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QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP STUDY ON SOME DIHYDROPTERIDINE REDUCTASE INHIBITORS

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The dihydropteridine reductase (DHPR) inhibitory potencies of some 4-phenyltetrahydropyridines, 4-phenylpiperidines, and 4-phenylpyridines, are analyzed in relation to their physico-chemical and molecular properties. They are found to have significant correlation with Hammett constant σ and the van der Waals volume V_w. The correlation is linear with σ and parabolic with V_w. Hence, it is argued that DHPR inhibition involves dispersion interaction and is enhanced by electron donation from the substituents but hindered by steric effects produced by large substituents. It is also found that these electronic and steric effects are significant only when they are produced by substituents being at specific position in the molecules.

KEY WORDS: Dihydropteridine reductase inhibitors; 4-phenylpiperidines; 4-phenylpyridines; 4-phenyltetrahydropyridines; quantitative structure-activity relationship study.

INTRODUCTION

The enzyme, dihydropteridine reductase (DHPR) (EC 1·6·99·7), catalyzes the conversion of dihydrobiopterin to tetrahydrobiopterin, the required cofactor for enzymatic hydroxylation of L-tyrosine to L-dopa. Hence DHPR plays an important role in the biosynthesis of dopamine. The dopamine is supposed to be involved in the central control of motor functions in the central nervous system[1]. It was observed[2,3] in patients suffering from parkinsonism that dopamine was largely absent from their striatum and substantia nigra. This observation had led to the establishment of a link between the lowering of the dopamine level in brain and parkinsonism.

The compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP,**Ia**) was found to possess the nigrostriatal neurotoxic effect which causes irreversible parkinsonism in humans and primates by selective destruction of neurons in the substantia nigra.[4,5] To establish whether the toxic effects of MPTP in humans is related to the formation and/or clearance of metabolites, Gessner *et al.*[6] made a study on some hydroxylated derivatives of 4-phenyl-1,2,3,6-tetrahydropyridine (**I**), 4-phenylpiperidine (**II**), and 4-phenylpyridine (**III**). Since at present no simple model exists to evaluate the neurotoxic effects of such compounds, they were tested *in vitro* as inhibitors of DHPR[6]. The series of compounds studied[6] are listed in Table I and their inhibitory activities against DHPR of human liver and rat striatal synaptosomes are given in Table II.

In their study, Gessner *et al.*[6] found that the inhibitory potency of hydroxylated

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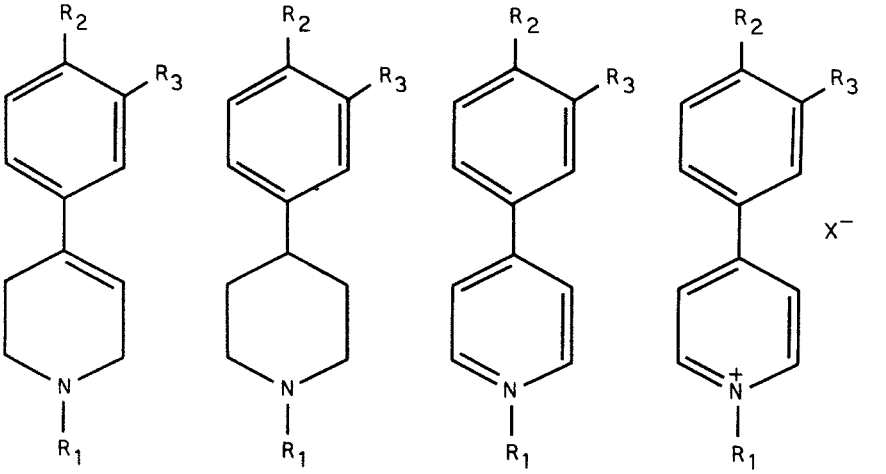

Ia : $R_1=CH_3$
$R_2=R_3=H$

derivatives increased with the number of hydroxyl substituents present on the phenyl ring and with oxidation of the nitrogen-containing ring. It is now desirable to determine the physico-chemical and molecular properties of the molecules that actually govern their DHPR inhibitory potency and the way in which the potency depends upon the physico-chemical properties. This quantitative structure-activity relationship (QSAR) study would provide a clear understanding of the mechanism of DHPR inhibition and a rationale for the better selection of the substituents. The present paper discusses the result of a QSAR study based on the data of Gessner *et al.*[6]

MATERIALS AND METHODS

In the QSAR study all the data of Gessner *et al.*[6] on DHPR inhibitors were analyzed in relation to physico-chemical and molecular properties of molecules characterized by parameters such as π, σ, and V_w. The parameter π is the hydrophobic constant of the substituent and is defined[7] as, $\pi = \log(P_X/P_H)$, where P_X and P_H are lipid-water partition coefficients of the substituted and unsubstituted reference compounds, respectively. It characterizes the hydrophobic interaction of the substituent with the receptor of the drug molecule and/or its share to the lipid solubility of the molecule on which depends the ability of the latter to cross the cell membrane and reach the receptor site. In evaluating the value of this parameter, the lipid is usually modelled by octanol.

The paramater σ is the Hammett constant[8] and refers to the electronic characteristics of substituents. Its positive or negative value denotes the electron-withdrawing or electron-donating character of the substituents, respectively. It usually represents

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TABLE I

Inhibitors of Dihydropteridine reductase: 4-phenyl-tetrahydropyridines (**I**), 4-phenylpiperidines (**II**), 4-phenylpyridines (**III**), and 4-phenylpyridinium salts (**IV**), and the physico-chemical constants of their important substituents.

Compound	R_1	R_2	R_3	π_2^a	$V_{w,2}^b$ ($10Å^3$)	σ_2^c
Ia	$-CH_3$	-H	-H	0.00	0.56	0.00
Ib	-H	-H	-H	0.00	0.56	0.00
Ic	-H	-Cl	-H	0.71	2.44	0.23
Id	$-CH_3$	-Cl	-H	0.71	2.44	0.23
Ie	$-CH_3$	-OH	-H	−0.67	1.37	−0.37
If	$-CH_3$	-OH	$-OCH_3$	−0.67	1.37	−0.37
Ig	-H	-OH	-H	−0.67	1.37	−0.37
Ih	-H	-OH	$-OCH_3$	−0.67	1.37	−0.37
Ii	$-CH_3$	-OH	-OH	−0.67	1.37	−0.37
Ij	$-COCH_3$	-OH	$-OCH_3$	−0.67	1.37	−0.37
Ik	$-COCH_3$	-OH	-OH	−0.67	1.37	−0.37
Il	-H	-OH	-OH	−0.67	1.37	−0.37
Im	$-COCH_3$	$-OCH_3$	$-OCH_3$	−0.02	3.04	−0.27
IIa	$-CH_3$	-H	-H	0.00	0.56	0.00
IIb	$-COCH_3$	-OH	$-OCH_3$	−0.67	1.37	−0.37
IIc	$-COCH_3$	-OH	-OH	−0.67	1.37	−0.37
IId	-H	-OH	-OH	−0.67	1.37	−0.37
IIe	$-CH_3$	-OH	$-OCH_3$	−0.67	1.37	−0.37
IIf	$-CH_3$	-OH	-OH	−0.67	1.37	−0.37
IIIa	-	-H	-H	0.00	0.56	0.00
IIIb	-	$-OCH_3$	$-OCH_3$	−0.02	3.04	−0.27
IIIc	-	-OH	-OH	−0.67	1.37	−0.37
IVa	$-CH_3$	-OH	-OH	−0.67	1.37	−0.37
IVb	$-CH_3$	-H	-H	0.00	0.56	0.00
IVc	$-CH_3$	-H	-H	0.00	0.56	0.00
IVd	$-CH_2-CH=CH_2$	-H	-H	0.00	0.56	0.00

[a] Hydrophobic constant for R_2-substituent. Taken from Reference 7.
[b] van der Waals volume for R_2-substituent. Calculated as described in Reference 10.
[c] Hammett constant for R_2-substituent. Taken from Reference 7.

the effects of hydrogen-bonding and charge-charge or charge-dipole interactions of compounds with the receptor. In the present analysis the value of σ and thos of π have been taken from the compilation of Hansch and Leo.[7]

The parameter V_w is the van der Waals volume and represents the size of the substituent or of the whole molecule, which is an important aspect of drug-receptor interaction. It characterizes the dispersion interaction between the drug molecule and the receptor and has been found to be very useful in QSAR studies.[9] In the present study it has been calculated as suggested by Moriguchi *et al.*[10] For a detailed study of the significance of various physico-chemical and molecular parameters and the importance of QSARs in enzymatic studies especially, readers should consult a recent article by Gupta.[11] The least square method is adopted to dervive all QSAR equations.[12]

RESULTS AND DISCUSSIONS

The multiple regression analysis using the least square method revealed significant correlations between the inhibition potencies of DHPR inhibitors and their physico-

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TABLE II
Observed and calculated DHPR inhibitory potencies of compounds shown in Table I. Observed values are those obtained by Gessner *et al.*[6]

Compound	pI_{50}[a] (hum.liv.)[b]		pI_{50}[a] (rat. str.)[c]	
	Obsd.	Calc. Eqn.(13)	Obsd.	Calc. Eqn.(14)
Ia	2.52	2.27	2.34	2.36
Ib	1.92	2.27	2.18	2.36
Ic	2.47	2.52	2.40	2.30
Id	2.57	2.52	2.19	2.30
Ie	5.52	5.61	5.59	5.52
If	5.03	5.61	5.24	5.52
Ig	5.23	5.61	5.47	5.52
Ih	5.14	5.61	5.00	5.52
Ii	5.47	5.61	5.60	5.52
Ij	5.30	5.61	4.04	5.52
Ik	6.55	5.61	6.60	5.52
Il	5.44	5.61	5.72	5.52
Im	2.96	2.62	2.64	2.67
IIa	1.64	1.38	1.92	1.55
IIb	4.57	4.71	4.36	4.72
IIc	5.28	4.71	5.51	4.72
IId	4.36	4.71	4.42	4.72
IIe	4.72	4.71	4.70	4.72
IIf	4.38	4.71	4.22	4.72
IIIa	2.62	2.27	2.72	2.36
IIIb	2.28	2.62	2.70	2.67
IIIc	6.42	5.61	6.55	5.52
IVa	6.20	5.61	5.80	5.52
IVb	2.00	2.27	1.92	2.36
IVc	2.25	2.27	2.47	2.36
IVd	2.03	2.27	2.13	2.36

[a] $pI_{50} = -\log I_{50}$ (I_{50}: molar concentration of inhibitor, inhibiting 50% of the enzyme activity).
[b] Against DHPR extracted from human liver
[c] Against DHPR extracted from rat striatal synaptosomes.

chemical and molecular properties. For the first 19 compounds of Table I, i.e., the derivatives of 4-phenyl-1,2,3,6-tetrahydropyridine (**I**) and 4-phenylpiperidine (**II**), the correlations obtained between the inhibition parameter pI_{50}(-log I_{50}; I_{50} being the molar concentration of the inhibitor, inhibiting 50% of the enzyme activity) and the hydrophobic constants π_1, π_2, and π_3 of R_1, R_2-, and R_3- substituents, respectively, were,

$$pI_{50}(\text{hum. liv}) = 3.44 - 0.28(\pm 0.81)\pi_1 - 2.45(\pm 0.88)\pi_2 - 0.96(\pm 1.32)\pi_3 - 0.95(\pm 0.81)I$$
$$n = 19,\ r = 0.896,\ s = 0.73,\ F_{4,14} = 12.26 \quad (1)$$

$$pI_{50}(\text{rat str.}) = 3.27 - 0.01(\pm 0.84)\pi_1 - 2.45(\pm 0.91)\pi_2 - 1.31(\pm 1.37)\pi_3 - 0.90(\pm 0.84)I$$
$$n = 19,\ r = 0.895,\ s = 0.75,\ F_{4,14} = 12.11 \quad (2)$$

where n is the number of data points, r is the correlation coefficient, s is the standard deviation, F is the F-ratio between the variances of calculated and observed activities,

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and the data within the parentheses are 95% confidence intervals. The parameter 'I' was used to indicate a difference between the derivatives of **I** and those of **II** with a value of zero for the former and unity for the latter.

Eqns (1) and (2) both represent significant correlations but indicate that π_1 and π_3 are insignificant at 95% confidence level. Hence if these two constants are deleted, the significance of correlations is hardly affected (Eqns. 3 and 4). Eqns (3) and (4) thus show that only π_2 is important in governing the DHPR inhibition. However, in all the

$$pI_{50}(\text{hum.liv.}) = 3.48 - 2.75(\pm 0.82)\pi_2 - 0.86(\pm 0.82)I$$
$$n = 19,\ r = 0.871,\ s = 0.75,\ F_{2,16} = 25.06 \quad (3)$$

$$pI_{50}(\text{rat.str.}) = 3.38 - 2.80(\pm 0.88)\pi_2 - 0.75(\pm 0.87)I$$
$$n = 19,\ r = 0.861,\ s = 0.80,\ F_{2,16} = 22.86 \quad (4)$$

equations the coefficient of π_2 has been negative, hence there would be a decrease in the inhibitory activity with an increase in the hydrophobicity of the R_2-substituent. This leads to the suggestion that not the hydrophobicity but the hydrophilicity of the R_2-substituent would be important in DHPR inhibition.

The hydrophilicity should be the function of the electronic parameter. We consequently find that the inhibition parameter is equally well correlated with σ_2 also (Eqns.5 and 6) and π_2 and σ_2 are also found to be mutually well correlated (r = 0.95). Since σ is the Hammett constant whose negative value denotes electron-donating

$$pI_{50}(\text{hum.liv.}) = 3.04 - 5.99(\pm 1.77)\sigma_2 - 0.75(\pm 0.80)I$$
$$n = 19,\ r = 0.874,\ s = 0.75,\ F_{2,16} = 25.84 \quad (5)$$

$$pI_{50}(\text{rat.str.}) = 2.95 - 5.99(\pm 1.98)\sigma_2 - 0.61(\pm 0.89)I$$
$$n = 19,\ r = 0.848,\ s = 0.84,\ F_{2,16} = 20.54 \quad (6)$$

character, Eqns. (5) and (6) both show that an increase in the electron-donating character of R_2-substituent will increase the inhibitory activity of the molecules. Like π_1 and π_3, σ_1 and σ_3 were also not found to be significant (Eqns. 7 and 8).

$$pI_{50}(\text{hum.liv.}) = 3.01 + 0.10(\pm 1.60)\sigma_1 - 5.69(\pm 2.77)\sigma_2 + 1.35(\pm 10.92)\sigma_3 - 0.75(\pm 0.88)I$$
$$n = 19,\ r = 0.875,\ s = 0.79,\ F_{4,14} = 9.81 \quad (7)$$

$$pI_{50}(\text{rat.str.}) = 2.96 - 0.35(\pm 1.79)\sigma_1 - 6.25(\pm 3.09)\sigma_2 - 0.63(\pm 12.18)\sigma_3 - 0.61(\pm 0.98)I$$
$$n = 19,\ r = 0.851,\ s = 0.88,\ F_{4,14} = 7.90 \quad (8)$$

Since π and σ were mutually correlated, they could not be used together in the regression analysis. However, the parameter V_w could be successfully incorporated, along with its square term, in Eqns. (5) and (6) and made a very significant improvement in the correlations (Eqns. 9 and 10). This parameter did not have any cor-

$$pI_{50}(\text{hum.liv.}) = 0.12 - 4.08(\pm 1.20)\sigma_2 + 4.61(\pm 1.59)V_{w,2} - 1.33(\pm 0.46)V_{w,2}^2 - 0.76(\pm 0.45)I$$
$$n = 19,\ r = 0.968,\ s = 0.41,\ F_{4,14} = 45.09 \quad (9)$$

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$$pI_{50}(\text{rat.str.}) = 0.33 - 4.05(\pm 1.73)\sigma_2 + 4.39(\pm 2.28)V_{w,2} - 1.31(\pm 0.66)V_{w,2}^2 - 0.69(\pm 0.65)I$$
$$n = 19,\ r = 0.937,\ s = 0.59,\ F_{4,14} = 21.59 \quad (10)$$

relation with π ($r = 0.18$) or σ($r = 0.12$). Hence we find that along with the electron-donating capability of the substituent, its size will also affect the DHPR inhibition. In fact, the parameter V_w itself, without σ, was found to have a good relationship with the inhibitory potency of the molecules (Eqns. 11 and 12). Since there is a parabolic correlation with V_w, the size of the substituent leads to an optimization in the

$$pI_{50}(\text{hum.liv.}) = 7.47(\pm 2.83)V_{w,2} - 2.21(\pm 0.80)V_{w,2}^2 - 0.58(\pm 0.95)I - 0.92$$
$$n = 19,\ r = 0.837,\ F_{3,15} = 11.69 \quad (11)$$

$$pI_{50}(\text{rat.str.}) = 7.23(\pm 3.11)V_{w,2} - 2.19(\pm 0.88)V_{w,2}^2 - 0.50(\pm 1.04)I - 0.71$$
$$n = 19,\ r = 0.811,\ s = 0.95,\ F_{3,15} = 9.59 \quad (12)$$

inhibitory activity, and the values of V_w corresponding to optimum activities against the enzymes from both the sources, as calculated from Eqns. 11 and 12, are almost identical (1.69×10 and 1.65×10 Å^3, respectively). Beyond these values, V_w will lead to a decrease in the inhibition potency due to the steric hindrance produced by larger R_2-substituents.

The positive coefficient of V_w in all the correlations, however, shows that untill V_w reaches its optimum value, there would be an increase in the inhibitory activity with an increase in the V_w value. Since V_w is not correlated with π, this increase in the inhibitory activity with the increase in V_w may be due to the dispersion interaction between the R_2-substituent and the receptor site.

The indicator parameter 'I' is not uniformly very significant in all the equations. However, in Eqns. (9) and (10) with which we are particularly concerned, the role of 'I' cannot be ignored. In these equations, it is significant at 95% confidence level and its negative coefficient shows that further hydrogenation of the nitrogen-containing ring will reduce the inhibitory activity approximately by a factor of 5. If 'I' is omitted from these equations, their r-values reduce to 0.938 and 0.913, respectively, and s-values increase to 0.55 and 0.67, respectively.

With 'I' equal to zero, the remaining 7 compounds of Table I, i.e., three 4-phenylpyridines (**III**) and four 1-methyl-4-phenylpyridinium salts (**IV**), could be successfully included in Eqns. (9) and (10) so as to have the new correlations,

$$pI_{50}(\text{hum.liv.}) = 5.25(\pm 1.31)V_{w,2} - 1.54(\pm 0.37)V_{w,2}^2 - 4.05(\pm 1.14)\sigma_2 - 0.89(\pm 0.45)I - 0.19$$
$$n = 26,\ r = 0.968,\ s = 0.44,\ F_{4,21} = 75.28 \quad (13)$$

$$pI_{50}(\text{rat.str.}) = 4.63(\pm 1.67)V_{w,2} - 1.38(\pm 0.47)V_{w,2}^2 - 4.26(\pm 1.45)\sigma_2 - 0.81(\pm 0.57)I + 0.20$$
$$n = 26,\ r = 0.947,\ s = 0.56,\ F_{4,21} = 43.64 \quad (14)$$

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TABLE III
Predicted DHPR inhibitory activities of some compounds with prospective R_2-substituents having large negative value of σ.

R_2	σ_2	$V_{w,2}$ ($10Å^3$)	pI_{50} (hum.liv.), Eqn.(13)		pI_{50} (rat. str.), Eqn.(14)	
			a	b	a	b
NH_2	−0.66	1.77	6.06	6.95	6.07	6.88
$NHNH_2$	−0.55	2.86	3.56	4.45	3.69	4.50
$NHCH_3$	−0.84	3.39	2.41	3.30	2.81	3.62
$NH(CH_3)_2$	−0.83	5.01	−10.11	−9.23	−8.47	−7.66

[a] For the derivatives of (**II**) for which the indicator parameter 'I' is equal to unity.
[b] For the derivatives of (**I**), (**III**), and (**IV**) for which the indicator parameter 'I' is equal to zero.

Eqns (9), (10), (13) and (14) account for 88 to 93% of the variance in the inhibitory activity and the F-value in all of them is significant at 99% level [$F_{4,14}$ (0.01) = 5.03; $F_{4,21}$ (0.01) = 4.37]. On the basis of these equations, we can finally say that small substituents with large negative value of σ will lead to good inhibitors of DHPR.

The hydroxyl group appears to be the ideal substituent. In Table III, we have predicted using Eqns. (13) and (14) the inhibitory potency of compounds with prospective R_2-substituents having large negative value of σ. Except for the first compound, all others are expected to possess very low DHPR inhibitory activity simply due to large steric effects. Hence the size of the substituent appears to play a very dominant role in DHPR inhibition by this class of inhibitors.

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