	c-C_4H_7	0.72	0.14	−0.03	0.17	3.82	2.64	4.69	1.44	0.01
9	c-C_5H_9	0.49	0.31	0.05	0.26	4.15	2.68	4.97	1.85	0.00
10	c-C_6H_{11}	5.65	−0.75	−0.01	−0.74	3.49	3.16	6.17	2.26	0.00
11	$CH_2CH(CH_3)_2$	0.54	0.27	−0.39	0.66	4.45	3.16	4.92	1.80	−0.01
12	$CH(CH_3)CH_2CH_3$	0.86	0.07	−0.21	0.28	3.39	3.16	4.92	1.80	−0.01
13	$CH(CH_2CH_3)CH_2CH_3$	2.72	−0.44	−0.40	−0.04	4.46	3.49	4.92	2.30	−0.01
14	$CH_2CH(CH_2CH_3)(CH_2)_3CH_3$	15.85	−1.20	−0.97	−0.23	5.96	3.18	8.22	3.80	−0.01
15	$CH_2CH{=}CH_2$	0.40	0.40	0.35	0.06	3.78	1.90	5.11	1.20	0.02
16	$CH_2C_6H_5$	25.17	−1.40	−1.32	0.08	6.02	2.56	5.28	2.18	0.03
17	$CH_2CH_2OCH_3$	2.53	−0.40	−0.33	0.07	4.44	1.92	6.03	0.02	0.00
18	$CH_2CH_2OCH_2CH_3$	7.59	−0.88	−0.34	−0.54	4.81	1.92	6.85	0.52	0.00
19	$CH_2(CH_2)_6CH_3$	nd[b]		−2.63		7.39	1.93	10.27	4.00	−0.03

[a] Values were calculated by eq 3. [b] Very weakly active and the activity could not be detected. The activity value is that predicted by eq 3.

Table II. Anticytokinin Activity and Physicochemical Properties of 4-Anilino-2-(methylthio)pyrido[2,3-*d*]pyrimidines

		activity				physicochemical parameters				
			log $1/I_{50}$							
no.	benzene substit	I_{50}, μM	obsd	calcd[a]	Δ log $1/I_{50}$	W_{max}	W_u	L	π	σ_1
20	H	15.85	−1.20	−0.79	−0.42	3.11	1.71	6.28	1.68	0.12
21	*p*-F	2.62	−0.42	−0.73	0.31	3.11	1.71	6.87	1.82	0.13
22	*p*-Cl	8.51	−0.93	−0.55	−0.38	3.11	1.80	7.74	2.39	0.15
23	*p*-Br	5.75	−0.76	−0.59	−0.17	3.11	1.95	8.04	2.54	0.15
24	*p*-Et	7.15	−0.86	−0.48	−0.39	3.17	1.91	8.33	2.70	0.10
25	*p*-OMe	9.42	−0.97	−0.99	0.02	3.11	2.02	8.20	1.66	0.11
26	*p*-OEt	5.42	−0.73	−0.77	0.04	3.36	2.07	9.02	2.06	0.09
27	*p*-Ac	8.96	−0.95	−1.19	0.24	3.13	2.02	8.28	1.13	0.18
28	*p*-CN	7.56	−0.88	−1.02	0.14	3.11	1.71	8.45	1.10	0.20
29	*m*-NO_2	3.55	−0.55	−0.64	0.06	5.90	1.71	6.87	1.40	0.20
30	*m*-Ac	13.15	−1.12	−0.90	−0.22	4.93	2.00	7.28	1.13	0.17
31	*m*-COOH	9.20	−0.96	−0.62	−0.34	4.93	1.71	7.13	1.36	0.17
32	*p*-NO_2	insol[b]	−0.90			3.11	1.71	7.66	1.40	0.23

[a] Values were calculated by eq 3. [b] Insoluble. The activity value is that predicted by eq 3.

Table III. Cytokinin Activity and Physicochemical Properties of 4-Anilino-2-(methylthio)pyrido[2,3-*d*]pyrimidines

		activity				physicochemical parameters				
			log $1/E_{50}$							
no.	benzene substit	E_{50}, μM	obsd	calcd[a]	Δ log $1/E_{50}$	W_{max}	W_u	L	π	σ_1
33	*m*-F	3.39	−0.53	−0.55	0.02	3.67	1.71	6.28	1.82	0.16
34	*m*-Br	2.38	−0.38	−0.40	0.02	4.76	2.13	6.42	2.54	0.17
35	*m*-I	5.20	−0.72	−0.34	−0.39	5.15	2.15	6.72	2.80	0.16
36	*m*-Me	1.58	−0.20	−0.51	0.31	4.20	2.06	6.36	2.24	0.11
37	*m*-Et	1.91	−0.28	−0.34	0.06	4.20	2.10	7.34	2.70	0.11
38	*m*-OH	5.44	−0.74	−0.79	0.05	4.16	1.71	6.28	1.01	0.14
39	*m*-CN	2.15	−0.33	−0.74	0.41	5.07	1.71	6.45	1.10	0.19
40	*p*-I	2.21	−0.35	−0.61	0.26	3.11	2.15	8.45	2.80	0.15
41	*p*-COOH	12.48	−1.10	−0.92	−0.19	3.11	1.71	8.13	1.36	0.18
42	*o*-Me	3.59	−0.56	−0.48	0.08	4.33	2.04	6.28	2.24	0.10
43	3,5-Cl_2	0.84	0.08	−0.02	0.10	4.48	1.87	6.28	3.10	0.21
44	3,5-Me_2	2.82	−0.45	−0.28	−0.17	4.20	2.06	6.36	2.80	0.10
45	2,5-Me_2	0.96	0.02	−0.29	0.30	4.20	2.06	6.36	2.80	0.09
46	3,4-Me_2	0.92	0.04	−0.29	0.32	4.20	2.06	7.09	2.80	0.09
47	2,4-Cl_2	0.95	0.02	0.02	0.00	4.48	1.87	7.74	3.10	0.18
48	*o*-Cl	nd[b]		−0.30		4.48	1.87	6.28	2.39	0.15

[a] Values were calculated by eq 3. [b] Very weakly active and the activity could not be detected. The activity value is that predicted by eq 3.

and van der Waals radii in angstroms (10^{-1} nm) of the component atoms of a substituent. Their difference from the original STERIMOL B parameters is that the definition is made in consideration of the conformational correspondence between substituents.

The hydrophobicity of the N^4 substituents in terms of the π value derived from the 1-octanol/water partition coefficient was estimated according to published meth-

Table IV. Development of Equation 1

const	W_{max}	$(W_{max})^2$	W_u	π	r^2	s	$F_{x,y}$[a]
−2.25 (1.67)[b]	1.34 (1.89)	−0.17 (2.22)			0.37	0.51	$F_{2,15} = 4.39$
−2.06 (1.46)	1.57 (2.41)	−0.20 (2.79)	−0.38 (2.01)		0.50	0.46	$F_{1,14} = 4.03$
−2.31 (2.07)	1.95 (3.68)	−0.28 (4.44)	−0.67 (3.79)	0.45 (3.09)	0.72	0.37	$F_{1,13} = 9.53$

[a] F statistic for the significance of the addition of each variable. [b] The figures in the parentheses indicate the t levels.

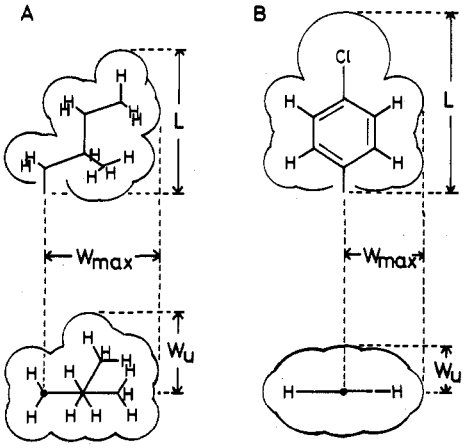

Figure 1. Schematic representation of the steric parameters for alkyl (A) and aromatic (B) side chains. Substituents used as models are 2-methylbutyl and *p*-chlorophenyl, respectively.

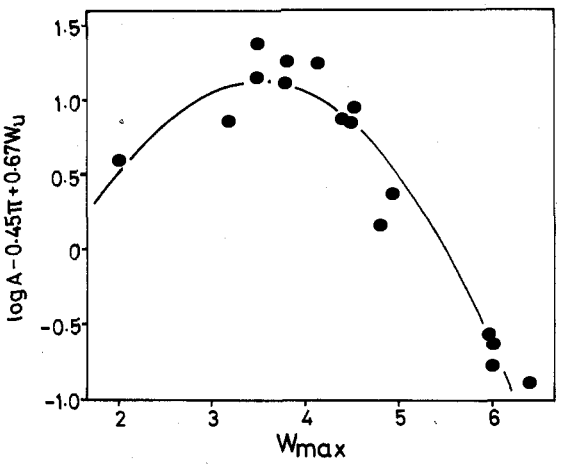

Figure 2. Parabolic relationship of anticytokinin activity of 4-alkylamino derivatives to W_{max} expressed by eq 1.

ods.[6,7] The electronic effect directed toward the N^4 atom was expressed by σ_I, whose values were taken from the literature.[8,9] The values for alkyl chains larger than *n*-butyl were approximated by that of *n*-butyl, those for cycloalkyls larger than cyclopropyl by that of cyclopropyl, and that for ethoxyethyl by that of methoxyethyl. For phenyl derivatives whose σ_I values are not available, we calculated the values from the following regression equation, where X denotes aromatic substituents, σ is Hammett's electronic constant, n is the number of compounds analyzed, r^2 is the squared correlation coefficient, s is the standard deviation, and the figures in the parenthesis indicate the t values:

$$\sigma_I(C_6H_4X) = \underset{(13.487)}{0.121}\sigma(X) + \underset{(39.249)}{0.121}$$

$$n = 15,\ r^2 = 0.94,\ s = 0.01$$

The equation was derived by using reported σ_I values for monosubstituted phenyls.[9]

Results

All of the N^4-alkyl derivatives tested showed anticytokinin activity, and the most active members were the *n*-butyl (**3**) and *n*-propyl (**2**) derivatives. The activity of compounds having smaller or larger substituents was weaker, suggesting participation of a steric factor. Considering the substituent parameters listed in Table I, multiple regression analysis gave eq 1 as the best correlation, where A expresses $1/I_{50}$. In this equation and in eq 2 and 3, n is the number of compounds included in the analysis, r^2 is the squared correlation coefficient, and s is the standard deviation. The figures in parentheses indicate the t values.

$$\log A = \underset{(3.68)}{1.95}W_{max} - \underset{(4.44)}{0.28}(W_{max})^2 - \underset{(3.79)}{0.67}W_u + \underset{(3.09)}{0.45}\pi - \underset{(2.07)}{2.32} \quad (1)$$

$$n = 18,\ r^2 = 0.72,\ s = 0.37$$

Table V. Squared Correlation Matrix for Variables Considered for the Development of Equation 1

	W_{max}	W_u	L	π
W_u	0.10			
L	0.70	0.06		
π	0.45	0.07	0.45	
σ_I	0.09	0.05	0.32	0.24

The parabolic relation of the activity to W_{max} is shown graphically in Figure 2, and it indicates that there is an optimum steric condition for the binding of alkyl derivatives to the receptor. The negative coefficient of the W_u term shows that the bulkier the N^4-alkyl substituents in this direction, the lower the activity. The eelctronic σ_I was not significant over the 95% level. The length parameter L is rather highly correlated with the W_{max} parameter, r^2 being 0.70. The replacement of the W_{max} by L, however, resulted in a poor correlation, and it was not significant over the 95% level when added to eq 1. Tables IV and V show the development of eq 1 and the correlation matrix of the parameters considered, respectively.

Of the N^4-phenyl derivatives, some of the compounds (compounds **20–31** in Table II) showed cytokinin agonistic activity, whereas others (compounds **33–47** in Table III) showed agonistic activity. Their activity in terms of I_{50} or E_{50} was much lower than that of the alkyl derivatives, and the range of the variation was narrower. Some compounds, like unsubstituted phenyl **20**, *p*-Cl **22**, *p*-Br **23**, *p*-I **40**, and *p*-COOH **41**, showed weak cytokinin activity in the absence of kinetin and weak anticytokinin activity in its presence. Thus the classification of these compounds as cytokinins or anticytokinins was rather difficult and somewhat arbitrary.

Examination of the biological data and physicochemical parameters suggested that the agonists and antagonists could be analyzed together. In eq 2, thus obtained for the phenyl series of compounds, the dependent variable, log

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Table VI. Development of Equation 2

const	W_{max}	$(W_{max})^2$	π	r^2	s	$F_{x,y}$[a]
−9.56 (4.16)[b]	4.46 (3.76)	−0.54 (3.61)		0.46	0.30	$F_{2,23} = 9.60$
−7.41 (3.67)	3.09 (2.89)	−0.37 (2.74)	0.25 (3.39)	0.62	0.25	$F_{1,22} = 13.04$

[a] F statistic for the significance of the addition of each variable. [b] The figures in the parentheses indicate the t levels.

Table VII. Squared Correlation Matrix for Variables Considered in the Development of Equation 2

	W_{max}	W_u	L	π
W_u	0.02			
L	0.35	0.01		
π	0.01	0.12	0.01	
σ_I	0.06	0.22	0.00	0.01

A, means log $1/I_{50}$ for compounds **20–31** and log $1/E_{50}$ for compounds **33–47**. The significance of the $(W_{max})^2$ term

$$\log A = 3.09W_{max} - 0.37(W_{max})^2 + 0.25\pi - 7.41 \quad (2)$$
$$(2.89) \quad (2.74) \quad (3.39) \quad (4.18)$$
$$n = 27,\ r^2 = 0.62,\ s = 0.25$$

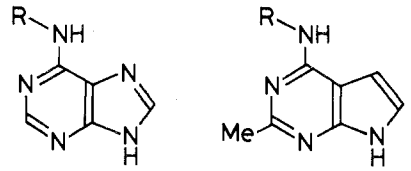
suggests that there is also an optimum steric condition for the phenyl series of compounds in terms of the maximum width of the N^4 substituents. The positive coefficient of the π term means that hydrophobic substituents favor activity irrespective of the quality of activity, agonistic or antagonistic. The lack of significance of the W_u term in eq 2 is probably due to the small variation in its value in the phenyl series of compounds. The electronic σ_I term was also insignificant, its level of significance being below 90%. Table VI shows the development of eq 2 and Table VII the independence of the variables considered.

The overall profiles of eq 1 and 2 are very similar to each other; i.e., the π terms are commonly important with very close coefficient values and the activities are parabolically related to the W_{max}. Thus, we combined them and explored the common correlations to obtain eq 3 as the best one. In this equation the W_{max} parameter was separately

$$\log A = 2.11W_{max}^{R} - 0.29(W_{max}^{R})^2 + 1.15W_{max}^{Ph} -$$
$$(5.34) \quad (6.46) \quad (2.59)$$
$$0.12(W_{max}^{Ph})^2 - 0.63W_u + 0.41\pi - 2.78 \quad (3)$$
$$(2.13) \quad (4.80) \quad (5.56) \quad (3.27)$$
$$n = 45,\ r^2 = 0.69,\ s = 0.30$$

considered for the alkyl and phenyl series of compounds and that for the former was marked by a superscript R and that for the latter by Ph. The correlations with the single, merged W_{max} gave poorer results, probably reflecting a somewhat different mode of interaction between the two series of compounds due to the different molecular shape. The situation is very similar to that observed for another class of cytokinin agonistic and antagonistic compounds N^4-substituted 4-amino-2-methylpyrrolo[2,3-d]pyrimidines.[3] The thickness W_u was found to be commonly significant through both series. The development of eq 3 is shown in Table VIII.

The compounds whose activity could not be determined exactly because of very low activity or sparing solubility were not included in the analyses, but their predicted activities were calculated by eq 3 and are listed in Tables I–III.



R: Alkyl or substituted phenyl

Figure 3. Structures of N^6-substituted adenylate cytokinins (left) and N^4-substituted 4-aminopyrrolo[2,3-d]pyrimidine anticytokinins (right).

Discussion

The final eq 3 indicates that the activity of the alkyl series of compounds is sterically governed by the maximum width W_{max} parameter and the thickness W_u in the direction parpendicular to the W_{max}. The optimum W_{max} value is calculated to be 3.6 Å. The corresponding optimum maximum width of N^6-alkyl substituents of adenylate cytokinins and N^4-alkyl substituents of another class of anticytokinins, 2-methylpyrrolo[2,3-d]pyrimidines (Figure 3), have been reported to be 5.2 and 4.5, respectively.[1,3] The somewhat smaller optimum value estimated for the present series of compounds may reflect constraints inherent in the bulkier molecular shape due to the fused 6–6 membered ring system of the base moiety. The competitive nature of the anticytokinins has been shown kinetically;[3,4] i.e., they are compounds that share the site of action with each other and with cytokinins. The N^4 substituents of the pyrido[2,3-d]pyrimidines are thus considered to be located closer to the spatial wall in the W_{max} direction of the common receptor cavity than those of the smaller fused 5–6 membered heterocycles, like adenine and pyrrolo[2,3-d]pyrimidine. The bulkier SMe group at the 2-position may aggravate this, as inferred from the smaller optimum W_{max} (4.5) value for the 2-methylpyrrolo[2,3-d]pyrimidine derivatives than that (5.2) of 2-unsubstituted adenines. The negative coefficient of the W_u term in eq 3 is in agreement with this view; i.e., the thickness in the direction perpendicular to W_{max} seems to obstruct the closer accommodation or binding to the receptor cavity.

It was necessary to separate the W_{max} parameter into the W_{max}^{R} and W_{max}^{Ph} parameters for obtaining the best correlation eq 3 for the combined set of compounds. This result may be interpreted to reflect a fundamentally similar but slightly different mode of steric interaction with the receptor between the two series of compounds, explaining why all of the alkyl series of compounds exhibited antagonistic activity while the phenyl derivatives showed both agonistic and antagonistic activities depending on the structure and position of the benzene substituents. A different geometry of the binding between alkyl and phenyl derivatives has been documented in the analysis of the activity of 4-substituted 2-methylpyrrolo[2,3-d]pyrimidine

Table VIII. Development of Equation 3

const	W_{max}^{R}	$(W_{max}^{R})^2$	W_{max}^{Ph}	$(W_{max}^{Ph})^2$	W_u	π	r^2	s	$F_{x,y}$[a]
−0.59 (7.06)[b]	0.45 (3.86)	−0.08 (3.59)					0.27	0.44	$F_{2,42} = 7.63$
−1.16 (6.04)	0.65 (5.32)	−0.12 (5.07)				0.27 (3.22)	0.41	0.40	$F_{1,41} = 10.35$
−0.34 (1.29)	0.97 (7.46)	−0.17 (7.23)			−0.60 (4.10)	0.43 (5.26)	0.59	0.34	$F_{1,40} = 16.79$
−2.78 (3.27)	2.11 (5.34)	−0.29 (6.46)	1.15 (2.59)	−0.12 (2.13)	−0.63 (4.80)	0.41 (5.56)	0.69	0.30	$F_{2,38} = 6.59$

[a] F statistic for the significance of the addition of each variable. [b] The figures in the parentheses indicate the t levels.

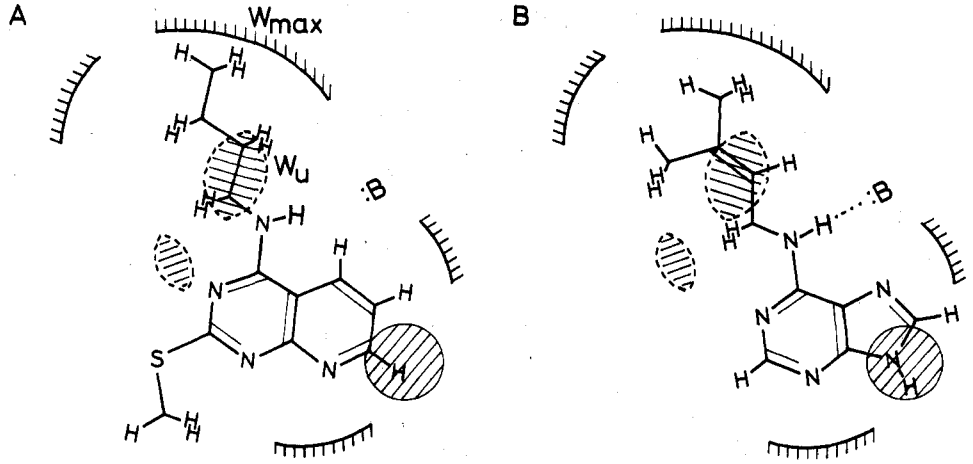


Figure 4. Receptor map for cytokinins. The compounds used as models are 4-(*n*-butylamino)-2-(methylthio)pyrido[2,3-*d*]pyrimidine (A) and 6-[(3-methyl-2-butenyl)amino]purine (6-isopentenyladenine) (B). The stippled solid lines show the steric interaction sites or spatial walls, the broken ovals are those located upward (or downward) from the plane of the page, the striped circle expresses the hydrophobic region of the receptor, and :B is the hydrogen acceptor site. The affixes W_{max} and W_u indicate the interaction sites of the present pyrido[2,3-*d*]pyrimidine derivatives suggested by eq 3 (and/or eq 1).

series of compounds.[3] Alteration of activity in the phenyl series from agonistic to antagonistic may reflect the conformational change of the receptor between active and inactive forms caused by the different mode of their binding from the alkyl derivatives. The aspect that, in hormonal action, agonist binding causes a conformational change of an otherwise inactive receptor to the active form and that antagonists are species that bind similarly to the receptor but do not cause the effective conformational change have been well documented in the previous study.[3]

The positive coefficient of the common term π may indicate the importance of the hydrophobicity of the molecule in the transport process. The electronic σ_I term was not significant. Previously a hydrogen-bonding interaction via the bridged NH group with a basic site on the receptor surface has been supposed for cytokinin-active adenine and urea derivatives[1] and pyrrolo[2,3-*d*]pyrimiidne anticytokinins,[3] an electron-withdrawing substituent enhancing activity. However, the simultaneous existence of moderately active compounds having an oxygen atom in place of NH as the bridged atom and thus lacking the hydrogen-donating capability has led us to suggest that the steric dimension of the molecule is of prime importance for activity, in other words the accommodation to the receptor cavity is the dominant factor, and the electronic effect, if any, intensifies the binding.[3] In view of these previous results and the fact that the hydrogen-bonding interaction is sensitive to the geometry of binding and distance, it is suggested that the present series of compounds binds to the receptor with less proper geometry for the electrostatic interaction than the other two. This seems fundamentally due to the difference in the molecular shape of the base moiety, since the structural variation of the side chains are essentially the same through these three classes of compounds.

On the basis of information obtained from the present and previous[1,3] structure–activity studies, an inclusive cytokinin receptor map was drawn in terms of physicochemical parameters, to which the *n*-butyl derivative **3**, one of the most active members in the present class, and 6-isopentenyladenine, a representative of the class of adenylate cytokinins, were accommodated (Figures 4A and 4B, respectively). The stippled solid lines represent the steric interaction sites or receptor walls located on the page plane and the broken ovals are those located upward (or downward). The affixes W_{max} and W_u show those interact significantly with the present series of compounds as suggested by eq 3 (and/or eq 1). In Figure 4A, the compound was accommodated so as to reflect this fact, the W_{max} and W_u regioins of the N^4 substituent being faced to the corresponding walls. The spatial walls without affixes and the basic site expressed by :B have been suggested by the previous studies,[1,3] and they do not interact significantly with the present compounds. The striped circle expresses the hydrophobic region of the receptor suggested by the previous study on *N,N'*-diphenylureas.[1] The drawings indicate the internal consistency between the results of quantitative structure–activity analyses obtained on the different classes of compounds.

Comparison of Figures 4A and 4B exhibits a somewhat different arrangement of the adenine and pyrido[2,3-*d*]-pyrimidine derivatives on the receptor, which is considered to originate from the different structural shapes of the base moiety. The smaller optimum W_{max} value and the lack of significance of the length L along the bond axis for the present series of compounds are understandable from the model drawings; the closer location of N^4 substituents to the receptor wall expressed by W_{max} makes the spatial wall in the L direction distant. The hydrogen atom at the N^6 atom of the adenine derivatives (Figure 4B) is thought to be located right in front of the basic site of the receptor at a proper distance, whereas that at the N^4 atom of pyrido[2,3-*d*]pyrimidine derivatives is not.

The spatial wall suggested by the W_u term in eq 3 and supposed to exist upward (or downward) from the zigzag alkyl plane may provide an explanation for the higher activity of the trans than cis isomers of zeatin [6-[(3-hydroxymethyl-2-butenyl)amino]purine] and 6-[(3-chloro-2-butenyl)amino]purine,[10] which could not be fully explained in the previous study[1] by the change of the W_{max} value in the cis form to a less suitable one. The deformation or distortion suggested by Hecht et al.[10] of the flat butenyl plane by steric interference of the 3-substituents with α-methylene in the cis form may cause unfavorable contact with the receptor wall denoted by W_u in Figure 4. Structure–activity insights summarized in the schematic drawings of Figure 4 can explain the reported activity of many other cytokinin derivatives. Thus, results may be of use for understanding the physiological aspects of cy-

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Table IX. Properties of Previously Unreported 4-Substituted 2-(Methylthio)pyrido[2,3-*d*]pyrimidines[a]

no.	mp, °C	formula	yield, %	λ_{max}, nm 0.2 N HCl	λ_{max}, nm 0.2 N NaOH	recrystn[b]
1	230	$C_9H_{10}N_4S$	74	264, 289 324, 337	267 335	A
2	178–179	$C_{11}H_{14}N_4S$	69	265, 291 326, 339	269 337	A
5	146–148	$C_{14}H_{20}N_4S$	54	265, 291 326, 339	269 336	A
6	155–158	$C_{15}H_{22}N_4S$	52	265, 292 326, 339	270 338	A
7	203–206	$C_{11}H_{10}N_4S$	14	267, 288 330, 341	271 341	A
13	189–191	$C_{13}H_{18}N_4S$	67	265, 292 326, 339	269 338	A
15	193–194	$C_{11}H_{12}N_4S$	57	264, 289 325, 338	268 336	A
17	135–138	$C_{11}H_{14}N_4OS$	41	264, 288 326, 338	267 336	A
18	135–138	$C_{12}H_{16}N_4OS$	32	265, 288 326, 339	268 335	A
19	92–93	$C_{16}H_{24}N_4S$	49	265, 292 326, 339	270 339	A
21	215–218[c]	$C_{14}H_{11}N_4SF \cdot H_2O$	71	238, 343	255, 372	A
22	267–269[c]	$C_{14}H_{11}N_4SCl \cdot {}^1/_2H_2O$	47	249, 344	257, 374	A
23	273–275[c]	$C_{14}H_{11}N_4SBr \cdot {}^1/_2CH_3OH$	38	248, 348	258, 374	B
24	219–244[c]	$C_{16}H_{16}N_4S \cdot H_2O$	51	249, 349	252, 369	A
25	221–224[c]	$C_{15}H_{14}N_4OS \cdot H_2O$	68	252, 353	254, 372	A
26	212–215[c]	$C_{16}H_{16}N_4OS \cdot {}^4/_5H_2O$	83	252, 355	255, 371	A
27	233–237[c]	$C_{16}H_{14}N_4OS \cdot {}^6/_7H_2O$	68	261, 353	406	A
28	242–244[c]	$C_{15}H_{11}N_5S \cdot H_2O$	85	257, 350	295 sh, 387	B
29	259–260[c]	$C_{14}H_{11}N_5O_2S$	40	248, 344	259, 372	A
30	220–223[c]	$C_{16}H_{14}N_4OS \cdot H_2O$	62	243, 344	255, 375	A
31	292	$C_{15}H_{12}O_2N_4S \cdot H_2O$	18	233, 344	370	A
32	223–224	$C_{14}H_{11}N_5O_2S \cdot {}^2/_3CH_2OH$	31	285, 363	246 sh, 448	A
33	243–245[c]	$C_{14}H_{11}N_4SF \cdot H_2O$	37	246, 343	256, 369	C
34	223–225	$C_{14}H_{11}N_4SBr \cdot {}^5/_6H_2O$	45	247, 346	255, 373	B
35	262–263	$C_{14}H_{11}N_4SI \cdot H_2O$	25	231, 345	255, 374	A
36	220	$C_{15}H_{14}N_4S \cdot {}^1/_3CH_3OH$	32	248, 345	253, 367	B
37	211–213[c]	$C_{16}H_{16}N_4S \cdot H_2O$	44	247, 347	252, 369	A
38	234	$C_{14}H_{12}ON_4S \cdot {}^1/_2H_2O$	76	249, 343	270 sh, 360	C
39	227–228[c]	$C_{15}H_{11}N_5S \cdot H_2O$	24	292, 344	272, 376	A
40	280–282[c]	$C_{14}H_{11}N_4SI \cdot H_2O$	34	250, 349	258, 372	B
41	>300	$C_{15}H_{12}O_2N_4S \cdot H_2O$	34	234, 257 355	376	A
42[d]	178–180[c]	$C_{21}H_{17}N_7O_7S$	77	233, 343	361	C
43	224–228[c]	$C_{14}H_{10}N_4SCl_2 \cdot H_2O$	56	248, 290 345	254, 273 378	A
44	229–232	$C_{16}H_{16}N_4S \cdot H_2O$	35	249, 348	251, 371	A
45	173–176	$C_{16}H_{16}N_4S \cdot H_2O$	61	233, 262 284 sh, 339	362	A
46	234–235[c]	$C_{16}H_{16}N_4S \cdot H_2O$	51	250, 352	252, 369	A
47	233–235[c]	$C_{14}H_{10}N_4SCl_2 \cdot H_2O$	46	256 sh, 284 sh 340	258 sh 365	A
48	125–128[c]	$C_{14}H_{11}N_4SCl \cdot H_2O$	60	234, 261 284, 340	363	A

[a] Analyses were carried out for C, H, and N and the results agreed with the calculated values within ±0.3%. [b] A, ethanol plus various amounts of water depending on the solubility of compounds; B, methanol plus various amounts of water; C, water. [c] Decomposed. [d] Isolated as picrate.

tokinin action in terms of interaction with the receptor, as well as for developing new structures that fit it. The comparative and quantitative interpretations of the common substituents in different classes of active compounds provide us with an insight into the role of the base moiety as well. The direct analysis of the compounds having various base moieties is virtually impossible. It is hampered by the difficulty of the synthesis and a fewer number of active structures.

Experimental Section

UV and ^1H NMR spectra were recorded on Shimadzu UV-200 and Hitachi R-22 spectrometers, respectively. All melting points were corrected.

Synthetic Procedure. 4-(Alkylamino)- and 4-anilino-2-(methylthio)pyrido[2,3-*d*]pyrimidines were prepared by refluxing a 1-butanol solution in which 4-chloro-2-(methylthio)pyrido[2,3-*d*]pyrimidine[4] and appropriate alkylamines or anilines were dissolved in a 1:1.5 molar ratio. The end of the reaction was determined by TLC. The reaction mixture was evaporated in vacuo to dryness and the residue was washed with water to give a solid, which was recrystallized from an appropriate solvent. Some compounds that could not be readily crystallized were isolated as picrate salts.

The physicochemical properties of previously unknown derivatives are summarized in Table IX. The analytical results for C, H, and N of these compounds were within ±0.3% of the theoretical values.

Bioassay Procedure. Compounds to be tested were added in different concentrations to the basal medium as specified previously.[11] The pH of the medium was adjusted to 5.6 with 1 N NaOH and autoclaved at 1.0 kg/cm^2 for 15 min. Three callus

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pieces of ca. 10 mg of fresh weight derived from *Nicotiana tabacum* L. var. Wisconsin No. 38 were implanted on the agar surface and maintained at 28 °C in darkness for 4 weeks, and then the average fresh weight was determined. The standard deviation of the activity measurements was within ±30%.

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Registry No. 1, 94993-30-3; **2**, 94993-31-4; **3**, 72564-68-2; **4**, 72564-67-1; **5**, 94993-32-5; **6**, 94993-33-6; **7**, 94993-34-7; **8**, 72564-71-7; **9**, 72564-70-6; **10**, 72564-73-9; **11**, 72564-69-3; **12**, 72564-72-8; **13**, 94993-35-8; **14**, 72564-65-9; **15**, 94993-36-9; **16**, 72564-63-7; **17**, 94993-37-0; **18**, 94993-38-1; **19**, 94993-39-2; **20**, 72564-74-0; **21**, 94993-40-5; **22**, 94993-41-6; **23**, 94993-42-7; **24**, 94993-43-8; **25**, 94993-44-9; **26**, 94993-45-0; **27**, 94993-46-1; **28**, 94993-47-2; **29**, 94993-48-3; **30**, 94993-49-4; **31**, 94993-50-7; **32**, 94993-51-8; **33**, 94993-52-9; **34**, 94993-53-0; **35**, 94993-54-1; **36**, 94993-55-2; **37**, 94993-56-3; **38**, 94993-57-4; **39**, 94993-58-5; **40**, 94993-59-6; **41**, 94993-60-9; **42**, 94993-62-1; **43**, 94993-63-2; **44**, 94993-64-3; **45**, 94993-65-4; **46**, 94993-66-5; **47**, 94993-67-6; **48**, 94993-68-7; CH_3NH_2, 74-89-5; $CH_3(CH_2)_2NH_2$, 107-10-8; $CH_3(CH_2)_5NH_2$, 111-26-2; $CH_3(CH_2)_6NH_2$, 111-68-2; c-$C_3H_5NH_2$, 765-30-0; $CH_3CH_2(CH_3CH_2)CHNH_2$, 616-24-0; $CH_2{=}CHCH_2NH_2$, 107-11-9; $CH_3O(CH_2)_2NH_2$, 109-85-3; $CH_3CH_2O(CH_2)_2NH_2$, 110-76-9; $CH_3(CH_2)_7NH_2$, 111-86-4; $C_6H_5NH_2$, 62-53-3; F-*p*-$C_6H_4NH_2$, 371-40-4; Cl-*p*-$C_6H_4NH_2$, 106-47-8; Br-*p*-$C_6H_4NH_2$, 106-40-1; Et-*p*-$C_6H_4NH_2$, 589-16-2; MeO-*p*-$C_6H_4NH_2$, 104-94-9; EtO-*p*-$C_6H_4NH_2$, 156-43-4; Ac-*p*-$C_6H_4NH_2$, 99-92-3; CN-*p*-$C_6H_4NH_2$, 873-74-5; NO_2-*m*-$C_6H_4NH_2$, 99-09-2; Ac-*m*-$C_6H_4NH_2$, 99-03-6; COOH-*m*-$C_6H_4NH_2$, 99-05-8; NO_2-*p*-$C_6H_4NH_2$, 100-01-6; F-*m*-$C_6H_4NH_2$, 372-19-0; Br-*m*-$C_6H_4NH_2$, 591-19-5; I-*m*-$C_6H_4NH_2$, 626-01-7; Me-*m*-$C_6H_4NH_2$, 108-44-1; Et-*m*-$C_6H_4NH_2$, 587-02-0; HO-*m*-$C_6H_4NH_2$, 591-27-5; CN-*m*-$C_6H_4NH_2$, 2237-30-1; I-*p*-$C_6H_4NH_2$, 540-37-4; COOH-*p*-$C_6H_4NH_2$, 150-13-0; Me-*o*-$C_6H_4NH_2$, 95-53-4; 3,5-Cl_2-$C_6H_3NH_2$, 626-43-7; 3,5-Me_2-$C_6H_3NH_2$, 108-69-0; 2,5-Me_2-$C_6H_3NH_2$, 95-78-3; 3,4-Me_2-$C_6H_3NH_2$, 95-64-7; 2,4-Cl_2-$C_6H_3NH_2$, 554-00-7; Cl-*o*-$C_6H_4NH_2$, 95-51-2; 4-chloro-2-(methylthio)pyrido[2,3-*d*]pyrimidine, 72564-62-6.

Cyclic Melanotropins. 9.[1] 7-D-Phenylalanine Analogues of the Active-Site Sequence

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The cyclic melanotropin Ac-Ser[1]-Tyr[2]-Ser[3]-Cys[4]-Glu[5]-His[6]-Phe[7]-Arg[8]-Trp[9]-Cys[10]-Lys[11]-Pro[12]-Val[13]-NH_2 is a highly potent agonist as determined in several melanocyte bioassays. In linear melanotropins, a D-Phe[7] substitution leads to increased potency and often prolonged biological activity. In order to determine if this substitution would have the same effect in cyclic melanotropins, we have prepared a series of these analogues. The D-Phe[7]-substituted cyclic melanotropins Ac-[Cys[4],D-Phe[7],Cys[10]]-α-MSH_{4-10}-NH_2 and Ac-[Cys[4],D-Phe[7],Cys[10]]-α-MSH_{4-11}-NH_2 were both more potent than their cyclic L-Phe[7]-containing counterparts in either the frog or lizard skin bioassay by more than a factor of 10. Neither peptide, however, exhibited prolongation of biological activity in either assay. Substitution of D-Phe[7] into the cyclic 4–12 and 4–13 sequences led to a slight or no increase in potency in both assays relative to the L-Phe[7] counterparts, but the activity of the melanotropins was ultraprolonged in each assay. Ac-[Cys[4],D-Phe[7],Cys[10]]-α-MSH_{4-12}-NH_2 was about equipotent to Ac-[Cys[4],D-Phe[7],Cys[10]]-α-MSH_{4-13}-NH_2, again demonstrating, as with certain linear and cyclic L-Phe[7]-containing melanotropins, that the C-terminal amino acid valine is not required for biological activity or for superpotency. Similar to the linear D-Phe[7] analogues that possessed ultraprolonged melanotropic activity, the 4–12 and 4–13 cyclic D-Phe[7] analogues also displayed the phenomenon of superagonism, which is a time-dependent increase in efficacy over that produced by an equipotent concentration of the native hormone. Cyclization of certain linear melanotropins resulted in analogues with increased resistance to biological degradation by serum enzymes or purified proteolytic enzymes. Further, incorporation of a D-Phe[7] into in the cyclic analogues led to melanotropins that were totally resistant to enzymatic inactivation by trypsin.

α-Melanocyte stimulating hormone (α-melanotropin, α-MSH) is a linear tridecapeptide that is synthesized in cells of the pars intermedia and the brain.[2] α-MSH stimulates melanin biosynthesis and melanosome dispersion within integumental melanophores,[3,4] and it appears to have numerous other physiological functions as well.[5,6] It has been proposed that a reverse turn is important for the biological activity of α-MSH at certain receptors partially on the basis of the much higher activity of linear analogues containing a D-Phe in the 7-position[7,8] (D-amino acids can stabilize reverse turns[9]). The importance of a reverse turn to the increased biological activity of α-MSH also has been suggested by the superpotency of cyclic

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