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Thyroxine Analogues. 23. Quantitative Structure–Activity Correlation Studies of in Vivo and in Vitro Thyromimetic Activities[1,2]

Stephen W. Dietrich, Michael B. Bolger, Peter A. Kollman, and Eugene C. Jorgensen*

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143.
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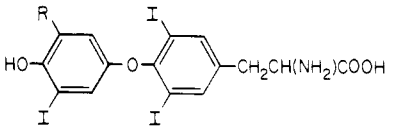
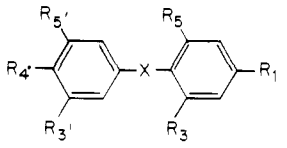
Quantitative structure–activity correlation studies of thyroid hormone analogues have been utilized to examine (1) in vivo rat antigoiter activities; (2) in vitro binding affinities to intact rat hepatic nuclei, solubilized rat hepatic nuclear protein receptors, and the plasma protein thyroxine binding globulin; and (3) correlations between in vivo antigoiter activities and in vitro binding to nuclear receptors. These studies provide a more precise elucidation of the relative importance of the physicochemical factors which influence thyromimetic activities. In particular, they (1) provide the first systematic QSAR examination of drug–receptor interactions and of the dependence of in vivo activity on such interactions; (2) demonstrate the importance of the interactive effects of the 3′ and 5′ substituents and of the 4′-OH with each other as well as with nuclear receptors in influencing binding affinity; (3) support the hypothesis that binding to nuclear receptors is the first step in initiating the events which lead to subsequent hormonal expression; (4) show that the free energy of binding to nuclear receptors can be factored into the contributing physicochemical properties of the substituents; and (5) suggest factors that need to be considered in designing new analogues.

Over the last 30 years, a large number of classical analogue structure–activity studies,[3-11] as well as a number of physicochemical studies utilizing x-ray crystallography,[12-16] NMR spectroscopy,[17,18] and theoretical MO calculations,[19] have consistently supported the structural and stereochemical dependence of thyromimetic activity for the thyroid hormones, 3,5,3′-triiodothyronine (T_3, **1**) and thyroxine (T_4, **2**), and their analogues (**3**). Recent studies have shown that (1) there are high-affinity, low-capacity binding sites for the thyroid hormones and analogues strongly associated with the chromatin of most cell nuclei[20-22] and (2) these nuclear binding sites are nonrandomly localized in chromatin fractions containing most of the endogenous RNA polymerase and template activity for RNA synthesis.[22] For rat hepatic cells these nuclear receptors have been solubilized with retention of binding affinity for the thyroid hormones and analogues[23] and have been characterized as acidic, nonhistone proteins of approximately 60 000 molecular weight.[20,21] In view of these studies and the qualitative correlation of in vivo activity with in vitro binding to such nuclear receptors,[24] it appears that this association between hormones and receptor is responsible for initiating the events which lead to subsequent hormonal effects. This has refocused attention on the role that the structural and stereochemical features of the hormone play in the hormone–receptor interactions. The actual study of such interactions has become possible as a result of the recent development of in vitro assays which measure the binding affinities of thyroid hormones and analogues to isolated intact rat hepatic nuclei,[24,25] to solubilized rat hepatic nuclear nonhistone proteins,[23,26,27] and to various purified plasma proteins.[28,29] The apparent equilibrium binding affinities measured in such in vitro assay systems reflect the thermodynamics of binding rather than actual biological activity. Their in vitro measurement avoids the difficulties arising from distribution, metabolism, and the sequence of events between binding and biological response in vivo and provides a unique opportunity to examine the physical origins of the binding interactions without such complications.



1, R = H
2, R = I



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On the basis of experimental, theoretical, and analogue activity studies,[3,4,18,19,30-33] the crucial features of the qualitative structure–activity relationships of the thyroid hormones and analogues can be briefly summarized as follows for the in vivo rat antigoiter assay and for in vitro binding to nuclear receptors.

(1) Thyromimetic activity is directly related to the ability of the 3 and 5 substituents to confine the diphenyl ether thyronine nucleus to two approximately energetically equal, readily interconvertible conformers in which the two phenyl rings are approximately mutually perpendicular.

Table I. Substituent Parameters Used in Structure-Activity Correlations

Substituent	σ^a	$\pi_{3\text{-PA}}{}^b$	$\pi_{BZ}{}^c$	INTERACT$_3$,^{d}	$E_s{}^e$	3′ size > I^d
H	0.00	0.00	0.00	0.00	1.24	0.0
F	0.06		0.14	1.37[f]	0.78	0.0
Cl	0.23		0.71	2.30[f]	0.27	0.0
Br	0.23		0.86	1.68[f]	0.08	0.0
I	0.18		1.12	0.75[f]	-0.16	0.0
OH	-0.37	-0.49	-0.67			0.0
NO_2	0.78		-0.28	8.29[f]		0.0
CH_3	-0.17	0.51	0.56	-0.51[g]	0.0[h]	0.0
C_2H_5	-0.15	0.97	1.02			0.127
i-C_3H_7	-0.15	1.30	1.53	-0.99[i]	-0.47[h]	0.253
n-C_3H_7	-0.13		1.55	-0.72[i]		0.405
i-C_4H_9	-0.12[j]	1.81[k]	2.00[k]			1.160
s-C_4H_9(±)	-0.12[j]		2.00[l]	-1.01[i]		0.707
t-C_4H_9	-0.20	1.68	1.98	-1.57[g]		0.920
c-C_6H_{11}	-0.22		2.51			2.46
C_6H_5	-0.01	1.89	1.96			2.22
CF_3	0.54		0.88			0.0
2′,3′-$(CH)_4$	0.04		0.99[m]			0.0

[a] σ_p values from ref 35 unless otherwise noted. [b] From the 3-substituted phenoxyacetic acid system; from ref 36 unless otherwise noted. [c] From the benzene system; from ref 35 unless otherwise noted. [d] See text. [e] From ref 37 unless otherwise noted. [f] CNDO/2 estimate; ref 2 and 38. [g] Experimental values; ref 2 and 38. [h] From ref 39. [i] CNDO/2 interpolation between Me and t-Bu experimental values; ref 2 and 38. [j] From ref 40. [k] Estimated. [l] Estimated from ref 41. [m] 0.99 = 5.4 (1.32) for 2′,3′-$(CH)_4$, since it was assumed that only approximately three of the four carbons could be fitting into the 3′ substituent hydrophobic pocket.

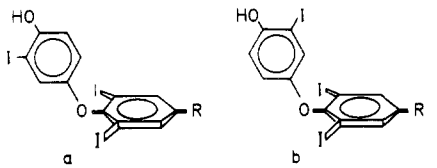


Figure 1. Distal (a) and proximal (b) conformers of T_3, R = $CH_2CH(NH_2)COOH$.

(2) For analogues monosubstituted ortho to the 4′-OH, it is the distal conformer (see Figure 1, a), with the 3′ substituent oriented away from the inner ring, and not the proximal conformer (see Figure 1, b), with the 3′ substituent oriented toward the inner ring, that is the active form of the analogue.

(3) Activity is optimal for lipophilic 3′ substituents and for a 4′-OH. (In in vivo studies the 4′-OH may be replaced by groups which can be metabolically converted to it, e.g., 4′-H or 4′-OCH_3.)

(4) Activity is inversely proportional to the size of the 5′ substituent.

(5) Activity is decreased by 2′ substitutions, although such substituents are capable of locking the diphenyl ether nucleus into a single conformation in which the 2′ substituent is distal to the inner ring.

For thyroxine analogues there are many similarities, but also significant differences, between the structure–binding affinity relationships for interactions with nuclear receptors and with plasma proteins. The qualitative structure–activity relationships for in vitro binding of analogues to the human plasma protein thyroxine binding globulin (TBG)[28,29] can be briefly summarized as follows for the 3′, 4′, and 5′ substituents: (1) binding is optimal for a 4′-OH; (2) maximal binding results from disubstitution ortho to the 4′-OH by electron-withdrawing 3′ and 5′ substituents.

On the basis of analogue in vivo antigoiter activities,[3,4] the binding affinities of analogues for rat hepatic intact nuclei[24,25] and solubilized nuclear protein,[26,33] and the pH dependence of in vivo binding of T_3 and T_4 to rat hepatic nonhistone nuclear proteins,[34] it appears that the 4′-OH is binding to nuclear receptors in its un-ionized form. In contrast, the relative binding affinities of analogues to TGB[28,29] indicate that it is probably the 4′-phenoxide ion that binds to this plasma protein.

Although the structure–activity relationships of the thyroid hormones and analogues have been investigated extensively over the last 30 years through the synthesis and testing of approximately 500 analogues, with very few exceptions such studies have involved qualitative rather than quantitative evaluation of activities. This lack of quantitative structure–activity studies has apparently been due to (1) lack of a substantial number of consistently reliable activities; (2) enormous variability of assay types; and (3) the complexity and number of physicochemical properties which affect the activities of the thyroid hormone analogues. The first two of these deficiencies have been in part eliminated by the extensive compilation and reevaluation of in vivo activities,[2-4] as well as by the recent extensive determination of accurate in vitro binding affinities to nuclear receptors[24-26] and plasma proteins.[29] The third set of problems has been clarified by the experimental, classical analogue and theoretical studies mentioned above.

In order to better understand the structure–activity relationships, as well as to investigate the specific molecular interactions involved in binding to nuclear receptors and to plasma proteins, we undertook a number of quantitative structure–activity correlation studies of various in vivo and in vitro thyromimetic activities. For the correlations of in vivo activities, we examined the rat antigoiter bioassay activities. We also examined the in vitro binding affinities of thyroid hormone analogues in three assay systems: (1) binding to intact rat hepatic nuclei; (2) binding to solubilized rat hepatic nuclei nuclear protein receptors; and (3) binding to the plasma protein thyroxine binding globulin. Lastly, we examined correlations between in vivo antigoiter activities and in vitro binding to nuclear receptors.

Substituent Parameters and Computational Details. Values for the substituent parameters utilized in these quantitative structure–activity relationship studies are presented in Table I. σ_p was used as an electronic parameter for 3′ and 5′ substituents, under the assumption[42a] that the electronic effect of a substituent on an ortho position should be comparable to that on the para. σ_{ortho} values for phenols[42b] were not utilized in that too many estimations of unknown values would have been necessary. $\sigma_{3'5'} = \sigma_{3'} + \sigma_{5'}$. Since the electronic effects of

3′ and 5′ substituents were assumed to be expressed through the ionization and/or hydrogen bonding of the 4′-OH, $\sigma_{3'5'}$ was set equal to 0.0 for 4′-H and 4′-OCH_3 analogues for correlations involving in vitro assays. This was not done for correlations involving in vivo activities, since it was assumed 4′-H and 4′-OCH_3 analogues would be metabolized to the corresponding 4′-OH analogues in vivo.

In agreement with the results of Kubinyi,[41] the choice of a system for π values was not found to be crucial to the overall results of the correlations; using π values from different systems did not substantially change the equations derived. The number of known π-substituent constants is largest for the benzene system (π_{BZ}), thus requiring the fewest estimations of unknown values. Hence, unless otherwise noted, $\pi = \pi_{BZ}$ was used for all 3, 5, 3′, and 5′ substituents. $\pi_{35} = \pi_3 + \pi_5$. $\pi_{3'5'} = \pi_{3'} + \pi_{5'}$.

We also used a number of indicator variables: $I(2')$ = an indicator variable for 2′ substitutions (there was not enough variation in 2′ substituents for use of a steric or hydrophobic parameter), 0 for 2′ substituent = H and 1 for 2′ substituent not = H [including 2′,3′-$(CH)_4$]; $I(4'\text{-H})$ = an indicator variable for 4′-H analogues, 1 for 4′ substituent = H and 0 for 4′ substituent not = H; $I(4'\text{-}OCH_3)$ = an indicator variable for 4′-OCH_3 analogues, 1 for 4′ substituent = OCH_3 and 0 for 4′ substituent not = OCH_3.

We developed the parameter INTERACT, which was derived from experimental data and molecular orbital calculations and is an estimate of the free-energy change (in kcal/mol) for orientation of the 4′-OH from cis to the 3′ substituent to cis to the 5′ substituent. Phenolic OH cis–trans energy differences for ortho-substituted phenols were used as estimates for these values. CNDO/2 MO calculations were utilized to estimate values whenever consistent, reliable experimental data were not available. Values of $\text{INTERACT}_{3'}$ listed in Table I are for a 3′ substituent with the 5′ substituent = H, $\text{INTERACT}_{5'}$ (i.e., for a 5′ substituent with the 3′ substituent = H) = $-\text{INTERACT}_{3'}$. Thus, INTERACT (3′,5′ disubstitution) = $\text{INTERACT}_{3'}$ + $\text{INTERACT}_{5'}$. Since the "INTERACT" effects of 3′ and 5′ substituents were assumed to be expressed through their influencing the hydrogen bonding capabilities of the 4′-OH by virtue of their orienting capabilities, INTERACT was set equal to 0.0 for 4′-H and 4′-OCH_3 analogues for correlations involving in vitro activities.

The parameter 3′ SIZE > I (based on bond distances, van der Waals radii, and conformational considerations[42c]) is an estimate of the *average* distance a 3′ substituent extends *out* from the 3′ position further than iodine. Iodine and smaller 3′ substituents were assigned values of 0.0 for this parameter. Utilizing the bond distances used in our molecular orbital studies of the thyroid hormones and analogues,[30,38] an estimate of 2.0 Å[43] for the van der Waals radii of a CH_2 or CH_3 (r_{vW,CH_3}) group, and van der Waals radii of Bondi,[44] 3′ SIZE > I values were calculated as follows. First, the distance (r_z) was calculated to the furthest out nonhydrogen atom of the 3′ substituent from the 3′-carbon atom. For 3′ substituent = H, r_z = 1.09 Å. For 3′ substituent = OH, r_z = distance to H. For 3′ substituent = c-C_6H_{11}, r_z = distance to $C_{4''}$ for a $C_{1''}$ equatorially substituted cyclohexane. For 3′ substituent = Ph, r_z = distance to H on $C_{4''}$ for a $C_{1''}$-substituted Ph. The appropriate heteroatom, H, or CH_2 van der Waals radius was then added to r_z to give $r_{3'}$ = approximate average van der Waals size of a 3′ substituent extending out from the 3′-carbon. 3′ SIZE > I values were finally calculated as follows.

$$3'\ \text{SIZE} > \text{I} = r_{3'} - r_{3'}(\text{I})$$
$$= r_{3'} - 4.12\ \text{Å}$$
$$= 0.0 \text{ if } r_{3'} - 4.12\ \text{Å} < 0.0$$

For acyclic 3′-alkyl substituents, 3′ SIZE > I values were calculated in a slightly different manner in order to take into account the conformational flexibility of the 3′-alkyl substituents.

First, we define $r_z(n)$ = calculated distance to the furthest carbon for an alkyl chain n carbons long in a fully extended, staggered conformation; $r_{3'}(n)$ = calculated $r_{3'}$ value for an alkyl chain n carbons long in a fully extended, staggered conformation = $r_z(n) + r_{vW,CH_3} = r_z(n) + 2.0$ Å; 3′ SIZE > I(n) = calculated 3′ SIZE > I value for an alkyl chain n carbons long in a fully extended, staggered conformation = $r_{3'}(n) - r_{3'}(\text{I}) = r_{3'}(n) - 4.12$ Å = 0.0 if $r_{3'} - 4.12$ Å < 0.00. In particular, calculated values are

$$3'\ \text{SIZE} > \text{I}(n = 1) = 0.00\ \text{Å}$$
$$3'\ \text{SIZE} > \text{I}(n = 2) = 0.38\ \text{Å}$$
$$3'\ \text{SIZE} > \text{I}(n = 3) = 1.74\ \text{Å}$$

A threefold conformational rotation for branching at the carbon α to the 3′ carbon was then examined in order to calculate the *average* distance a particular 3′ acyclic alkyl substituent extends out from the 3′-carbon. For example, a 3′-CH_3 substituent, for the three staggered α-carbon rotamers, extends out (in a particular direction) as a carbon chain n = 1 carbons long for all three rotamers. Hence

$$3'\ \text{SIZE} > \text{I}(CH_3) = [3'\ \text{SIZE} > \text{I}(n = 1) + 3'\ \text{SIZE} > \text{I}(n = 1) + 3'\ \text{SIZE} > \text{I}(n = 1)]/3$$
$$= (0.00\ \text{Å} + 0.00\ \text{Å} + 0.00\ \text{Å})/3 = 0.00\ \text{Å}$$

A 3′-Et substituent, for the three staggered α-carbon rotamers, extends out (in a particular direction) as a carbon chain n = 1 carbons long for $^2/_3$ of the rotamers and as a carbon chain n = 2 carbons long for $^1/_3$ of the rotamers. Hence

$$3'\ \text{SIZE} > \text{I}(\text{Et}) = [3'\ \text{SIZE} > \text{I}(n = 1) + 3'\ \text{SIZE} > \text{I}(n = 1) + 3'\ \text{SIZE} > \text{I}(n = 2)]/3$$
$$= (0.00\ \text{Å} + 0.00\ \text{Å} + 0.38\ \text{Å})/3 = 0.127\ \text{Å}$$

Similarly

$$3'\ \text{SIZE} > \text{I}(i\text{-Pr}) = [3'\ \text{SIZE} > \text{I}(n = 1) + 3'\ \text{SIZE} > \text{I}(n = 2) + 3'\ \text{SIZE} > \text{I}(n = 2)]/3$$
$$= (0.00\ \text{Å} + 0.38\ \text{Å} + 0.38\ \text{Å})/3 = 0.253\ \text{Å}$$

$$3'\ \text{SIZE} > \text{I}(n\text{-Pr}) = [3'\ \text{SIZE} > \text{I}(n = 1) + 3'\ \text{SIZE} > \text{I}(n = 1) + 3'\ \text{SIZE} > \text{I}(n = 3)]/3$$
$$= (0.00\ \text{Å} + 0.00\ \text{Å} + 1.74\ \text{Å})/3 = 0.580\ \text{Å}$$

For 3′-*i*-Bu, the 3′ SIZE > I (n = 3) value was multiplied by two to take into account the branching at the β-carbon atom,

$$3'\ \text{SIZE} > \text{I}(s\text{-Bu}) = [3'\ \text{SIZE} > \text{I}(n = 1) + 3'\ \text{SIZE} > \text{I}(n = 2) + 3'\ \text{SIZE} > \text{I}(n = 3)]/3$$
$$= (0.00\ \text{Å} + 0.38\ \text{Å} + 1.74\ \text{Å})/3 = 0.707\ \text{Å}$$

$$3'\ \text{SIZE} > \text{I}(i\text{-Bu}) = [3'\ \text{SIZE} > \text{I}(n = 1) + 3'\ \text{SIZE} > \text{I}(n = 1) + 2 \times 3'\ \text{SIZE} > \text{I}(n = 3)]/3$$
$$= (0.00\ \text{Å} + 0.00\ \text{Å} + 2 \times 1.74\ \text{Å})/3$$
$$= 1.160\ \text{Å}$$

An adjustment was also necessary for 3′-*t*-Bu.

$$3'\ \text{SIZE} > \text{I}(t\text{-Bu}) = [3'\ \text{SIZE} > \text{I}(n = 2) + 3'\ \text{SIZE} > \text{I}(n = 2) + r_{\text{vW,CH}_3}]/3 = (0.38\ \text{Å} + 0.38\ \text{Å} + 2.00\ \text{Å})/3 = 0.920\ \text{Å}$$

For 3′-*t*-Bu, $r_{\text{vW,CH}_3}$ = 2.0 Å was used in place of 3′ SIZE > I (n = 2) for the third α-carbon branch. The 3′-*t*-Bu is the only 3′-alkyl substituent with a third nonhydrogen α-carbon branch, and apparently this extra steric bulk, by interaction with the receptor and/or with the 4′-OH, adds an extra negative steric influence to this group. Although this is an adjustment factor to account for the apparent steric bulk of the 3′-*t*-Bu substituent, the necessity of its inclusion to "fit" the relative activity of this substituent provides additional insight into the strict conformational and size requirements of 3′ substituents; i.e., the third nonhydrogen α-carbon branch of this substituent does add an additional negative steric interaction beyond that attributable to the average distance the 3′ substituent extends out from the 3′-carbon further than iodine (see below).

All regression correlations of this study were performed utilizing standard multiple regression techniques.[45-48] For presentation of the regression equations and associated statistical data, values in parentheses after regression coefficients are 95% confidence intervals, r = the multiple least-squares regression coefficient, n = the number of data points used in the calculation of the regression equation, s = the overall standard deviation of the regression, $F_{\text{DFN,DFD}}$(calcd) = the calculated F statistic value for DFN degrees of freedom in the numerator and DFD degrees of freedom in the denominator, and (Z%) = the percent confidence level at which $F_{\text{DFN,DFD}}$(calcd) is significant: (<75%) for F(calcd) < F(75%); (>99.9%) for F(calcd) > F(99.9%). For $F(X\%) \leq F(\text{calcd}) < F(Y\%)$, ($Z$%) is obtained by interpolation linearly with log (%)[46]

$$\log(Z) = \log(Y) + [\log(X) - \log(Y)]\,\frac{F(\text{calcd}) - F(Y\%)}{F(X\%) - F(Y\%)}$$

On the basis of the qualitative in vivo structure–activity relationships of the thyroid hormone analogues (in particular, only 3′ substituent "size" > I decreases activity, all 5′ substituent "size" decreases activity), for analogues with $R_{3'} \neq R_{5'}$, the larger substituent was assumed to be the 3′ substituent and the smaller substituent was assumed to be the 5′ substituent.

Because of the complexity and number of physicochemical properties influencing thyromimetic activity and the large number of positions at which substituents can be varied (3, 5, 2′, 3′, 4′, 5′), careful consideration was given to the substituent parameters which were tested in the correlation studies in order to avoid chance correlations due to examining too many independent variables as compared to the often limited number of analogues available for analysis.[49] With this in mind, the known qualitative structure–activity relationships were useful in the actual selection of substituent parameters which might reflect the physicochemical origins of thyromimetic activity.

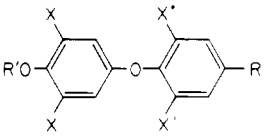
Results and Discussion

Rat Antigoiter Bioassay Activities. To date, there have been only a few quantitative SAR studies of in vivo activities of the thyroid hormones and analogues[41,42a,50-52] and none using in vitro activities. These previous studies, however, did not specifically restrict analysis to data from a single assay type in a single animal type; the studies examined thyroxine-like activity in amphibia,[50] mammalia,[50] rodents,[42a,51] and the rat.[41,52] Although various metabolic, antigoiter, and metamorphosis activities are often similar, significant deviations do occur between specific assay types.[3,4] In the previous studies, DL corrections were simply L = 2 × DL or were not made at all, and DL- or L-T_4 was used as the reference compound. In addition, activities had not been corrected from a weight to a molar basis. In order to conduct our studies of in vivo thyromimetic activity with the largest, most accurate possible set of experimental data for a single assay, we conducted our in vivo quantitative SAR correlations solely with rat antigoiter bioassay activities. Unless otherwise noted, all activities were corrected to a molar basis[2,53] and for comparison of L analogues with L-T_3 as the reference compound (see ref 2 for details).

The first attempt to quantitatively study thyromimetic activity (and one of the early quantitative studies of structure–activity relationships) was a study of Bruice et al.,[50] who derived equations relating thyroxine-like activity in amphibia and mammalia of the type of eq 1

$$\log(\%\ \text{thyroxine-like activity}) = k\Sigma f + c \qquad (1)$$

where $\Sigma f = f_X + f_X + f_{X'} + f_{X'} + f_{OR'}$ and f_X, $f_{X'}$, and $f_{OR'}$ are empirical constants for **4**.



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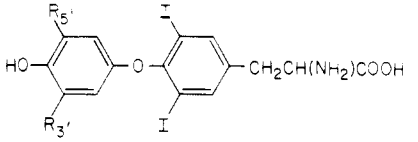
For the action of thyroxine analogues on rodents, Hansch and Fujita[42a] developed eq 2 for structure **5**. $R_{3'}$ and $R_{5'}$ = various halogen combinations. π values are from



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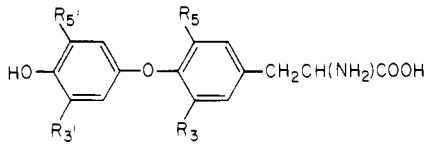
the 2-substituted phenol system. $\sigma = \sigma_p$ values. Thyroxine-like activity = A, relative to L-T_4 = 100.

$$\log(A) = -1.134\,(\pi_{3'5'})^2 + 7.435\,\pi_{3'5'} - 16.323\,\sigma_{3'5'} - 0.287 \qquad (2)$$

$$n = 9;\ r = 0.884;\ s = 0.660$$

Although the accuracy of biological data available and our conception of the SAR of thyroid hormone analogues have both changed immensely since this study, it was in part on the basis of eq 2 (predicting activity to be optimal for moderately lipophilic, electron-donating 3′,5′ substituents) that more extensive examination of the thyromimetic activities of 3′,5′-alkyl-substituted analogues was encouraged.

Much more recently Kubinyi and Kehrhahn[41,52] developed a large series of equations considering thyroxine-like activity of thyroxine analogues in the rat, as an example of a mixed approach to quantitative structure–activity relationships based on Hansch and Free–Wilson analysis. For analogues of the type **6**, they utilized π values from the benzene system; σ_p values for 3′ and 5′ substituents; $E_{s(3')}^{\text{cor}} = E_{s(3')} - E_{s(I)} = 0$ for $E_{s(3')} > E_I$ = an approximate measure of 3′ substituent size > I; $E_s' = E_{s(5')} + E_{s(3')}^{\text{cor}}$ = sum of 3′ and 5′ steric influences on activity; [I] and [CH_3] = Free–Wilson parameters for group contributions of I and CH_3, respectively, based on a_{Br} = 0.00.



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With these parameters, their results can be summarized with eq 3–5 for a wide variety of substituent types.

$$\log (A) = +1.673\ (\pm 0.324)\ \pi_{3'5'} - 1.242\ (\pm 0.969)\ \sigma_{3'5'} + 1.714\ (\pm 0.600)\ E_{s(3')}^{cor} + 0.856 \quad (3)$$

$n = 10; r = 0.984; s = 0.201$

$$\log (A) = +1.908\ (\pm 0.517)\ \pi_{3'5'} - 2.151\ (\pm 1.517)\ \sigma_{3'5'} + 1.871\ (\pm 0.700)\ E_{s(5')} - 1.598 \quad (4)$$

$n = 13; r = 0.946; s = 0.347$

$$\log (A) = +1.569\ (\pm 0.251)\ \pi_{3'5'} - 1.582\ (\pm 0.555)\ \sigma_{3'5'} + 1.493\ (\pm 0.299)\ E_s' + 0.176\ (\pm 0.159)\ [I] - 0.563\ (\pm 0.195)\ [CH_3] - 1.348 \quad (5)$$

$n = 23; r = 0.965; s = 0.250$

All three of these equations predict in vivo thyromimetic activity to be proportional to the sum of 3′ and 5′ substituent lipophilicities and electron-donating capabilities. In addition, it is predicted that (1) utilizing eq 3 and compound **6** ($R_3 = R_5 = I$; $R_{5'} = H$), 3′ substituent steric bulk greater than iodine (estimated by $E_{s(3')}^{cor}$) reduces activity; (2) utilizing eq 4 and compound **6** ($R_3 = R_5 = I$; $R_{3'}$ = substituents not sterically "larger" than I), any 5′ substituent bulk (estimated by $E_{s(5')}$) reduces activity; and (3) utilizing eq 5 and compound **6**, activity is reduced by the sum of steric bulk of 3′ substituents larger than iodine and of 5′ substituents (estimated by E_s') and is of the order $I > Br > CH_3$ for R_3 and R_5 substituents.

The QSAR studies of Hansch[42a] and Kubinyi[41,52] are important and represent the evolving understanding of in vivo thyromimetic activity structure–activity relationships. The choice and significance of the substituent parameters utilized in these previous studies will be examined below.

As an examination of the possibility of a parabolic dependence of in vivo thyromimetic activity on lipophilicity, a preliminary study[25] of in vivo rat antigoiter bioassay activities (BA) of 3,5-diiodothyronines (**7**, Table II) yielded eq 6, utilizing π values derived from the 3-substituted phenoxyacetic acid system, a simple L = 2 × DL correction factor, L-T_4 = 100% as reference compound, BA values *not* corrected to a molar basis, and $R_{3'}$ = substituents all with approximately the same electronic contributions in order to restrict analysis to an inspection of the π/π^2 parabolic relationship

$$\log (BA) = +1.358\ (\pm 0.541) + 2.405\ (\pm 1.076)\ \pi_{3'} - 1.192\ (\pm 0.652)\ \pi_{3'}^2 \quad (6)$$

$n = 8; r = 0.936; s = 0.383$

log (BA) maximized for ideal $\pi_{3'} = 1.01$; squared independent variable cross-correlation matrix π_3–$\pi_{3'}^2$ element = 0.847. The stepwise development of eq 6 is presented in Table III. Equation 6 is highly significant and supports the study of Hansch and Fujita,[42a] which predicts a parabolic dependence of activity on compound lipophilicity. Two factors, however, raise the question of whether this π/π^2 relationship truly represents a distribution phenomenon or, rather, whether it represents a steric effect for large 3′ substituents. (1) As first noted at the time of this study,[25] activity rises linearly with $\pi_{3'}$, up to $\sim\pi_{3'\text{-i-Pr}}$, but then *sharply* drops for larger $\pi_{3'}$ values (and as can be graphically seen in Figure 31.4, p 849, ref 3). This deviation from a distribution-related π/π^2 parabola can be seen in Table II from the deviations of log $(BA)_{calcd}$ from log $(BA)_{obsd}$. (2) As will be shown quantitatively below, and as was observed previously,[25] binding of analogues to intact rat hepatic nuclei (where distribution should not be a factor) also peaks at $\sim\pi_{3'\text{-i-Pr}}$ and then *sharply* decreases for larger $\pi_{3'}$ values.

We developed the essentially equivalent eq 8–11 as the simplest, most general equations for predicting in vivo thyromimetic rat antigoiter bioassay activities (BA) for structures of the type **8** (Table IV). The representative stepwise development of eq 8 is presented in Table V. The independent variable squared cross-correlation matrix for the variables used in these equations is presented in Table VI.

$$\log (BA) = -2.836\ (\pm 0.547) + 1.354\ (\pm 0.243)\ \pi_{3S} + 1.344\ (\pm 0.234)\ \pi_{3'} - 1.324\ (\pm 0.338)\ 3'\ \text{SIZE} > I - 0.359\ (\pm 0.316)\ \pi_{5'} - 0.658\ (\pm 0.444)\ \sigma_{3'5'} - 0.890\ (\pm 0.409)\ I(4'\text{-OCH}_3) \quad (8)$$

$n = 36; r = 0.938; s = 0.304$

$$\log (BA) = -3.324\ (\pm 0.716) + 1.521\ (\pm 0.325)\ \pi_{3S} + 1.447\ (\pm 0.309)\ \pi_{3'} - 1.394\ (\pm 0.470)\ 3'\ \text{SIZE} > I - 0.423\ (\pm 0.422)\ \pi_{5'} - 0.949\ (\pm 0.614)\ \sigma_{3'5'} - 0.927\ (\pm 0.585)\ I(4'\text{-OCH}_3) \quad (8a)$$

$n = 44; r = 0.897; s = 0.445$

$$\log (BA) = -3.093\ (\pm 0.620) + 1.341\ (\pm 0.242)\ \pi_{3S} + 1.325\ (\pm 0.231)\ \pi_{3'} - 1.316\ (\pm 0.338)\ 3'\ \text{SIZE} > I + 0.242\ (\pm 0.218)\ E_{s(5')} - 0.721\ (\pm 0.435)\ \sigma_{3'5'} - 0.882\ (\pm 0.410)\ I(4'\text{-OCH}_3) \quad (9)$$

$n = 36; r = 0.938; s = 0.306$

$$\log (BA) = -2.836\ (\pm 0.547) + 1.354\ (\pm 0.243)\ \pi_{3S} + 1.344\ (\pm 0.234)\ \pi_{3'5'} - 1.324\ (\pm 0.338)\ 3'\ \text{SIZE} > I - 1.703\ (\pm 0.435)\ \pi_{5'} - 0.658\ (\pm 0.444)\ \sigma_{3'5'} - 0.890\ (\pm 0.409)\ I(4'\text{-OCH}_3) \quad (10)$$

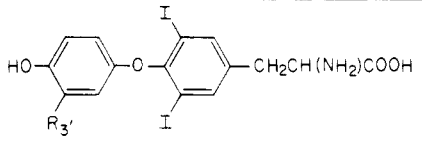
$n = 36; r = 0.938; s = 0.304$

$$\log (BA) = -3.916\ (\pm 0.772) + 1.288\ (\pm 0,256)\ \pi_{3S} + 1.226\ (\pm 0.232)\ \pi_{3'5'} - 1,257\ (\pm 0.356)\ 3'\ \text{SIZE} > I + 1.062\ (\pm 0.301)\ E_{s(5')} - 0.932\ (\pm 0.468)\ \sigma_{3'5'} - 0,839\ (\pm 0.436)\ I(4'\text{-OCH}_3) \quad (11)$$

$n = 36; r = 0.928; s = 0.327$

On the basis of eq 8 and 9, we arrive at the following conclusions concerning in vivo rat antigoiter bioassay activities.

Table II. Data Used in the Formulation of Equation 6 Correlating Rat Antigoiter Activities (BA) for Thyroid Hormone Analogues (7)



7

Data point no.	$R_{3'}$	BA (%), obsd[a]	Log (BA) Obsd	Calcd[b]	Dev
1	OH	1.5	0.176	−0.107	0.283
2	H	10	1.000	1.358	−0.358
3	Me	85	1.929	2.274	−0.345
4	Et	550	2.740	2.569	0.172
5	*i*-Pr	1000	3.000	2.469	0.531
6	*t*-Bu	120	2.079	2.033	0.046
7	*i*-Bu	60	1.778	1.805	−0.027
8	Ph	22	1.342	1.644	−0.302

[a] Uncorrected to a molar basis. L-T_4 = 100%, assuming L = 2 × DL; see ref 25. [b] Calculated with eq 6.

(1) Activity is enhanced by bulky, lipophilic 3 and 5 substituents (π_{35}). This is consistent with the concept of thyromimetic activity being directly related to the ability of the 3 and 5 substituents (by virtue of their size or bulk) to constrain the diphenyl ether thyronine nucleus to the two approximately energetically equal, readily interconvertible proximal and distal conformers. Because of the near collinearity of π and group size for 3 and 5 substituents, however, it is not possible to rule out or confirm an inherent hydrophobic effect for the 3 and 5 substituents.

(2) Although directly related to 3′ substituent lipophilicity ($\pi_{3'}$), activity is also decreased by 3′ substituent steric bulk which extends out from the 3′ position further than iodine (3′ SIZE > I). Kubinyi[41] used $E_{s(3')}^{cor}$ derived from $E_{s(3')}$ (see above and eq 3) as an estimate of 3′ substituent size or steric bulk greater than iodine. E_s is a measure of the steric effect of a substituent on a reaction site or binding position located *ortho to or on the next atom to* the substituent. For essentially symmetrical substituents (e.g., H, F, Br, I, CH_3, *t*-Bu), $E_{s(3')}$ is also a good measure of how far a substituent can extend out from say the 3′ position. For substituents with conformational flexibility to move by internal rotations away from the "ortho" position (e.g., *n*-Pr, c-C_6H_{11}, *i*-Bu, Ph), however, E_s will not reflect substituent "size" extending out from the position. Indeed, Kubinyi[41] was forced to exclude analogues with 3′-*i*-Bu and 3′-Ph substituents from his correlations utilizing $E_{s(3')}^{cor}$. The ability of 3′ SIZE > I to account for the negative, "greater than iodine" steric effects of Et, *i*-Pr, *n*-Pr, *t*-Bu, Ph, and *s*-Bu 3′ substituents indicates that this parameter more accurately (than $E_{s(3')}^{cor}$) represents this effect.

(3) Activity is enhanced by electron-donating 3′ and 5′ substituents ($\sigma_{3'5'}$), as previously observed by both Hansch[42a] and Kubinyi.[41]

(4) Activity is decreased by 5′ substituent lipophilicity ($\pi_{5'}$) or bulk ($E_{s(5')}$). The almost complete lack of orthogonality between $\pi_{5'}$ and $E_{s(5')}$ (see Table VI) allows prediction of the detrimental effect of 5′ substituents by either parameter (eq 8 and 9), although in most correlations $\pi_{5'}$ was found to be a slightly better predictor of the negative 5′ substituent effects than $E_{s(5')}$.

(5) Activity correlates well with an indicator variable [$I(4'\text{-}OCH_3)$] for the less active 4′-OCH_3 analogues which are metabolized to the naturally occurring 4′-OH analogues in vivo.

Following the example of Kubinyi[41] (eq 3–5), eq 10 and 11 utilize $\pi_{3'5'}$ instead of $\pi_{3'}$ alone. Inspection of the equations shows, however, that eq 10 is merely a linear combination of the variables of eq 8 and hence (since $\pi_{5'}$ and $E_{s(5')}$ are so well correlated) eq 11 is essentially equivalent to eq 9; i.e.

$$\log (BA) = a(\pi_{3'5'}) - b(\pi_{5'})$$

is equivalent to

$$\log (BA) = a(\pi_{3'}) - (b - a)(\pi_{5'})$$

and due to the $\pi_{5'}$–$E_{s(5')}$ lack of orthogonality

$$\log (BA) = c(\pi_{3'5'}) + d(E_{s(5')})$$

is [assuming $c(\pi_{5'}) \approx -e(E_{s(5')})$] essentially equivalent to

$$\log (BA) = c(\pi_{3'}) + (d - e)(E_{s(5')})$$

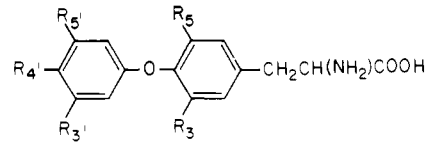
As only negative effects on activity are observed for 5′ substituents, the $+\pi_{3'}/-\pi_{5'}$ (or $+E_{s(5')}$) model, and not the $+\pi_{3'5'}/-\pi_{5'}$ (or $+E_{s(5')}$) model, makes more intuitive sense and is favored by the principle of parsimony; all things being equal, one accepts the simplest model.[54] Synthesis and testing of analogues with 5′ substituents which are considerably more orthogonal in $\pi_{5'}$ and $E_{s(5')}$ (see below) should help to resolve this ambiguity.

Because the coefficients of the E_s terms are nearly identical in eq 3 and 4 both E_s values were combined by Kubinyi[41] as $E_s' = E_{s(5')} + E_{s(3')}^{cor}$. Combination of originally separate variables in this manner can be misleading since this implies that the magnitudes and mechanisms of the effects described by the two original variables are equivalent and additive. Two examples of where such equivalence would hold are (1) if the influence of the lipophilicities of substituents X and Y on activity is distribution-related, then one would be justified in using $\pi_{XY} = \pi_X + \pi_Y$ as a measure of this effect; and (2) if the electronic influences of the 3′ and 5′ substituents of the thyroid hormone analogues on thyromimetic activity are expressed through their combined influences on the degree of ionization of the 4′-OH, then one is justified in using $\sigma_{3'5'} = \sigma_{3'} + \sigma_{5'}$ as a measure of this effect. Such equivalence may not hold, however, for analogues not yet studied. In addition, if the original model is wrong, then the relative importance of the variables may be different than in the original model. This is especially evident from our in vivo studies which show that Kubinyi's[41] $\pi_{3'5'}/E_{s(3')}^{cor}/E_{s(5')}$ model is essentially equivalent to a $\pi_{3'}/E_{s(3')}^{cor}/E_{s(5')}$ model; the former model, but not the latter model, allows the (potentially misleading) $E_s' = E_{s(3')}^{cor} + E_{s(5')}$ combination. The similar $\pi_{3'}$ (or $\pi_{3'5'}$) and π_{35} regression coefficients of eq 8–11 initially suggest that this could be used instead. Although essentially equivalent equations can be obtained in this manner, we favor the use of the equations which maintain the 3′ and 3,5 substituent lipophilicity contri-

Table III. Stepwise Development of Equation 6

Eq no.	Intercept	$\pi_{3'}$	$\pi_{3'}^2$	r	s	$F_{DFN,DFD}$(calcd)
7	1.186	0.594		0.572	0.813	$F_{1,6}$ = 2.91 (83.6% vs. mean)
6	1.358	2.405	−1.192	0.936	0.383	$F_{2,5}$ = 17.62 (99.4% vs. mean)
						$F_{1,6}$ = 22.09 (99.4% vs. eq 7)

Table IV. Data Used in the Formulation of Equations 8-11 Correlating Rat Antigoiter Bioassay Activities (BA) for Thyroid Hormone Analogues (8)



8

Data point no.	R_3	R_5	$R_{3'}$	$R_{5'}$	$R_{4'}$	BA_{obsd}[a]	Log (BA) Obsd	Calcd[b]	Dev
1	Me	H	I	H	OH	0.12	−0.921	−0.691	−0.230
2	Me	Me	Me	H	OH	0.54	−0.268	−0.455	0.188
3	Me	Me	Me	Me	OH	0.36	−0.444	−0.544	0.101
4	Me	Me	*i*-Pr	H	OH	3.60	0.556	0.500	0.056
5	Me	Me	*n*-Pr	H	OH	2.36	0.373	0.313	0.060
6	Me	Me	*s*-Bu	H	OH	2.91	0.464	0.512	−0.048
7	Me	Me	I	H	OH	0.90	−0.046	0.067	−0.113
8[c]	Me	I	H	H	OH	0.093	−1.032	−0.562	−0.469
9	Me	I	I	H	OH	6.24	0.795	0.825	−0.030
10[c]	Cl	Cl	Cl	H	OH	0.091	−1.041	−0.111	−0.930
11	I	Cl	I	Cl	OH	2.27	0.356	0.622	−0.266
12	Br	Br	*i*-Pr	H	OH	30.0	1.477	1.313	0.164
13	Br	Br	Br	H	OH	4.63	0.666	0.497	0.169
14[c]	Br	Br	Br	Br	OH	0.065	−1.187	0.036	−1.224
15	Br	Br	I	H	OH	16.87	1.227	0.879	0.348
16	Br	Br	I	I	OH	1.97	0.294	0.358	−0.064
17	I	Br	I	Br	OH	2.83	0.452	0.771	−0.319
18[c]	I	Br	I	H	OH	71.98	1.857	1.231	0.626
19[c]	I	H	I	H	OH	0.056	−1.252	0.067	−1.319
20	I	I	H	H	OH	0.81	−0.092	0.196	−0.287
21	I	I	Me	H	OH	14.47	1.160	1.060	0.100
22	I	I	Me	Me	OH	9.04	0.956	0.972	−0.015
23	I	I	Et	H	OH	93.5	1.971	1.498	0.473
24	I	I	*i*-Pr	H	OH	142.1	2.153	2.016	0.136
25	I	I	*i*-Pr	I	OH	55.36	1.743	1.496	0.247
26	I	I	*n*-Pr	H	OH	39.5	1.597	1.829	−0.232
27	I	I	*i*-Bu	H	OH	7.74	0.889	1.428	−0.539
28	I	I	*s*-Bu	H	OH	79.9	1.902	2.027	−0.125
29	I	I	*t*-Bu	H	OH	21.7	1.336	1.771	−0.435
30	I	I	Ph	H	OH	3.50	0.544	−0.102	0.646
31	I	I	NO_2	H	OH	0.18	−0.745	−0.694	−0.050
32	I	I	OH	H	OH	0.27	−0.569	−0.461	−0.107
33	I	I	F	H	OH	1.12	0.049	0.344	−0.295
34[c]	I	I	F	F	OH	0.43	−0.366	0.255	−0.621
35[c]	I	I	I	F	OH	6.03	0.780	1.493	−0.713
36	I	I	Cl	H	OH	4.88	0.688	0.999	−0.310
37	I	I	Cl	Cl	OH	3.80	0.580	0.592	−0.013
38	I	I	Br	H	OH	23.78	1.376	1.200	0.176
39[c]	I	I	Br	Br	OH	1.58	0.199	0.740	−0.542
40	I	I	I	H	OH	100.0	2.000	1.583	0.417
41	I	I	I	I	OH	18.1	1.258	1.062	0.195
42	I	I	*i*-Pr	H	OCH_3	19.0	1.279	1.127	0.152
43	I	I	*t*-Bu	H	OCH_3	2.35	0.371	0.881	−0.510
44	I	I	I	H	OCH_3	11.25	1.051	0.693	0.358

[a] See ref 2. Corrected to a molar basis, assuming L = DL/0.59. L-T_3 = 100 = reference compound. [b] Calculated using eq 8. [c] Not used in calculating eq 8-11.

Table V. Stepwise Development of Equation 8

Eq no.	Intercept	π_{35}	$\pi_{3'}$	3′ SIZE > I	$\pi_{5'}$	$\sigma_{3'5'}$	$I(4'\text{-}OCH_3)$	r	s	$F_{DFN,DFD}$(calcd)
12	0.047		0.628					0.511	0.698	$F_{1,34}$ = 12.01 (99.8% vs. mean)
13	−1.792	0.924	0.692					0.755	0.540	$F_{1,33}$ = 23.66 (>99.9% vs. eq 12)[a]
14	−2.454	1.129	1.158	−0.987				0.863	0.423	$F_{1,32}$ = 21.83 (>99.9% vs. eq 13)[b]
15	−2.698	1.237	1.262	−1.058			−0.707	0.894	0.381	$F_{1,31}$ = 8.42 (99.3% vs. eq 14)
16	−2.766	1.303	1.281	−1.221		−0.790	−0.798	0.926	0.326	$F_{1,30}$ = 12.36 (99.8% vs. eq 15)
8	−2.836	1.354	1.344	−1.324	−0.359	−0.658	−0.890	0.938	0.304	$F_{1,29}$ = 5.41 (97.2% vs. eq 16)

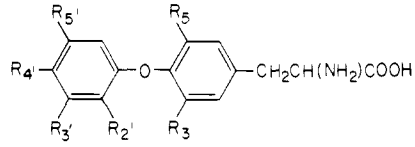
[a] $F_{1,33}$ (99.9%) = 13.04. [b] $F_{1,32}$ (99.9%) = 13.12.

butions to activity as separate parameters. As mentioned above, the π_{35} parameter (or its contribution to $\pi_{353'5'}$) probably reflects the ability of these substituents, due to their bulk, to confine the diphenyl ether thyronine nucleus to the proximal and distal conformers, rather than a distribution phenomenon. The use of the π_{35} parameter

Table VI. Independent Variable Squared Cross-Correlation Matrix for Equations 8-11

	$\pi_{3'}$	$\pi_{5'}$	$\pi_{3'5'}$	$\sigma_{3'5'}$	$E_{s(5')}$	3' SIZE > I	π_{35}	$I(4'\text{-}OCH_3)$
$\pi_{3'}$	1.000	0.001	0.745	0.046	0.007	0.416	0.009	0.044
$\pi_{5'}$		1.000	0.225	0.114	0.949	0.052	0.006	0.024
$\pi_{3'5'}$			1.000	0.000	0.176	0.205	0.002	0.012
$\sigma_{3'5'}$				1.000	0.055	0.098	0.004	0.013
$E_{s(5')}$					1.000	0.059	0.002	0.025
3' SIZE > I						1.000	0.024	0.009
π_{35}							1.000	0.042
$I(4'\text{-}OCH_3)$								1.000

Table VII. Data Used in the Formulation of Equations 17 and 24 Correlating in Vitro Binding Affinity (BN) and Free Energy of Binding [ΔG(BN)] to Intact Rat Hepatic Nuclei for Thyroid Hormone Analogues (9)



9

Data point no.	R_3	R_5	$R_{3'}$	$R_{5'}$	$R_{4'}$	$R_{2'}$	BN_{obsd}[a]	Log (BN) Obsd	Log (BN) Calcd[b]	Log (BN) Dev	$-\Delta G$(BN), kcal/mol[c] Obsd	$-\Delta G$(BN), kcal/mol[c] Calcd[d]	$-\Delta G$(BN), kcal/mol[c] Dev
1[e]	I	I	H	H	OH	H	0.3	−0.523	0.472	−0.995	8.883	10.294	−1.411
2	I	I	Cl	H	OH	H	6.2	0.792	1.286	−0.494	10.749	11.449	−0.700
3	I	I	Me	H	OH	H	13.5	1.130	1.114	0.016	11.228	11.205	0.023
4	I	I	Et	H	OH	H	21.0	1.322	1.487	−0.165	11.500	11.734	−0.234
5	I	I	*i*-Pr	H	OH	H	104.0	2.017	1.918	0.099	12.486	12.346	0.140
6	I	I	*t*-Bu	H	OH	H	38.5	1.586	1.622	−0.037	11.874	11.926	−0.052
7	I	I	*i*-Bu	H	OH	H	20.0	1.301	1.353	−0.052	11.470	11.544	−0.074
8	I	I	Ph	H	OH	H	2.0	0.301	0.016	0.285	10.052	9.648	0.404
9	I	I	c-C_6H_{11}	H	OH	H	1.4	0.146	0.355	−0.209	9.832	10.128	−0.296
10	I	I	Me	H	OH	Me	1.1	0.041	0.194	−0.153	9.684	9.900	−0.217
11	I	I	H	Me	OH	Me	0.1	−1.000	−0.937	−0.063	8.206	8.296	−0.089
12[e]	I	I	H	I	OH	Me	0.3	−0.523	−1.426	0.903	8.883	7.602	1.281
13	I	I	*f*	H	OH	*f*	8.0	0.903	0.687	0.216	10.906	10.600	0.306
14	I	I	I	H	OH	H	100.0	2.000	1.756	0.244	12.462	12.116	0.346
15	I	I	I	I	OH	H	12.5	1.097	0.778	0.319	11.181	10.728	0.452
16	I	I	Cl	Cl	OH	H	4.5	0.653	0.666	−0.013	10.551	10.569	−0.018
17	I	I	Me	Me	OH	H	6.2	0.792	0.625	0.167	10.749	10.511	0.237
18	I	I	*i*-Pr	*i*-Pr	OH	H	1.4	0.146	0.582	−0.436	9.832	10.450	−0.618
19	I	I	I	H	H	H	0.4	−0.398	−0.293	−0.105	9.060	9.209	−0.149
20	I	I	CF_3	H	H	H	0.2	−0.699	−0.568	−0.131	8.633	8.819	−0.186
21	I	I	Me	H	H	H	0.2	−0.699	−0.935	0.236	8.633	8.298	0.335
22	Br	Br	*i*-Pr	H	OH	H	36.0	1.556	1.045	0.512	11.832	11.106	0.726
23	Me	Me	Me	H	OH	H	0.1	−1.000	−0.768	−0.232	8.206	8.536	−0.329
24	Me	Me	Me	Me	OH	H	0.1	−1.000	−1.257	0.257	8.206	7.842	0.364
25	Me	Me	*i*-Pr	H	OH	H	0.7	−0.155	0.036	−0.191	9.405	9.676	−0.271
26	I	H	I	H	OH	H	0.5	−0.301	−0.126	−0.175	9.198	9.446	−0.249
27	I	H	I	I	OH	H	0.1	−1.000	−1.104	0.104	8.206	8.059	0.148

[a] From ref 24 and 25. No DL/L correction. On a molar basis. BN = $(K_A/K_{T_3}) \times 100$, where K_A = equilibrium association constants for analogue A. BN = relative affinity [relative to BN(T_3) = 100 as reference compound]. [b] Calculated using eq 17. [c] See text for derivation. [d] Calculated using eq 24. [e] Not used in calculating eq 17 and 24. [f] 2',3'-$(CH)_4$.

separate from the $\pi_{3'}$ (or $\pi_{3'5'}$) parameter assures that this distinction is maintained. Even with the use of the $\pi_{353'5'}$ parameter, we could find no parabolic dependence of in vivo activity on lipophilicity (in agreement with the results of Kubinyi[41]), even though the analogue $\pi_{353'5'}$ values encompass a range of 3.32 π units.

Comparing eq 8a (for all the data points of Table IV) with eq 8 (with eight deleted data points), it can be seen that deletion of the poorly fit data points does not lead to an overly significant change in the regression equation. The poor prediction of the activities of the analogues in Table IV which were not included in the regression calculation of eq 8–11 can be almost entirely ascribed to questionable analogue purity, structural identity, and/or activity determinations of these "early" analogue (see below). The correlations of eq 8–11 can really be considered quite good correlations considering that the synthesis and testing of the analogues were conducted by a large number of different investigating groups during an over 30-year period.

For the correlations presented below for in vitro binding of analogues to rat hepatic intact nuclei and solubilized nuclear protein, essentially identical equations could be obtained in almost every case for a $\pi_{3'}/\pi_{5'}$ model, for a $\pi_{3'}/E_{s(5')}$ model, for a $\pi_{3'5'}/\pi_{5'}$ model, or for a $\pi_{3'5'}/E_{s(5')}$ model (just as described above for the correlations involving in vivo antigoiter activities). Since in most cases the best correlations were obtained with the $\pi_{3'}/\pi_{5'}$ model, it is the model for which equations are presented, although the other models (because of lack of $\pi_{5'}$ and $E_{s(5')}$ orthogonality) cannot be ruled out (but see concluding remarks at the end of this paper).

An unsuccessful attempt was made to expand eq 8–11 to include 4'-deoxy analogues by inclusion of the $I(4'\text{-H})$ indicator available. This failure is apparently due to (1) uncertainty in the antigoiter activities of some of the

4′-deoxy analogues[2] and (2) unequal in vivo hydroxylation of different 4′-deoxy analogues (possibly because of varying 3′ and 5′ substituent bulk affecting the ease of in vivo hydroxylation).[2,53] The antigoiter activities of 4′-deoxy analogues certainly deserve further study, especially with respect to 3′ and 5′ substituent influences on in vivo hydroxylation.

Binding to Intact Rat Hepatic Nuclei. The measurement of the in vitro binding affinities of thyroid hormone analogues to intact isolated rat hepatic nuclei is based on the ability of the analogues to displace (under equilibrium conditions) radioactively labeled L-T_3 from the nuclei. The relative analogue binding affinities apparently reflect analogue binding to the nuclear receptors. Utilizing the data of Table VII, the correlation eq 17 for in vitro binding of analogues to intact rat hepatic nuclei (BN) was derived for structures of type **9**. The stepwise development of eq 17 and independent variable squared cross-correlation matrix are presented in Tables VIII and IX, respectively.

$$\log (\mathrm{BN}) = -3.292\ (\pm 0{,}660) + 1.680\ (\pm 0.290)\ \pi_{35} + 1.147\ (\pm 0.362)\ \pi_{3'} - 1.218\ (\pm 0.307)\ 3'\ \mathrm{SIZE} > \mathrm{I} - 0.873\ (\pm 0.289)\ \pi_{5'} - 0.920\ (\pm 0.432)\ I(2') - 2.049\ (\pm 0.411)\ I(4'\text{-H}) \quad (17)$$

$n = 25; r = 0.969; s = 0.280$

Just as was found for the correlations of in vivo rat antigoiter activities, binding of analogues to intact rat hepatic nuclei is enhanced by large, lipophilic 3,5 substituents (π_{35}) and is decreased by 5′ substituent size or lipophilicity (estimated here by $\pi_{5'}$) and by 2′ substitution [$I(2')$]. Of interest is that the 3′ substituent apparently binds in a hydrophobic pocket ($\pi_{3'}$) approximately the size of iodine (3′ SIZE > I). That this same size-limited, 3′ substituent hydrophobic effect is observed for in vivo and in vitro activities suggests that it is reflecting receptor binding and not distribution in both assay systems. The inherent loss of 4′-OH binding for 4′-deoxy analogues to the intact nuclei can be seen from the indicator variable [$I(4'\text{-H})$]. In contrast to the in vivo quantitative SAR, addition of a $\sigma_{3'5'}$ parameter is *not* significant. The large deviations between the calculated and observed log (BN) values for the two omitted data points (no. 1 and no. 12 of Table VII) appear to be due to the questionable purities or binding affinity determinations for the samples tested.

QSAR studies represent extrathermodynamic linear free energy correlations of activity with the physicochemical properties of the analogues. In vitro equilibrium association constants, K_A, such as measured for binding to intact nuclei or proteins, permit direct correlation of the apparent free energy of binding (ΔG) with the physicochemical properties of the analogues.

$$\Delta G = -RT \ln (K_A) \quad (23)$$

Using eq 23, $K_{T_3} = 6.1 \times 10^8\ \mathrm{M}^{-1}$ at $T = 310$ K,[55] and the log (BN) data of Table VII, eq 17 can be converted [with the resulting $-\Delta G$(BN) data of Table VII] to the equivalent eq 24.

$$-\Delta G(\mathrm{BN}) = +4.955\ (\pm 0.936) + 2.384\ (\pm 0.412)\ \pi_{35} + 1.626\ (\pm 0.514)\pi_{3'} - 1.727\ (\pm 0.435)\ 3'\ \mathrm{SIZE} > \mathrm{I} - 1.239\ (\pm 0.409)\ \pi_{5'} - 1.305\ (\pm 0.612)\ I(2') - 2.906\ (\pm 0.584)\ I(4'\mathrm{H}) \quad (24)$$

$n = 25; r = 0.969; s = 0.396$

Table VIII. Stepwise Development of Equation 17

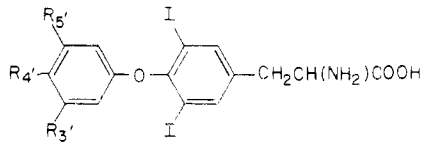
Eq no.	Intercept	π_{35}	$\pi_{3'}$	3′ SIZE > I	$\pi_{5'}$	$I(2')$	$I(4'\text{-H})$	r	s	$F_{\mathrm{DFN,DFD}}$(calcd)
18	−1.753	1.070						0.499	0.870	$F_{1,23} = 7.63$ (98.8% vs. mean)
19	−2.003	1.284					−1.471	0.697	0.736	$F_{1,22} = 10.11$ (99.5% vs. eq 18)
20	−2.370	1.215	0.436				−1.323	0.742	0.704	$F_{1,21} = 3.08$ (90.4% vs. eq 19)
21	−3.598	1.475	1.411	−1.121			−1.509	0.872	0.526	$F_{2,20} = 11.52$ (>99.9% vs. eq 19)[a]
										$F_{1,21} = 17.54$ (>99.9% vs. eq 20)[b]
22	−3.422	1.503	1.453	−1.288	−0.778		−1.782	0.934	0.396	$F_{1,19} = 16.41$ (>99.9% vs. eq 21)[c]
17	−3.292	1.680	1.147	−1.218	−0.873	−0.920	−2.049	0.969	0.280	$F_{1,18} = 20.05$ (>99.9% vs. eq 22)[d]

[a] $F_{2,20}(99.9\%) = 9.95$. [b] $F_{1,21}(99.9\%) = 14.59$. [c] $F_{1,19}(99.9\%) = 15.08$. [d] $F_{1,18}(99.9\%) = 15.38$.

Table IX. Independent Variable Squared Cross-Correlation Matrix for Equations 17 and 24

	$\pi_{3'}$	$\pi_{5'}$	π_{35}	3′ SIZE > I	$I(2')$	$I(4'\text{-H})$
$\pi_{3'}$	1.000	0.019	0.007	0.621	0.145	0.028
$\pi_{5'}$		1.000	0.006	0.047	0.003	0.043
π_{35}			1.000	0.042	0.040	0.040
3′ SIZE > I				1.000	0.031	0.031
$I(2')$					1.000	0.018
$I(4'\text{-H})$						1.000

Table X. Data Used in the Formulation of Equations 25, 26, and 33 Correlating in Vitro Binding Affinity (BS) and Free Energy of Binding [ΔG(BS)] to Solubilized Rat Hepatic Nuclear Protein Receptors for Thyroid Hormone Analogues (10)



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Data point no.	$R_{3'}$	$R_{5'}$	$R_{4'}$	BS_{obsd}[a]	Log (BS) Obsd	Calcd[b]	Dev	Calcd[c]	Dev	$-\Delta G$(BS), kcal/mol[d] Obsd	Calcd[e]	Dev
1	H	H	H	0.01	−2.000	−1.844	−0.156	−1.775	−0.225	6.969	7.275	−0.306
2	Me	H	H	0.225	−0.648	−0.906	0.258	−0.910	0.262	8.812	8.455	0.357
3[f]	*i*-Pr	H	H	0.492	−0.308	0.183	−0.491	0.108	−0.416	9.276	9.843	−0.567
4	*t*-Bu	H	H	0.335	−0.475	−0.476	0.001	−0.467	−0.008	9.048	9.059	−0.011
5	F	H	H	0.0136	−1.866	−1.610	−0.257	−1.559	−0.308	7.151	7.570	−0.419
6	Cl	H	H	0.118	−0.928	−0.654	−0.274	−0.678	−0.250	8.430	8.771	−0.341
7	Br	H	H	0.24	−0.620	−0.403	−0.217	−0.446	−0.174	8.851	9.088	−0.237
8	I	H	H	0.23	−0.638	0.032	−0.671	−0.044	−0.594	8.825	9.636	−0.810
9[f]	H	H	OH	0.082	−1.086	−0.304	−0.782	−0.222	−0.864	8.215	9.392	−1.178
10	Me	H	OH	3.30	0.518	0.634	−0.116	0.538	−0.020	10.403	10.430	−0.027
11	*i*-Pr	H	OH	89.15	1.950	1.723	0.227	1.630	0.321	12.355	11.918	0.437
12	*n*-Pr	H	OH	23.97	1.380	1.435	−0.055	1.359	0.020	11.577	11.549	0.028
13	*t*-Bu	H	OH	8.45	0.927	1.064	−0.138	1.073	−0.146	10.960	11.159	−0.199
14	F	H	OH	0.164	−0.785	−0.069	−0.716	−0.105	−0.681	8.625	9.553	−0.928
15	Cl	H	OH	3.73	0.572	0.886	−0.314	0.834	−0.262	10.475	10.832	−0.357
16	Br	H	OH	15.89	1.201	1.137	0.064	1.136	0.065	11.334	11.245	0.089
17	I	H	OH	100.0	2.000	1.572	0.428	1.596	0.404	12.423	11.872	0.551
18	Me	H	OCH_3	0.17	−0.770	−0.713	−0.057	−0.680	−0.090	8.646	8.769	−0.122
19	*i*-Pr	H	OCH_3	6.82	0.834	0.376	0.458	0.338	0.496	10.833	10.156	0.677
20	*t*-Bu	H	OCH_3	0.27	−0.569	−0.283	−0.286	−0.237	−0.332	8.920	9.372	−0.452
21	I	H	OCH_3	1.29	0.111	0.225	−0.115	0.186	−0.075	9.846	9.949	−0.102
22	NO_2	H	OH	0.225	−0.648	−0.773	0.125	−0.852	0.204	8.812	8.534	0.278
23	*s*-Bu(±)	H	OH	78.29	1.894	1.549	0.345	1.522	0.371	12.278	11.772	0.506
24	Me	Me	H	0.145	−0.839	−1.261	0.422	−1.347	0.508	8.552	7.859	0.693
25	I	I	OH	13.85	1.141	0.863	0.279	0.980	0.162	11.252	11.032	0.220
26	*i*-Pr	*i*-Pr	OH	1.10	0.041	0.754	−0.713	0.179	−0.138	9.752	9.940	−0.188
27	Cl	Cl	OH	3.71	0.569	0.436	0.134	0.762	−0.192	10.472	10.734	−0.262
28	Br	Br	OH	5.07	0.705	0.592	0.113	0.876	−0.172	10.657	10.891	−0.234
29	*i*-Pr	Cl	OH	52.56	1.721	1.274	0.447	1.624	0.097	12.042	11.910	0.132
30	*i*-Pr	Br	OH	21.95	1.341	1.178	0.163	1.436	−0.095	11.525	11.654	−0.129
31	*i*-Pr	I	OH	12.41	1.094	1.014	0.080	1.080	0.014	11.187	11.168	0.020
32	NO_2	H	H	0.038	−1.420	−2.313	0.893	−2.208	0.788	7.759	6.685	1.074
33	Me	Me	OH	0.845	−0.073	0.280	−0.353	−0.120	0.046	9.596	9.533	0.063
34[f]	Me	Me	OCH_3	0.335	−0.475	−1.068	0.593	−1.117	0.642	9.048	8.173	0.875
35[f]	*s*-Bu(±)	H	OCH_3	1.29	0.111	1.549	−1.438	1.522	−1.412	9.896	11.772	−1.875

[a] From ref 26 and 33. No DL/L correction. On a molar basis. BS = relative binding affinity [relative to BS(L-T_3) = 100 as reference compound] = $(K_A/K_{T_3}) \times 100$, where K_A = equilibrium association constant for analogue A. [b] Calculated using eq 25. [c] Calculated using eq 26. [d] See text for derivation. [e] Calculated using eq 33. [f] Not used in calculating eq 25, 26, and 33.

Equation 24 allows estimation of the kcal/mol contributions to the free energy of binding of various analogue structural features and physicochemical properties.

(1) Each π unit of the 3′ substituent is predicted to contribute 1.63 kcal/mol to the free energy of binding. This amounts to about 0.8 kcal/mol per CH_2 unit of a 3′-alkyl substituent, in excellent agreement with experimental estimations of hydrophobic bonding energetics.[56] For example, the experimental free energies for transfer of benzene, toluene, and ethylbenzene from liquid water to liquid hydrocarbon are approximately −4.6, −5.4, and −6.2 kcal/mol, respectively.[56]

(2) Each π unit of the 3 and 5 substituents contributes considerably more to the free energy of binding than would be expected on purely hydrophobic bonding, especially when compared to the smaller regression coefficient for $\pi_{3'}$. Since π and size are extremely well correlated for the 3 and 5 substituents used in deriving this equation, this probably reflects the ability of the 3 and 5 substituents, by virtue of their bulk, to confine the diphenyl ether thyronine nucleus to the proximal and distal conformers, although inherent hydrophobic bonding by the 3 and 5 substituents cannot be excluded.

(3) The 4′-OH apparently contributes about 2.9 kcal/mol

to the free energy of binding, a reasonable value for net hydrogen bond formation between the 4′-OH and a nuclear receptor.

Binding to Solubilized Rat Hepatic Nuclear Protein. Utilizing the data of Table X, the correlation eq 25 and 26 for in vitro binding affinities of analogues to solubilized rat hepatic nuclear protein (BS) were derived for analogues of structure **10**. The stepwise development of eq 25 and 26 and the independent variable squared cross-correlation matrix are presented in Tables XI and XII, respectively.

$$\log (BS) = -0.304\ (\pm 0.380) + 1.675\ (\pm 0.420)\ \pi_{3'} - 2.118\ (\pm 0.876)\ 3'\ \text{SIZE} > \text{I} - 0.634\ (\pm 0.406)\ \pi_{5'} - 1.540\ (\pm 0.375)\ I(4'\text{-H}) - 1.347\ (\pm 0.489)\ I(4'\text{-OCH}_3) \quad (25)$$

$$n = 31;\ r = 0.946;\ s = 0.400$$

$$\log (BS) = -0.222\ (\pm 0{,}430) + 1.546\ (\pm 0.445)\ \pi_{3'} - 1.904\ (\pm 0.825)\ 3'\ \text{SIZE} > \text{I} - 0.780\ (\pm 0.391)\ \pi_{5'} - 1.553\ (\pm 0.366)\ I(4'\text{-H}) - 1.323\ (\pm 0.447)\ I(4'\text{-OCH}_3) + 0.958\ (\pm 0.793)\ \sigma_{3'5'} - 0.114\ (\pm 0.109)\ \text{INTERACT} \quad (26)$$

$$n = 31;\ r = 0.960;\ s = 0.364$$

Utilizing eq 25, we can draw the following conclusions concerning in vitro binding of analogues to solubilized rat hepatic nuclear protein.

(1) Just as was found for binding of analogues to intact rat hepatic nuclei, the 3′ substituent apparently binds in a size-limited (3′ SIZE > I), hydrophobic ($\pi_{3'}$) pocket, while any 5′ substituent bulk or lipophilicity (estimated here by $\pi_{5'}$) is detrimental to binding.

(2) Indicator variables again reflect the inherent loss of 4′-OH hydrogen bonding with the receptor due to replacement with a 4′-H [I(4′-H)] or with a 4′-OCH_3 [I(4′-OCH_3)].

Addition of a $\sigma_{3'5'}$ term alone or of an INTERACT term alone to eq 25 is not significant (see Table XI). Simultaneous inclusion of both terms, however (to give eq 26), is significant at the 95.5% confidence level. The signs of the regression coefficients indicate that binding is enhanced by electron-withdrawing substituents which orient the 4′-OH toward the 5′ position. This *suggests* that the 4′-OH donates a hydrogen bond to the 5′ side of the nuclear receptor. This also suggests that the negative effect of 5′ substitution might be due to interference with 4′-OH hydrogen bond formation with the receptor and/or to direct steric interaction of the 5′ substituent with the receptor. These results are consistent with the results of our CNDO/2 and ab initio molecular oribital studies,[2,30] which support the model of 3′ and 5′ substituents interacting with and affecting the hydrogen bonding of the 4′-OH to the nuclear receptor.

The in vitro binding affinity to solubilized nuclear receptors of analogue no. 28 of Table X is fairly accurately predicted by eq 26, but the in vivo antigoiter activity of the same analogue (no. 39 of Table IV) is poorly predicted by eq 8. It is noteworthy that a sample of this 3,5-diiodo-3′,5′-dibromo analogue was newly synthesized and purified for testing in the in vitro assay after the in vivo activity of another sample of this analogue was found to be inconsistently low according to the in vivo SAR model. Data points no. 3 and no. 9 were omitted in deriving eq

Table XI. Stepwise Development of Equations 25 and 26

Eq no.	Intercept	$\pi_{3'}$	3′ SIZE > I	$\pi_{5'}$	I(4′-H)	I(4′-OCH_3)	$\sigma_{3'5'}$	INTERACT	r	s	$F_{\text{DFN,DFD}}$ (calcd)
27	0.689				−1.737				0.709	0.812	$F_{1,29}$ = 29.25 (>99.9% vs. mean)[a]
28	−0.165	0.760			−1.360				0.820	0.669	$F_{1,28}$ = 14.65 (>99.9% vs. eq 27)
29	−0.036	0.829			−1.533	−1.138			0.884	0.556	$F_{1,27}$ = 13.51 (99.9% vs. eq 28)
30	−0.393	1.433	−0.634		−1.388	−1.086			0.923	0.467	$F_{1,26}$ = 12.41 (99.9% vs. eq 29)
25	−0.304	1.675	−2.118	−0.634	−1.540	−1.347			0.946	0.400	$F_{1,25}$ = 10.35 (99.6% vs. eq 30)
31	−0.429	1.755	−2.114	−0.651	−1.465	−1.327	0.523		0.951	0.391	$F_{1,24}$ = 2.24 (82.9% vs. eq 25)
32	−0.182	1.567	−2.038	−0.678	−1.599	−1.352		−0.045	0.948	0.402	$F_{1,24}$ = 0.81 (<75% vs. eq 25)[c]
26	−0.222	1.546	−1.904	−0.780	−1.553	−1.323	0.958	−0.114	0.960	0.364	$F_{1,23}$ = 4.67 (95.7% vs. eq 31)
											$F_{1,23}$ = 6.26 (97.6% vs. eq 32)
											$F_{2,23}$ = 3.62 (95.5% vs. eq 25)

[a] $F_{1,29}(99.9\%) = 13.39$. [b] $F_{1,28}(99.9\%) = 13.50$. [c] $F_{1,24}(75\%) = 1.39$.

Table XII. Independent Variable Squared Cross-Correlation Matrix for Equations 25, 26, and 33

	$\pi_{3'}$	$\pi_{5'}$	$\sigma_{3'5'}$	3' SIZE > I	I(4'-H)	I(4'-OCH_3)	INTERACT
$\pi_{3'}$	1.000	0.038	0.119	0.602	0.121	0.036	0.291
$\pi_{5'}$		1.000	0.006	0.007	0.083	0.052	0.099
$\sigma_{3'5'}$			1.000	0.103	0.020	0.007	0.329
3' SIZE > I				1.000	0.025	0.025	0.086
I(4'-H)					1.000	0.061	0.000
I(4'-OCH_3)						1.000	0.000
INTERACT							1.000

25 and 26 when it was found that the structures of the tested compounds were in error. The necessity of omitting data points no. 34 and no. 35 in deriving eq 25 and 26 could be due to analogue purity or to certain 4'-OCH_3 analogue binding interactions which our model fails to take into account.

As was done for the binding of analogues to intact nuclei, we converted eq 26 [using eq 23, $K_{T_3} = 1.29 \times 10^9$ M^{-1} at $T = 298$ K,[22] and the log (BS) data of Table X] with the resulting $-\Delta G$(BS) data of Table X to the equivalent eq 33.

$$\Delta G(\text{BS}) = +9.392\ (\pm 0.587) + 2.108\ (\pm 0.607)\ \pi_{3'} - 2.597\ (\pm 1.125)\ 3'\ \text{SIZE} > \text{I} - 1.064\ (\pm 0.533)\ \pi_{5'} - 2.117\ (\pm 0.499)\ I(4'\text{-H}) - 1.804\ (\pm 0.610)\ I(4'\text{-OCH}_3) + 1.306\ (\pm 1.080)\ \sigma_{3'5'} - 0.155\ (\pm 0.149)\ \text{INTERACT} \quad (33)$$

$n = 31$; $r = 0.960$; $s = 0.496$

Comparing eq 24 and 33, there are slight quantitative differences in the ΔG contributions of the outer ring substituents to the free energy of binding of analogues to intact nuclei and to solubilized nuclear protein receptors, although the qualitative picture remains unchanged. Of particular interest, eq 33 predicts (1) a 4'-OH net contribution to binding of ~2.1 kcal/mol, consistent with 4'-OH hydrogen bond formation with the receptor, and (2) a 3'-substituent hydrophobic interaction with the receptor of ~2.1 kcal/mol/π unit. The slight differences in the substituent contributions to the ΔG of binding for eq 24 and 33 could be due to experimental error and/or to actual differences between the two assay systems.

These free energies for binding of analogues to solubilized rat hepatic nuclear protein receptors provide an additional means of more closely inspecting the interactive effects of the 3' and 5' substituents on the contribution of the 4'-OH to binding to nuclear receptors. The contribution [ΔG(X)] of substituent X present at a certain position on a molecule to the free energy of binding can be determined relative to substituent Y present at the same position by

$$\Delta G(\text{X}) = \Delta G(\text{AX}) - \Delta G(\text{AY}) = -RT \ln [K_{\text{AX}}/K_{\text{AY}}]$$

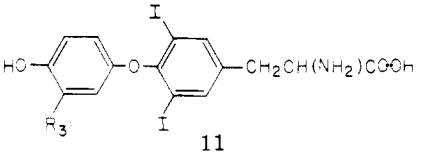
where ΔG(AX) = the apparent free energy of binding of the analogue containing substituent X at a certain position, ΔG(AY) = the apparent free energy of binding of the analogue containing the reference substituent Y at the same position, and K_{AX} and K_{AY} = the corresponding analogue apparent equilibrium association constants. The validity of the additivity assumption for substituent contributions to the free energy of binding is best verified if ΔG(X) values determined from structurally different pairs of analogues are essentially the same. When two or more groups are far apart on a molecule, the additivity assumption has been found to be valid.[26,32] When the groups are close together (e.g., 3',4' disubstitution), interactions between them can result in significant deviations from additivity.

Table XIII. Data Used in the Formulation of Equation 35 Correlating $-\Delta G$(4'-OH) for Thyroid Hormone Analogues

Data point no.	$R_{3'}$[a]	$-\Delta G$(4'-OH), kcal/mol[b] Obsd[c]	Calcd[d]	Dev
1[e]	H	1.25	2.37	1.12
2	Me	1.59	1.33	-0.26
3[f]	*i*-Pr	3.08	2.01	-1.07
4	*t*-Bu	1.91	2.14	0.23
5	F	1.47	1.51	0.04
6	Cl	2.05	2.12	0.07
7	Br	2.48	2.76	0.28
8	I	3.60	3.26	-0.34
9	NO_2	1.05	1.03	-0.02

[a] See structures 11 and 12. [b] See text and eq 34 for derivation. [c] From data of Table X. [d] Calculated using eq 35. [e] Not used in calculating eq 35; purity of 4'-OH analogue of Table X questionable. [f] Not used in calculating eq 35; purity of 4'-H analogue of Table X questionable.

Utilizing this procedure for the partitioning of the free energy of binding into substituent contributions, the free energy of binding of a 4'-OH analogue (11) minus the free energy of binding of the corresponding 4'-deoxy analogue (12) provides an estimate of the contribution of the 4'-OH



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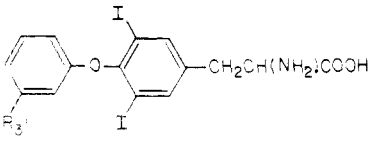

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to the free energy of binding.

$$\Delta G(4'\text{-OH}) = \Delta G(11) - \Delta G(12) \quad (34)$$

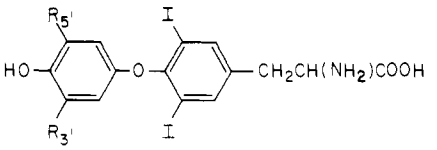
For a variety of 3' substituents, it can be seen [from the $-\Delta G$(4'-OH) values presented in Table XIII] that the free energy of binding of the 4'-OH to nuclear receptors is not constant from compound to compound; i.e., there are interactive effects between the 4'-OH and the 3' substituent which affect the value of $-\Delta G$(4'-OH). *Assuming* that the contribution of the 4'-OH to the free energy of binding to nuclear receptors is due to its forming an intermolecular hydrogen bond with the receptor, we utilized $\sigma_{3'}$ (as an estimate of the electronic influence of the 3' substituent on the hydrogen bond strength), INTERACT$_{3'}$ (as an estimate of the intramolecular hydrogen bonding and/or steric influence of the 3' substituent on the orientation of the 4'-OH), and the data of Table XIII to derive

Table XIV. Stepwise Development of Equation 35

Eq no.	Intercept	$\sigma_{3'}$	INTERACT$_{3'}$	r	s	$F_{DFN,DFD}$ (calcd)
36	2.095	−0.465		0.183	0.896	$F_{1,5}$ = 0.17 (<75% vs. mean)[a]
37	2.208		−0.106	0.404	0.833	$F_{1,5}$ = 0.98 (<75% vs. mean)[a]
35	2.370	9.206	−1.028	0.960	0.286	$F_{1,4}$ = 45.09 (99.6% vs. eq 36)
						$F_{1,4}$ = 38.50 (99.6% vs. eq 37)
						$F_{2,4}$ = 23.40 (99.3% vs. mean)

[a] $F_{1,5}(75\%) = 1.69$.

Table XV. Data used in the Formulation of Equation 38 Correlating in Vitro Binding to Purified Thyroxine Binding Globulin (TBG) for Thyroid Hormone Analogues (13)



13

Data point no.	$R_{3'}$	$R_{5'}$	TBG_{obsd}[a]	Log (TBG) Obsd	Log (TBG) Calcd[b]	Log (TBG) Dev
1	I	I	100.0	2.000	2.016	−0.016
2	I	H	9.0	0.954	0.959	−0.005
3[c]	H	H	0.07	−1.155	−0.098	−1.057
4	Me	Me	0.29	−0.538	−0.272	−0.266
5	OH	H	0.06	−1.222	−1.351	0.129
6	Me	H	0.28	−0.553	−0.185	−0.368
7	Et	H	1.59	0.201	−0.018	0.220
8	*t*-Bu	H	0.67	−0.174	−0.468	0.294
9	*i*-Bu	H	0.10	−1.000	−0.532	−0.468
10	Ph	H	0.04	−1.398	−1.461	0.063
11	*i*-Pr	H	3.53	0.548	0.131	0.417

[a] From ref 29. TBG = relative binding affinity to TBG [relative to TBG (T_4) = 100] = $(K_A/K_{T_4}) \times 100$, where K_A = equilibrium association constant for analogue A. No DL/L correction. On a molar basis. [b] Calculated using eq 38. [c] Not used in calculating eq 38; analogue purity questionable.

Table XVI. Stepwise Development of Equation 38

Eq no.	Intercept	$\pi_{3'5'}$	3' SIZE > I	$\sigma_{3'5'}$	r	s	$F_{DFN,DFD}$(calcd)
39	0.220			3.481	0.731	0.770	$F_{1,8}$ = 9.17 (98.1% vs. mean)
40	0.592		−0.777	3.571	0.911	0.497	$F_{1,7}$ = 12.24 (99.0% vs. eq 39)
38	−0.098	0.563	−1.100	2.366	0.962	0.355	$F_{1,6}$ = 7.72 (96.5% vs. eq 40)

eq 35. The stepwise development of eq 35 is presented in Table XIV.

$$-\Delta G(4'\text{-OH}) = +2.370\ (\pm 0.357) + 9.206\ (\pm 4.118)\ \sigma_{3'} - 1.028\ (\pm 0.425)\ \text{INTERACT}_{3'} \quad (35)$$

$n = 7; r = 0.960; s = 0.286$

It can be seen from Table XIV that neither $\sigma_{3'}$ (eq 36) nor INTERACT$_{3'}$ (eq 37) alone correlates very well with the $-\Delta G(4'\text{-OH})$. Simultaneous inclusion of both terms is highly significant, however (eq 35). This result is consistent with eq 33, which also predicts the 4'-OH binding to be enhanced by electron-withdrawing 3' substituents which tend to orient the 4'-OH toward the 5' position consistent with 4'-OH hydrogen bond donation toward the 5' side of the molecule as it binds to the receptor. The magnitudes of the regression coefficients of eq 35 are significantly larger than the corresponding ones of eq 33. This could be due to (1) the high degree of correlation between $\sigma_{3'}$ and INTERACT$_{3'}$ for the compounds used to derive eq 37; independent variable squared cross-correlation matrix element = 0.942 for $\sigma_{3'}$–INTERACT$_{3'}$; and (2) the partial collinearity of $\sigma_{3'5'}$ and INTERACT with the other parameters for the compounds used to derive eq 33 (see Table XII). Further studies in this area obviously call for a set of compounds with $\sigma_{3'5'}$ and INTERACT, as well as the rest of the other variables, a great deal more orthogonal in order to provide better acceptability for the $+\sigma_{3'5'}/-$INTERACT model and to avoid the possibility of chance correlations (see comments at the end of this paper). Equation 35 does predict that the 4'-OH, without the interactive effects of the 3' and 5' substituents, is possibly forming a hydrogen bond with the nuclear receptor with a net intrinsic hydrogen bond strength of 2.37 kcal/mol. The large difference between ΔG(4'-OH) calculated and observed for the omitted data points no. 1 and no. 3 (Table XIII) is consistent with the questionable purity of analogues no. 9 and no. 3 of Table X. A similar type of analysis for analogues which contain 5' substituents could provide some insight into whether the 5' substituent, in addition to electronically and orientationally affecting the 4'-OH hydrogen bonding with the receptor, exerts its negative influence on thyromimetic activity by merely sterically interacting with the receptors and/or sterically interfering with 4'-OH hydrogen bonding with the receptor.

Binding to Thyroxine Binding Globulin. As shown above, in vivo rat antigoiter bioassay activities are enhanced by electron-donating 3' and 5' substituents. In contrast, in vitro binding to nuclear receptors is apparently enhanced by electron-withdrawing 3' and 5' substituents,

Table XVII. Independent Variable Squared Cross-Correlation Matrix for Equation 38

	$\pi_{3',5'}$	$\sigma_{3',5'}$	3′ SIZE > I
$\pi_{3',5'}$	1.000	0.316	0.259
$\sigma_{3',5'}$		1.000	0.001
3′ SIZE > I			1.000

as they influence the association of the 4′-OH with the nuclear receptor. This can be rationalized in part by inspecting the quantitative SAR for the in vitro binding of thyroid hormone analogues of structure **13** to the plasma protein thyroxine binding globulin (TBG), the principal transport and storage site for the thyroid hormones in human plasma. Utilizing the data of Table XV, eq 38, correlating log (TBG) = log (relative analogue binding affinity to TBG), was derived. The appropriate stepwise development of eq 38 and the independent variable squared cross-correlation matrix are presented in Tables XVI and XVII, respectively.

$$\log(\mathrm{TBG}) = -0.098\ (\pm 0.704) + 0.563\ (\pm 0.496)\ \pi_{3',5'} - 1.100\ (\pm 0.482)\ 3'\ \mathrm{SIZE} > \mathrm{I} + 2.366\ (\pm 1.676)\ \sigma_{3',5'} \quad (38)$$

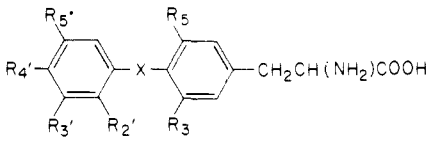
$n = 10; r = 0.962; s = 0.355$

Hydrophobic bonding by 3′ and 5′ substituents ($\pi_{3',5'}$), size-limited at least for the 3′ substituent (3′ SIZE > I), contributes only moderately to binding. Of greater importance, however, is the apparent binding of the 4′-phenoxide to this plasma protein, as indicated by the large, positive $\sigma_{3',5'}$ regression coefficient. It thus appears that in vivo activity is enhanced by electron-donating 3′ and 5′ substituents, which discourage plasma protein binding and encourage passage of the un-ionized 4′-OH across cell membranes.

In plasma, the thyroid hormones are primarily protein bound, with only extremely low concentrations actually free in the plasma.[4,57] This strong, specific binding to the plasma proteins presents a model in which the hormones and analogues are not free to distribute by a random walk process[42a] into cells, but rather in vivo thyromimetic activity is dependent on the low concentration of hormone or analogue free in the plasma. This model would account for (1) the lack of a parabolic dependence on analogue lipophilicity for in vivo activity and (2) the enhancement of in vivo activity by electron-donating 3′ and 5′ substituents, which (due to their influence on the degree of 4′-OH ionization) will decrease binding affinity for the plasma proteins and hence increase the concentration free in the plasma.

Correlations between in Vivo and in Vitro Activ-

Table XVIII. Data Used in the Formulation of Equation 41 Correlating in Vivo Antigoiter Activities (BA) with in Vitro Binding to Intact Rat Hepatic Nuclei (BN) for Thyroid Hormone Analogues (14)



14

Data point no.	R_3	R_5	$R_{3'}$	$R_{5'}$	$R_{4'}$	$R_{2'}$	X	BA_{obsd}[a]	BN_{obsd}[b]	Log (BN) obsd	Log (BA) Obsd	Log (BA) Calcd[c]	Log (BA) Dev
1	I	I	I	H	OH	H	O	100.00	100.00	2.000	2.000	1.701	0.299
2	I	I	H	H	OH	H	O	0.81	0.30	−0.523	−0.092	−0.140	0.049
3	I	I	Me	H	OH	H	O	14.47	13.5	1.130	1.160	1.067	0.094
4	I	I	Et	H	OH	H	O	40.8	21.0	1.322	1.611	1.207	0.404
5	I	I	*i*-Pr	H	OH	H	O	142.1	104.0	2.017	2.153	1.714	0.439
6	I	I	*t*-Bu	H	OH	H	O	21.7	38.5	1.586	1.337	1.399	−0.062
7	I	I	*i*-Bu	H	OH	H	O	7.74	20.0	1.301	0.889	1.191	−0.302
8	I	I	Ph	H	OH	H	O	2.03	2.0	0.301	0.308	0.461	−0.154
9[d]	I	I	c-C_6H_{11}	H	OH	H	O		1.4	0.146		0.348	
10	I	I	Cl	H	OH	H	O	4.88	6.2	0.792	0.688	0.820	−0.131
11	I	I	Cl	Cl	OH	H	O	3.80	4.5	0.653	0.580	0.718	−0.138
12	I	I	Me	Me	OH	H	O	9.04	6.2	0.792	0.956	0.820	0.136
13	I	I	I	I	OH	H	O	18.1	12.5	1.097	1.258	1.042	0.216
14	I	I	*i*-Pr	H	OH	H	O	83.4	100.0	2.000	1.916	1.701	0.214
15[d]	I	I	*i*-Pr	*i*-Pr	OH	H	O		1.4	0.146		0.348	
16	I	I	Me	H	H	H	O	2.71	0.2	−0.699	0.433	0.847	−0.414
17	I	I	CF_3	H	H	H	O	13.56	0.2	−0.699	1.132	0.847	0.286
18	I	I	I	H	H	H	O	27.12	0.4	−0.398	1.433	1.066	0.367
19	Me	Me	Me	H	OH	H	O	0.54	0.1	−1.000	−0.268	−0.489	0.221
20	Me	Me	Me	Me	OH	H	O	0.36	0.1	−1.000	−0.444	−0.489	0.045
21	Me	Me	*i*-Pr	H	OH	H	O	3.60	0.7	−0.155	0.556	0.128	0.428
22	I	I	Me	H	OH	Me	O	9.04	1.10	0.041	0.956	0.271	0.685
23	I	I	H	Me	OH	Me	O	0.18	0.1	−1.000	−0.745	−0.489	−0.256
24	I	I	H	I	OH	Me	O	0.36	0.30	−0.523	−0.444	−0.140	−0.303
25	I	I	*e*	H	OH	*e*	O	18.08	8.0	0.903	1.257	0.901	0.357
26	I	I	H	H	NH_2	H	O	0.27	0.76	−0.119	−0.569	0.154	−0.723
27	I	H	I	H	OH	H	O	0.25	0.50	−0.301	−0.602	0.021	−0.624
28	I	H	I	I	OH	H	O	0.125	0.1	−1.000	−0.903	0.489	−0.414
29	Br	Br	*i*-Pr	H	OH	H	O	30.0	36.0	1.556	1.477	1.378	0.100
30[d]	*i*-Pr	*i*-Pr	I	H	OH	H	O		0.2	−0.699		−0.269	
31	I	I	I	H	OH	H	CH_2	54.25	250.0	2.398	1.734	1.992	−0.258
32[d]	I	I	I	I	OH	H	CH_2		2.6	0.415		0.544	
33[d]	I	I	H	H	OH	H	S		1.3	0.114		0.324	
34	I	I	I	H	OH	H	S	13.82	100.0	2.000	1.140	1.701	−0.561

[a] From ref 2. No DL correction. On a molar basis. Relative to L-T_3 = 100. [b] See footnote *a*, Table XVII. [c] Calculated using eq 41. [d] Not used in calculating eq 41. [e] 2′,3′-$(CH_2)_4$.

Table XIX. Stepwise Development of Equations 41 and 43

Eq no.	Intercept	Log (BN)	Log (BS)	I(4′-H)	I(4′-OCH_3)	r	s	$F_{DFN,DFD}$(calcd)
44	0.404	0.635				0.820	0.522	$F_{1,27}$ = 55.43 (>99.9% vs. mean)[a]
41	0.241	0.730		1.116		0.917	0.371	$F_{1,26}$ = 27.64 (>99.9% vs. eq 44)[b]
45	0.597		0.548			0.779	0.692	$F_{1,24}$ = 37.08 (>99.9% vs. mean)[c]
46	0.383		0.720	0.984		0.915	0.454	$F_{1,23}$ = 32.75 (>99.9% vs. eq 45)[d]
43	0.274		0.757	1.159	0.623	0.937	0.402	$F_{1,22}$ = 7.38 (98.6% vs. eq 46)

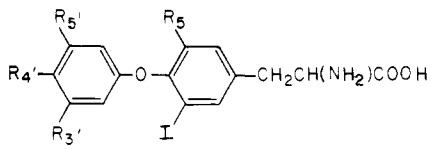
[a] $F_{1,27}(99.9\%) = 13.61$. [b] $F_{1,26}(99.9\%) = 13.74$. [c] $F_{1,24}(99.9\%) = 14.03$. [d] $F_{1,23}(99.9\%) = 14.19$.

Table XX. Independent Variable Squared Cross-Correlation Matrix for Equations 41 and 43

	Eq 41			Eq 43		
	Log (BN)	I(4′-H)		Log (BS)	I(4′-H)	I(4′-OCH_3)
Log (BN)	1.000	0.095	Log (BS)	1.000	0.210	0.001
I(4′-H)		1.000	I(4′-H)		1.000	0.064
			I(4′-OCH_3)			1.000

ities. Utilizing the data of Table XVIII, eq 41 correlating in vivo antigoiter activities (BA) with in vitro binding to intact rat hepatic nuclei (BN) was derived.[25] The stepwise development of eq 41 and the independent variable squared cross-correlation matrix are presented in Tables XIX and XX, respectively. Equation 42 was originally derived[26] for correlation of in vivo antigoiter activities (BA) with in vitro binding to solubilized rat hepatic nuclear protein (BS). Utilizing the data of Table XXI (expanded from the data used in the derivation of eq 42), eq 43 was derived for correlation of in vivo antigoiter activities (BA) with in vitro binding to solubilized rat hepatic nuclear protein (BS). The stepwise development of eq 43 and the independent variable squared cross-correlation matrix are

Table XXI. Data Used in the Formulation of Equation 43 Correlating in Vivo Antigoiter Activities (BA) with in Vitro Binding to Solubilized Rat Hepatic Nuclear Protein (BS) for Thyroid Hormone Analogues (15)



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Data point no.	R_5	$R_{3'}$	$R_{5'}$	$R_{4'}$	BA_{obsd}[a]	BS_{obsd}[b]	Log (BS) obsd	Log (BA) Obsd	Log (BA) Calcd[c]	Log (BA) Dev
1	I	H	H	H	1.24	0.01	−2.000	0.093	−0.082	0.175
2[d]	I	H	H	OH	0.81	0.082	−1.086	−0.092	−0.548	0.457
3	I	H	H	NH_2	0.036	0.0031	−2.502	−1.444	−1.620	0.176
4	I	I	H	H	27.12	0.230	−0.638	1.433	0.949	0.484
5	I	I	H	OH	100.0	100.0	2.000	2.000	1.788	0.212
6	I	I	H	OCH_3	11.25	1.29	0.111	1.051	0.981	0.070
7	I	Me	H	H	2.71	0.225	−0.648	0.433	0.942	−0.509
8	I	Me	H	OH	14.47	3.30	0.518	1.160	0.666	0.494
9[d]	I	Me	H	OCH_3		0.17	−0.770		0.314	
10	I	*i*-Pr	H	OH	142.1	89.15	1.950	2.153	1.750	0.402
11	I	*i*-Pr	H	OCH_3	19.0	6.82	0.834	1.279	1.528	−0.249
12	I	*s*-Bu(±)	H	OH	79.9	78.29	1.894	1.902	1.708	0.195
13[d]	I	*t*-Bu	H	H		0.335	−0.475		1.073	
14	I	*t*-Bu	H	OH	21.7	8.45	0.927	1.336	0.976	0.361
15	I	*t*-Bu	H	OCH_3	2.35	0.27	−0.569	0.371	0.466	−0.095
16	I	Me	Me	H	0.054	0.145	−0.839	−1.268	−0.361	−0.907
17[d]	I	Me	Me	OCH_3		0.335	−0.475		0.537	
18[d]	I	NO_2	H	H		0.038	−1.420		0.358	
19	I	NO_2	H	OH	0.18	0.225	−0.648	−0.745	−0.216	−0.528
20	I	Me	H	NH_2	0.036	0.031	−1.509	−1.444	−0.868	−0.576
21	I	Me	Me	NH_2	0.13	0.041	−1.387	−0.886	−0.776	−0.110
22	H	I	H	OH	0.25	0.688	−0.162	−0.602	0.151	−0.753
23	I	F	H	OH	0.65	0.164	−0.785	−0.187	−0.320	0.133
24	I	Cl	H	OH	4.88	3.73	0.572	0.688	0.707	−0.018
25	I	Br	H	OH	23.78	15.89	1.201	1.376	1.183	0.193
26	I	I	I	OH	18.1	13.85	1.141	1.258	1.138	0.120
27[d]	I	*i*-Pr	H	H		0.492	−0.308		1.199	
28	I	*n*-Pr	H	OH	39.5	23.97	1.380	1.597	1.318	0.278
29	I	Br	H	H	18.0	0.24	−0.620	1.255	0.963	0.292
30	I	Cl	H	H	7.78	0.118	−0.928	0.891	0.730	0.161
31	I	F	H	H	1.39	0.0136	−1.866	0.143	0.020	0.123
32[d]	I	*i*-Pr	*i*-Pr	OH		1.10	0.041		0.305	
33[d]	I	*s*-Bu(±)	H	OCH_3		1.29	0.111		0.981	
34	I	Cl	Cl	OH	3.80	3.71	0.569	0.580	0.705	−0.125
35[d]	I	Br	Br	OH	1.58	5.07	0.705	0.199	0.808	−0.609
36[d]	I	*i*-Pr	Cl	OH		52.56	1.721		1.576	
37[d]	I	*i*-Pr	Br	OH		21.95	1.341		1.289	
38[d]	I	*i*-Pr	I	OH		12.41	1.094		1.102	

[a] See footnote *a*, Table XVIII. [b] See footnote *a*, Table X. [c] Calculated using eq 43. [d] Not used in calculating eq 43.

presented in Tables XIX and XX, respectively.

$$\log (BA) = +0.241\ (\pm 0.170) + 0.730\ (\pm 0.134) \log (BN) + 1.116\ (\pm 0.484)\ I(4'\text{-H}) \quad (41)$$

$n = 29; r = 0.917; s = 0.371$

$$\log (BA) = +0.261\ (\pm 0.207) + 0.853\ (\pm 0.152) \log (BS) + 1.164\ (\pm 0.466)\ I(4'\text{-H}) + 0.609\ (\pm 0.437)\ I(4'\text{-OCH}_3) \quad (42)$$

$n = 22; r = 0.943; s = 0.410$

$$\log (BA) = +0.274\ (\pm 0.192) + 0.757\ (\pm 0.135) \log (BS) + 1.159\ (\pm 0.362)\ I(4'\text{-H}) + 0.623\ (\pm 0.387)\ I(4'\text{-OCH}_3) \quad (43)$$

$n = 26; r = 0.937; s = 0.402$

It thus appears that in vivo antigoiter activities (BA) correlate well with in vitro binding to intact rat hepatic nuclei (BN) and with in vitro binding to solubilized rat hepatic nuclear protein (BS), with adjustments made for in vivo metabolism of 4′-deoxy [I(4′-H)] and 4′-OCH_3 [I(4′-OCH_3)] analogues. [Data point no. 16 = 4′-H-3′,-5′-Me_2-T_2 of Tables XXI was assigned a value of 0.0 for I(4′-H) in that the two 3′ and 5′ CH_3 groups apparently sterically inhibit the in vivo hydroxylation of this analogue.] The deviations of the intercepts from 0.0 and of the log (BN) and log (BS) regression coefficients from 1.0 reflect not only the minimal accuracy of some of the in vivo data but also differences in analogue plasma protein binding, metabolism, elimination, and 4′-OH ionization effects in vivo.

These correlations of in vivo and in vitro activities do indicate, however, that distribution (as influenced by analogue lipophilicity), at least for the range of lipophilicities of the analogues examined, does not play a major role in influencing whole animal activity. This is consistent with our inability to find any parabolic dependence of in vivo antigoiter activity on analogue lipophilicity.

Addition of a $\sigma_{3'5'}$ term to eq 43 was not significant, even though log (BA) and log (BS) correlate with $-\sigma_{3'5'}$ (eq 8) and with $+\sigma_{3'5'}$ (eq 26), respectively. This is not completely surprising, however, since the $\sigma_{3'5'}$ values used in deriving eq 8 and 26 differ for the 4′-deoxy and 4′-OCH_3 analogues (see the discussion above concerning substituent constant choices).

These correlations between in vivo activities and in vitro binding to nuclear receptors provide strong support for the hypothesis that the latter is the first step in initiating the events which lead to subsequent thyroid hormone responses. These equations provide an additional bonus in that they now permit estimation of in vivo antigoiter activities based on in vitro binding affinities to nuclear receptors (or vice versa). We successfully utilized eq 42 to predict antigoiter activities (BA) of a number of analogues [and used these BA(calcd) values to determine what in vivo assay dose levels to use for these compounds] from their binding affinities to solubilized nuclear protein receptors (BS),[58] as can be seen from Table XXII. The largest deviations between the BA(calcd) and BA(obsd) values occur in Table XXII for the 4′-deoxy analogues and perhaps reflect varying degrees of in vivo hydroxylation of these analogues.

Conclusions and Future Areas of Study. The results of all of these quantitative structure–activity relationship correlations can be briefly summarized as follows.

Table XXII. Prediction of in Vivo Antigoiter Activities (BA) from in Vitro Binding Affinities to Solubilized Rat Hepatic Nuclear Protein (BS) Using Equation 42 for Thyroid Hormone Analogues (15, $R_{5'}$ = H)

R_3	$R_{4'}$	BS[a] (obsd)	BA[b] (calcd)	BA[c] (obsd)
n-Pr	OH	23.97	27.41	39.5
F	H	0.0136	0.68	1.39
Cl	H	0.118	4.30	7.78
Br	H	0.240	7.88	18.0
NO_2	OH	0.225	0.51	0.18

[a] From Table VII. [b] Calculated using eq 42. [c] References 2 and 58.

(1) The correlations confirm that both in vivo antigoiter activity and in vitro binding to nuclear receptors are enhanced by bulky, lipophilic 3 and 5 substituents and by size-limited, lipophilic 3′ substituents and are decreased by any 5′ substituent bulk or lipophilicity.

(2) In vivo activity is enhanced by electron-donating 3′ and 5′ substituents, which prevent plasma protein binding and encourage movement of the analogue into cells.

(3) In vitro binding probably involves hydrogen bond donation of the 4′-OH to the 5′ side of the nuclear receptor.

(4) The good correlations between in vivo antigoiter activities and in vitro nuclear receptor binding reflect that the latter is probably the first step in initiating subsequent hormonal responses (as expressed through protein synthesis).

(5) Except for 3′ and 5′ substituent influences on the degree of 4′-OH ionization (and hence on the degree of plasma protein binding), distribution, at least within the range of analogue lipophilicities studied, does not play a major role in determining whole animal activities.

(6) Binding of analogues to TBG is strongly influenced by the degree of 4′-OH ionization.

(7) The success of our use of the 3′ SIZE > I and INTERACT parameters in these QSAR studies indicates that they are reasonable estimates of the physical properties they were designed to predict. Hopefully these, or similarly derived parameters, may be of use in future QSAR studies of the thyroid hormone analogues or other systems for which the "traditional" substituent parameters are unable to account for particular physicochemical properties of the analogues.

(8) The free energy of in vitro binding of analogues to nuclear receptors can be partitioned into the individual substituent contributions (e.g., see eq 34). These substituent contributions can themselves be related to the physicochemical properties of each substituent (see eq 24, 33, and 35). These values can be interpreted in order to give insight into the nature of the receptor site and to provide direct comparison with experimental values from model systems (e.g., 3′-substituent hydrophobic bonding and 4′-OH hydrogen bonding).

A diagramatic representation of the overall picture of thyroid hormone analogue binding to nuclear receptor is presented in Figure 2.

As mentioned above, one of the difficulties involved in the QSAR examination of thyromimetic activities is the lack of orthogonality between some of the substituent constants. This is especially evident for (1) $\pi_{5'}$ and $E_{s(5')}$; (2) $\sigma_{3'5'}$ and INTERACT; and (3) π_{35} and $E_{s(35)}$. The complete synthesis and testing of each new thyroid hormone analogue require considerable effort, time, and expense. In order to obtain the maximal amount of information for the fewest number of new analogues, therefore, it will be necessary to ensure that future design of new analogues takes into account the orthogonality of substituent con-

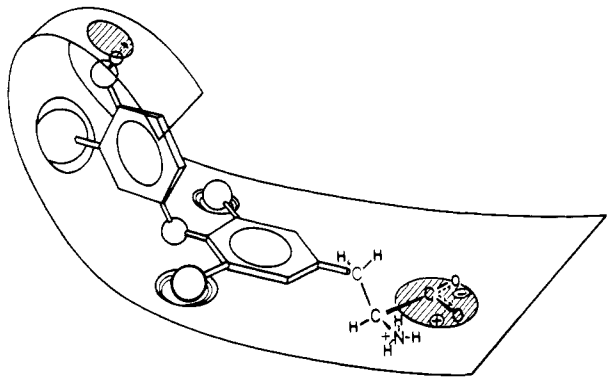

Figure 2. Diagramatic representation of thyroid hormone analogue binding to nuclear receptor.

stants. Presynthesis substituent constant cluster analysis[59] and examination of substituent constant squared cross-correlation matrices should certainly be utilized in such analysis. Some "intuitive" suggestions [based on the known qualitative and quantitative SAR's for the various thyromimetic activities of the thyroid hormone analogues, previously observed (see above) lack of orthogonality between substituent constants, and possible ease of synthesis] can be made, however, for a number of new substituent combinations: (1) 3′ substituents capable of forming strong intramolecular hydrogen bonds with the 4′-OH and with varying substituent "size" and lipophilicity; (2) 3′,5′ substituent combinations with $\sigma_{3'5'} \approx \sigma_{3'5'}$ as for 3′-*i*-Pr, 5′-halogen, but INTERACT ≈ −INTERACT as for 3′-*i*-Pr, 5′-halogen; (3) 3′ and/or 5′ substituent with π and E_s substituent constants not correlated; (4) various other 3′-alkyl substituents for further investigation of the π and "size" 3′-substituent requirements for thyromimetic activity; (5) 3,5 substituents with greater orthogonality of π_{35} and $E_{s(35)}$.

Although thyromimetic activities have been examined for a wide variety of 3,5 substituents, finite quantitative activities are available for only a limited number of 3,5 substituents (F, Cl, Br, I, CH_3) and for these π_{35} and $E_{s(35)}$ are well correlated. In addition to thyromimetic activity being influenced by the ability of the 3,5 substituents to "lock" the diphenyl ether nucleus into the proximal and distal conformations, 3,5 substituents larger than iodine (e.g., *i*-Pr, SPh) (larger than bromine for TBG binding[29]) also exert a negative steric influence on activity.[2-4] Determination of finite quantitative thyromimetic activities for a wider variety of 3,5 substituents is most necessary for determining the relative importance in influencing thyromimetic activities of 3,5 substituent lipophilicity, "size" or "bulk", and conformational flexibility (as this affects the diphenyl ether conformation).

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Absolute Configuration of Glycerol Derivatives. 4.[1] Synthesis and Pharmacological Activity of Chiral 2-Alkylaminomethylbenzodioxans, Competitive α-Adrenergic Antagonists[2]

Wendel L. Nelson,* John E. Wennerstrom,

Department of Pharmaceutical Sciences, University of Washington, Seattle, Washington 98195

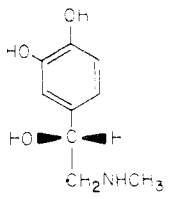
Donald C. Dyer, and Michael Engel

Department of Veterinary Anatomy, Pharmacology and Physiology, Iowa State University, Ames, Iowa 50011.
Received December 17, 1976

The optical isomers of α-adrenergic receptor antagonists prosympal (**2**), piperoxan (**3**), and dibozane (**4**) were prepared by methods establishing the absolute configuration of each. (2*S*)-3-(2′-Hydroxyphenoxy)-1,2-propanediol ditosylate (**10**) was prepared from (2*R*)-3-tosyloxy-1,2-propanediol acetonide (**6**). Intramolecular displacement afforded (2*S*)-tosyloxymethylbenzodioxan [(2*R*)-**11**]. Reaction of (2*R*)-**11** with the appropriate amine (diethylamine, piperidine, or piperazine) afforded the 2*S* isomers of **2**, **3**, and **12**, respectively. Reaction of (2*S*)-**12** with (2*R*)-**11** afforded the *SS* isomer of **4**. Reaction of (2*S*)-3-benzyloxy-1,2-propanediol ditosylate (**14**) with catechol (NaOMe) afforded (2*R*)-benzyloxymethylbenzodioxan (**15**). Subjecting **15** to hydrogenolysis, tosylation, and displacement with the appropriate amine afforded 2*R* isomers of **2**, **3**, and **12**. Reaction of (2*R*)-**12** with (2*S*)-**11** afforded (*RR*)-**4**. Reaction of (2*R*)-**12** with (2*R*)-**11** afforded *meso*-**4**. The *S* isomers were more effective antagonists to the α-adrenergic response of methoxamine-induced contraction of rabbit aortic strips by twofold in **2** and 18–19-fold in **3** and **4**. *meso*-**4** was as effective as the *SS* isomer of **4**. The results are interpreted in terms of a similar conformational distribution of aminoalkyl, oxygen, and aromatic functional groups of the (*S*)-benzodioxans and (*R*)-epinephrine.

The search for compounds which alter responses to the adrenergic neurotransmitters has long been a focal point in the search for useful therapeutic agents. Among the groups of compounds which are effective agents in reversing the pressor response to epinephrine (**1**) are the 2-alkylaminobenzodioxans.[3,4] Some of the compounds were used clinically as antihypertensive agents because of their α-adrenergic blocking properties but since have been discarded because of the usual adverse affects associated with blockade of α-adrenergic receptors.[3,5] However, some of the compounds, e.g., prosympal (**2**),[4,6a] piperoxan (**3**),[4,6] and dibozane (**4**),[4,6c,d] remain as very important pharmacological tools because their α-adrenergic receptor antagonism is a competitive blockade. Additionally, the alkylaminomethylbenzodioxan system remains as a focal point for the study of other amines, some of which have similar activity, e.g., guanoxan,[7] acetoxatrine,[8] and spiroxamide.[9]

Although the α-adrenergic receptor antagonist properties of the compounds have been widely studied, only a single report appears in the literature relative to the effect of absolute stereochemistry on these properties. (−)-Prosympal (**2**) was five to six times more potent than the (+) isomer in reversing the pressor response to epinephrine in cats and was also a more potent miotic by a factor of four- to eightfold.[10]



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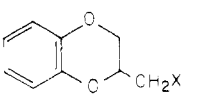
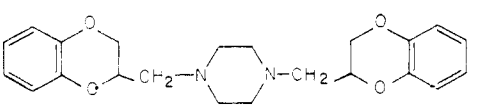


2, X = NEt_2
3, X = c-NC_5H_{10}



4

Our investigation of this system is related to our continuing interest in glycerol derivatives which can be readily synthesized as either *R* or *S* enantiomers and as such can be used in determination of absolute configuration of