Thyroid Hormone Uptake by Hepatocytes: Structure–Activity Relationships of Phenylanthranilic Acids with Inhibitory Activity

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The synthesis of a series of mono- and disubstituted *N*-phenylanthranilic acids is described. Substituents on the phenyl ring include Cl, CN, OH, CF_3, Br, I, CH_3, OCH_3, and OCF_2CF_2H. These compounds have been tested for their inhibitory effect on triiodothyronine (T_3) uptake by H4 hepatocytes. The nonsteroidal antiinflammatory drugs flufenamic acid, mefenamic acid, and meclofenamic acid and the structurally related compounds 2,3-dimethyldiphenylamine and diclofenac were also tested. The most potent compounds were found to be, in order of decreasing activity, meclofenamic acid (2,6-Cl_2, 3-CH_3), flufenamic acid (3-CF_3), mefenamic acid (2,3-$(CH_3)_2$), and the compounds with 3,5-Cl_2 and 3-OCF_2CF_2H substituents. The least potent compounds had 3-CN and 3-OH substituents. An analysis of quantitative structure–activity relationships (QSAR) for the series of phenylanthranilic acids showed that the inhibition of T_3 uptake is highly dependent on the hydrophobicity of the compound. The relationship between uptake inhibition and the calculated octanol–water partition coefficient (clogP) was found to be parabolic, with optimum inhibitory activity found when the clogP of the phenylanthranilic acid was 5.7. It was also found that the 1-carboxylic acid group of the phenylanthranilic acids was not a prerequisite for uptake inhibition to occur, but its removal or alteration resulted in reduced inhibition.

Introduction

The uptake of the naturally synthesized thyroid hormones L-triiodothyronine (T_3, 1) and L-thyroxine (T_4, 2) (Figure 1) from the bloodstream into the cell is an essential step in the complex process of thyroid hormone action. Inhibitors of cellular uptake may be useful therapeutic agents for the rapid treatment of disorders caused by thyroid hormone excess. Currently, however, the mechanism of cellular uptake and the role of this system in the regulation of thyroid hormone action remain unclear. In this paper we report the use of a series of phenylanthranilic acids to investigate the structural preferences of the thyroid hormone uptake system.

The passage of thyroid hormones into the cell was originally thought to occur by a process of passive diffusion through the cell membrane.[1] More recently, substantial evidence has accumulated that hormone uptake is the result of an active transport process as reviewed by Davis.[2] Recent evidence also suggests that thyroid hormone uptake may be similar to that of certain classes of amino acid uptake.[3,4] The transport of T_3 across cell membranes is energy-dependent and saturable. L-T_3 is transported in preference to L-T_4, other L-iodothyronines, and D-T_3.[2,5,6] These findings imply that thyroid hormone may bind to a structurally-specific site in order to be transported into a cell.

Cell membrane binding sites for T_3, which may represent part of the T_3 uptake mechanism, have been identified,[2,7,8] but these sites have not been completely characterized and at present their roles are uncertain. Several groups of investigators have identified compounds that inhibit T_3 uptake at concentrations ranging from 1 to 100 μM. The majority of these compounds appear to be small molecules containing multiple aromatic rings including phloretin, iopanoic acid, several dihydropyridines, calmodulin antagonists, and β-blockers.[6,9,10] The thyroid hormone uptake system is also inhibited at high concentrations by the phenylalanine and tryptophan but not by other, nonaromatic amino acids.[11] The presence of aromatic rings or similar planar functional groups in these inhibitors suggests that they are required for the inhibition of thyroid hormone uptake; however, at present there is not enough information to develop a structural model for inhibition.

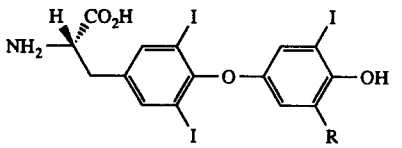

Figure 1. Structures of the thyroid hormones. R = H, triiodothyronine (1); R = I, thyroxine (2).

Although many of the newly identified inhibitors of cellular thyroid hormone uptake have well-known biological activities, it cannot be assumed that their inhibitory action on T_3 uptake is related to these properties. Our studies suggest that uptake inhibition by the dihydropyridine calcium channel blockers in cultured hepatic cells may not be due to calcium-dependent regulation of T_3 uptake,[12] which is consistent with the absence of voltage-dependent calcium channels in these cells.[13]

To investigate the mechanism of thyroid hormone uptake and to seek further evidence for the existence of a thyroid hormone membrane transporter protein, we have

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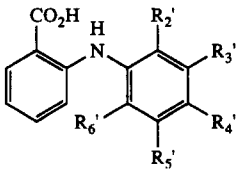


Figure 2. General structure of the substituted phenylanthranilic acids. $R_{3'} = CF_3$; flufenamic acid (**3**). $R_{2',6'} = Cl$, $R_{3'} = CH_3$; meclofenamic acid (**4**). $R_{2',3'} = CH_3$; mefenamic acid (**5**).

used a series of phenylanthranilic acids (Figure 2), including the clinically important nonsteroidal antiinflammatory drugs, flufenamic acid (**3**), meclofenamic acid (**4**), and mefenamic acid (**5**), to investigate the thyroid hormone uptake system. The phenylanthranilic acids were of special interest because it has been shown that compounds **3**–**5** inhibit T_3 binding to specific binding sites in isolated cell nuclei[14] and also inhibit T_4 binding to the serum transport proteins thyroxine-binding globulin and transthyretin.[15] It therefore seemed plausible that these compounds might act by a specific interaction with a thyroid hormone binding protein involved in T_3 transport through the cell membrane.

Results and Discussion

Chemistry. Structural details and physical properties of *N*-phenylanthranilic acids synthesized for this study are presented in Table I. These compounds were chosen to examine the effect of substituent position, size, and hydrophobicity on uptake inhibition. The thyroid hormone binding sites described above each have specific structural requirements for the nature and position of ring substitution,[16] and it was anticipated that the inhibition of thyroid hormone uptake by phenylanthranilic acids might display similar structural requirements. Particular attention was therefore paid to the 3′- and 5′-positions.

The compounds used in this study were synthesized by one of the two well-established procedures[17] shown in Scheme I. Method A, the classical Ullmann–Goldberg reaction, is a copper-catalyzed condensation of an aniline with an aryl halide in the presence of a base. This reaction was performed in refluxing DMF with 2-bromobenzoic acid as the aryl halide, *N*-methylmorpholine as the base, and Cu_2O as the catalyst. However, this method produces tarry byproducts, making purification of the products difficult which is often reflected in low isolated yields for these reactions. Method B, the reaction of the appropriate aniline with diphenyliodonium carboxylate,[17,18] which is also copper catalyzed ($Cu(OAc)_2$), requires milder reaction conditions (refluxing *i*-PrOH) and is superior, producing far cleaner products. Spectroscopic data (^1H NMR, IR, and mass spectroscopy) for the phenylanthranilic acids have been submitted as supplementary material.

Inhibition Studies. The degree of inhibition of [^{125}I]-T_3 uptake into rat hepatoma cells by a series of phenylanthranilic acids and several related compounds is shown in Table I. Each is reported as a percentage inhibition compared to a control containing no test compound. These measurements were performed with the compound being investigated at a single concentration (0.1 mM) in H4 rat hepatoma cells, with a minimum of five replicate measurements being made in each case. We have previously reported the dose–response curves of mefenamic, flufenamic, and meclofenamic acids which have also been included in this study.[6] These data were obtained by an identical method to that used in the present work. The dose–response curves for these compounds were found to have similar slopes, and we have assumed that the dose–response curves of the phenylanthranilic acids reported in this paper are parallel to those that have been previously determined.

Structure–Activity Relationships of B Ring Substitution. The variation of the chemical substituents on the phenylanthranilic acid B ring proved to have a marked effect of the inhibition of [^{125}I]T_3 uptake, with the inhibition values ranging from 6.6% to 74% at 0.1 mM. The most potent compound in the series is the nonsteroidal antiinflammatory drug meclofenamic acid (**4**) while the least potent was found to be the 3′-hydroxy compound (**24**).

In order to derive quantitative structure–activity relationships for the inhibition of T_3 uptake by the phenylanthranilic acids the single point percentage inhibition data was first transformed to make it proportional to log(IC_{50}) using the "logit" transformation[19,20] (logit ($\%I$) = log [$\%I/(100 - \%I)$]).

From the inhibition data of the phenylanthranilic acids in Table I, excluding compounds **25** and **26**, we have derived the quantitative structure–activity relationship given by eq 1 (Figure 3). In this equation clogP is the log of the calculated octanol–water partition coefficient of the unionized molecule,[21] n is the number of points, s is the standard error of estimate, and r is the correlation coefficient. Standard errors are given in brackets.

$$\text{logit}\ (\%I) = 3.92(0.62)\text{clogP} - 0.346(0.060)(\text{clogP})^2 - 11.0(1.6) \quad (1)$$

$$n = 22 \quad s = 0.164 \quad r^2 = 0.814$$

Transformation of the data with the logit function resulted in an improved fit of the structure–activity relationship to the inhibition data and substantially improved the predictive ability of the equation compared to correlation of clogP with untransformed inhibition data. The clogP values of the phenylanthranilic acids proved to be in good agreement with the experimental logP values available for some of the compounds.[22] Although clogP is the only term included, several other parameters were considered in the development of eq 1. Molecular size was represented by the calculated molecular refractivity (cMR).[21] There is a degree of colinearity between clogP and cMR (correlation = 0.80) for this group of compounds and a similar, but less satisfactory ($r^2 = 0.44$), relationship was also observed when inhibition was compared to cMR. The better defined relationship between logP and uptake inhibition seems to implicate lipophilicity rather than molecular volume as being of prime importance. The Hammett constants σ_p and σ_m[23] of the ortho and para substituents were used to estimate the electronic effects of B ring substitution with indicator variables making ortho substitution. These electronic parameters have been shown to be correlated with acidity of the carboxylic acid group.[22] However, the addition of extra variables to eq 1 failed to significantly improve the correlation, implying that compound acidity is not correlated with inhibitory activity. The small size of the data set precludes a more extensive search for correlated parameters.[24]

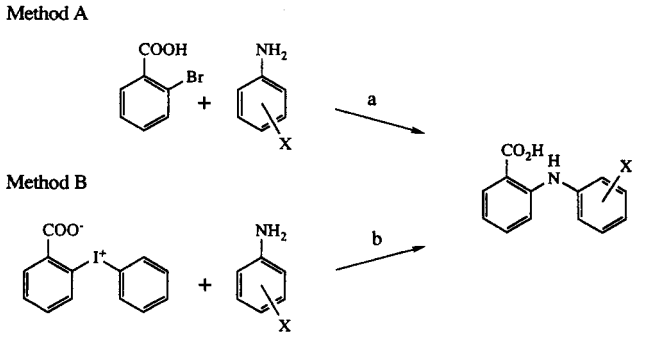
To confirm that eq 1 is a good predictor of thyroid hormone uptake inhibition, this quantitative structure–activity relationship was cross validated using the method

Table I. Data for the Compounds Tested for Inhibition of L-T_3 Uptake by H4 Rat Hepatoma Cells

no.	substitution[a]	method[b]	yield,[c] %	recryst[d]	anal.[e]	lit. mp, °C	mp, °C	clogP[f]	%I[g]	std err[h]	logit (%I)[i]	calclated logit (%I)[j]
3	3′-CF_3[k]							5.526	64.4	4.0	0.257	0.118
4	2′-Cl, 3′-CH_3, 6′-Cl[k]							5.746	74.2	0.6	0.459	0.088
5	2′-CH_3, 3′-CH_3[k]							5.287	60.9	3.7	0.192	0.022
6	H	A	76	C		186–187[l]	184.5–186.5	4.339	28.0	4.4	−0.140	−0.572
7	2′-CH_3, 6′-CH_3	B	56	C		209–211[l]	206–207	5.337	48.0	2.4	−0.035	0.080
8	2′-Cl	A	32	C		196–198[l]	196–199	4.827	44.6	3.7	−0.094	−0.202
9	2′-Cl, 3′-Cl	A	26	M		255.5–256.5[l]	255–256	5.457	56.6	3.0	0.115	0.108
10	2′-Cl, 5′-Cl	A	13	E		237.5–238[l]	239.5–241	5.577	43.2	3.7	0.099	0.084
11	2′-Cl, 6′-Cl	B	10	C		214–216[l]	220–222	5.247	54.6	2.9	0.140	−0.005
12	3′-Br	B	33	C		173–174[l]	175–176	5.307	48.1	2.2	−0.063	0.008
13	3′-Br, 5′-Br	B	34	E	C, H, N		261–263	6.207	39.1	3.5	−0.192	0.050
14	3′-CF_3, 4′-Cl	A	43	C	C, H, N		162–163	6.066	51.0	3.5	0.099	0.026
15	3′-CF_3, 4′-OCH_3	A	41	M/W	C, H, N		170–172	5.553	54.0	1.5	0.187	0.091
16	3′-CF_3, 5′-CF_3	A	34	M		203–204[l]	203–205	6.516	39.5	6.8	−0.087	−0.186
17	3′-CH_3	B	46	C/H		136–137[l]	139–141	4.838	30.0	2.7	−0.368	−0.131
18	3′-Cl	A	66	E/W		171–173[l]	170–172	5.157	51.4	3.7	0.024	0.000
19	3′-Cl, 4′-Cl	A	14	D		178–179[l]	176–178	5.787	55.6	3.2	0.084	0.000
20	3′-Cl, 5′-Cl	A	13	E		254–255[l]	249–250	5.907	60.6	2.3	−0.119	0.072
21	3′-CN	B	24	E		209–210[l]	209–210	4.303	11.6	3.7	−0.882	−0.493
22	3′-I	B	26	C		168–170[m]	171–173	4.587	40.0	2.3	−0.128	0.122
23	3′-OCF_2CHF_2	B	46	M/W	C, H, N		131–132	6.033	57.0	2.1	0.122	0.006
24	3′-OH	B	48	C		170.5–171.5[n]	171–172	3.672	6.6	3.9	−1.151	−1.648
25	1-CH_2CO_2H, 2-Cl, 6-Cl[k,o]							4.066	38.0	4.7	−0.213[j]	−0.793
26	1-H, 2′-CH_3, 3′-CH_3[k,o]							4.568	35.4	1.9	−0.269[j]	−0.328

[a] All compounds have a COOH group at position 1 unless otherwise stated. [b] Method of synthesis: (A) Ullmann–Goldberg reaction, (B) carboxyphenylation of aniline with diphenyliodonium carboxylate. [c] Percentage reaction yield. [d] Recrystallization solvent: C, chloroform; D, dichloromethane; E, ethanol; H, hexanes; M, methanol; W, water. [e] Microanalyses for elements stated were within 0.4% of the theoretical value. [f] Octanol–water partition coefficient calculated using program CLOGP version 3.54. [g] % Inhibition of [^{125}I]T_3 uptake by H4 rat hepatoma cells. [h] The standard error for %I. [i] The logit of the percentage inhibition data where logit (%I) = log[%I/(100 − %I)]. This transformation makes percentage inhibition values proportional to log(IC_{50}). [j] Predicted value of logit(%I) calculated using eq 1. [k] Compound obtained as reported in Experimental Section. [l] Reference 17. [m] Bourquin, et al. *Helv. Chim. Acta* **1958**, *41*, 1061–1068. [n] Taisho Pharmaceutical Co., Ltd. Patent 6809051, JP. 1969. [o] These compounds were not included in the derivation of eq 1.

Scheme I. Synthesis of Phenylanthranilic Acids[a]



[a] (a) *N*-Methylmorpholine, Cu_2O, DMF; (b) $Cu(OAc)_2$, *i*-PrOH.

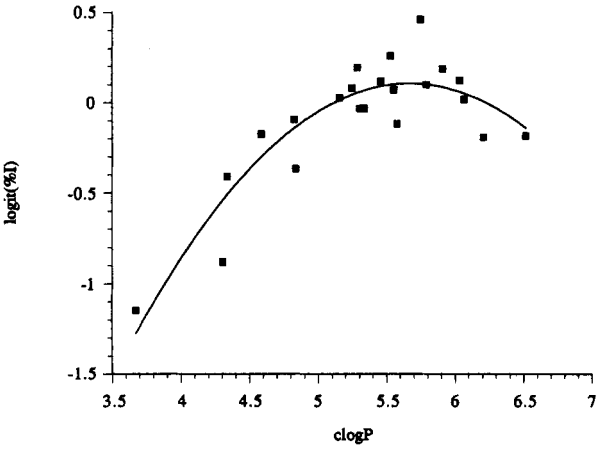


Figure 3. The relationship between the calculated octanol–water partition coefficient (clogP) and percentage inhibition of [^{125}I]T_3 uptake into H4 rat hepatoma cells (%I) for the 22 substituted phenylanthranilic acids of Table I. The logit transformation has been used to make %I proportional to log(IC_{50}) data.

of Cramer et al.[25] In this method the data set is divided into smaller groups (in this case five) which are successively removed from the data set. The regression is then recalculated with the reduced number of compounds. In each case the activity of the excluded compounds is calculated with the new equation and the predictive error is calculated. The "cross validated r^2" is calculated using the formula: $r^2 = (SD - PRESS)/SD$, where PRESS is the "predictive sum of squares", i.e. the squared sum, over all compounds, of the predictive errors, and SD is the sum of squared deviations from the mean of each biological activity. If a structure–activity relationship has genuine predictive ability, then the cross validated r^2 value must not fall substantially below that of the conventional r^2 value, reflecting the ability of a reduced set of compounds to predict the biological activity of the remaining members of the data set. It has been noted that care must be taken when dividing the data set into subgroups to ensure each contains compounds with a representative range of potencies.[26] This prevents the biasing of the data set that results if too many high- or low-potency compounds are removed. The predictive r^2 value obtained for the compounds in Table I is 0.72, which is in good agreement with the conventional r^2 value of 0.80. This supports the ability of the model described by eq 1 to predict the potency of new phenylanthranilic acids in the thyroid hormone uptake system.

While eq 1 is able to predict the inhibitory potency of phenylanthranilic acids in the thyroid hormone uptake system, it is apparent that it does not predict the potency of the thyroid hormones themselves. T_4 and T_3 have clogP values of 3.54 and 2.99, respectively, but inhibit hormone uptake with lower IC_{50} values than the phenylanthranilic

acids; T_3 has an IC_{50} value for thyroid hormone uptake inhibition of 1 μM and T_4 has an IC_{50} value of 20 μM, while the most potent phenylanthranilic acid inhibitor, meclofenamic acid (7), has an IC_{50} value of 25 μM.[6] The potency of the thyroid hormones would therefore be underestimated by eq 1. The failure of eq 1 to predict the inhibitory potency of the thyroid hormones may be due to the importance of structural features of either the hormones or the phenylanthranilic acids that were not considered in the structure–activity relationship or may result from different modes of interaction of each class of molecule with the uptake system itself.

Importance of the Carboxylic Acid Group. In order to determine the importance of the carboxylic acid group for uptake inhibition, 2,3-dimethyldiphenylamine (26) and diclofenac (25) were also tested. These two compounds differ from the phenylanthranilic acids respectively by having either no carboxylic acid group or by having a methylene group inserted between the acid group and the A ring. The ability of each of these compounds to inhibit uptake was compared to the phenylanthranilic acids having the same substitution pattern. It was found that mefenamic acid (5), which is one of the more potent inhibitors (60.9% inhibition), was more potent than the diphenylamine (26) which lacks the acid group and displays 35.4% inhibition. Comparison of (2′,6′-dichlorophenyl)anthranilic acid (11) with diclofenac (25) revealed that the insertion of the extra methylene group between the aromatic ring and the acid also reduces uptake inhibition, from 54.0 to 38.0%. In neither case did alteration or removal of the acid group result in complete loss of inhibition. This indicates that the carboxylic acid group, as found in phenylanthranilic acid, is not an absolute prerequisite for inhibition. In both cases the structural modification of the phenylanthranilic acids resulted in clogP values that were lower than the those of the parent phenylanthranilic acids. The loss in inhibition may, in each case, be accounted for by the lower hydrophobicity of the molecule. It can be seen from Table I that the inhibitory potency of diphenylamine (26) is well predicted by eq 1 but the potency of diclofenac (25) is underestimated.

Implications for the Mechanism of Uptake Inhibition. Equation 1 reveals a parabolic dependence on hydrophobicity, with maximum inhibition occurring at a clogP value of approximately 5.7. The value of clogP is dependent on changes in both the entropy and enthalpy of solvation as the test compound moves between an aqueous and lipid environment, and both have been identified to be of importance for the binding of thyroid hormones at other sites.[27,28] This correlation of uptake inhibition with the parameter clogP implies the presence of a lipophilic site of interaction between the hormone uptake system and substituted phenylanthranilic acids. Structure–activity relationships with terms that are parabolic in logP are frequently observed in complex biological systems such as cultured cells.[29] Such relationships have frequently been observed in systems, such as the current work, where the biologically active compound is of relatively low potency.[29] The behavior of the uptake system therefore appears to be similar to the thyroid hormone binding sites of the thyroid hormone nuclear receptor and the transport protein TBG where structure–activity relationships have identified hydrophobicity to be an important parameter in the binding of thyroid hormones and their analogues.[30] This relationship may be caused by hydrophobic stacking of the hormone aromatic rings with aromatic protein residues.[31] However, more information about the nature of the thyroid hormone uptake system is required before a detailed model for binding can be developed.

It is interesting to note that parabolic relationship between clogP and inhibition holds well regardless of the position of substitution and the size of the substituents on the B ring. This indicates steric interaction does not play a large part in the determination of inhibitory potency, at least for the substitution patterns and sizes we have considered. Most importantly there does not appear to be a preference for either 3′-substituted or 3′,5′-disubstituted phenylanthranilic acids as might be suggested by the presence of these substitution patterns in the thyroid hormones. Although the 3′-(trifluoromethyl) compound (3) is more potent than the 3′,5′-bis(trifluoromethyl) compound (16) and the 3′-bromo compound (12) is more potent than the 3′,5′-dibromo compound, the reverse situation is found for chlorine substitution where the 3′,5′-dichloro compound (20) is more potent than the 3′-chloro compound (18). Substitution at the 2′- and 6′-positions also fails to produce consistently potent compounds. Although meclofenamic acid (4) is the most potent compound identified, the 2′,6′-dichloro (11) compound and 2′,6′-dimethyl (7) compounds have substantially reduced inhibitory activities.

The role of the carboxylic acid group of the phenylanthranilic acids is less clear, although it is evident that it is not essential for inhibition to occur. Many of the compounds that have been reported to inhibit thyroid hormone uptake do not contain this type of functional group, and it could be that it does not have a specific role in the inhibition of T_3 uptake by phenylanthranilic acids but may play a part by contributing to gross molecular properties of the molecule, such as hydrophobicity.

Conclusion

We have described the inhibition of cellular thyroid hormone uptake by a series of substituted phenylanthranilic acids. Inhibitory activity was found to be largely independent of the substitution pattern on the B ring but a parabolic relationship clogP and potency was identified, with maximum activity occurring at a clogP value of 5.7. The relatively low potency of the phenylanthranilic acids means that further investigation of the thyroid hormone uptake system should be undertaken to identify the structural requirements of the proposed binding site. The importance of hydrophobicity in the inhibition of thyroid hormone uptake by phenylanthranilic acids may reflect the hydrophobic nature of the thyroid hormones themselves, and this physical property will be considered as we continue our investigations of other classes of thyroid hormone uptake inhibitors.

Experimental Section

Materials. Diclofenac, flufenamic acid, and mefenamic acid were purchased from Sigma. Other suppliers were unlabeled T_3 and T_4 (Henning, Berlin, Germany) and meclofenamic acid, (Warner-Lembert, Ann Arbor, MI).

T_3 Uptake. Early cellular uptake of T_3 was measured as previously reported.[6] H4 rat hepatoma cells (ATCC CRL 1548) were obtained from Flow Laboratories, Sydney, Australia. Cells were maintained in DMEM (Dulbecco's Modification of Eagle's

Medium) with 10% fetal calf serum at 37 °C and for uptake experiments were subcultured into 2-mL wells (at confluence 2 × 10^6 cells/well) (Nunclone plates, A/S Nunc, Roskilde, Denmark). After further culture in serum-free DMEM overnight, this medium was replaced and the uptake of 10^{-11} M [^{125}I]T_3 (>3200 μCi/μg, Amersham International, Amersham UK) was measured at 2 min at 37 °C. Nondisplaceable uptake was determined from duplicate incubations containing 10^{-5} M unlabeled T_3. At 2 min the displaceable uptake was 3.3 ± 0.2% of the added counts representing 84% of the total uptake. Uptake was linear over the time period 0–10 min. Possible degradation or metabolism of [^{125}I]T_3 was evaluated by LH-20 column chromatography, as previously described;[6] there is no evidence of degradation of [^{125}I]T_3 at 2 min. The test substances were included together with [^{125}I]T_3 for the 2-min incubation. Test substances were dissolved in 100% DMSO or 100% ethanol or appropriate mixtures of both for stock solutions and added to DMEM to give a final concentration of 0.1 mM. Controls contained equivalent amounts of solvent. After incubation, uptake was terminated by rapidly decanting the medium and washing the cells five times in phosphate buffered saline, 2 mL, pH 7.4 at room temperature. Cells were then harvested with 2 mL of NaOH (0.1 M) and uptake measured as the cell-associated radioactivity.

Chemistry. Melting points were determined on a Reichert hot-stage microscope and are uncorrected. Microanalyses were performed by the Australian Microanalytical Service, Melbourne. Infrared spectra were recorded as KBr disks on a Hitachi 270-30 grating spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Bruker AMX-WB300 at a ^{1}H frequency of 300 MHz. Mass spectra were recorded on a JEOL JMS-DX300 mass spectrometer in electron impact mode unless otherwise stated.

Method A: The Ullmann–Goldberg Reaction. 2-[(2,5-Dichlorophenyl)amino]benzoic Acid (10). 2-Bromobenzoic acid (2.0 g, 10 mmol), 2,5-dichloroaniline (1.6 g, 10 mmol), *N*-methylmorpholine (1.5 g, 15 mmol), and cuprous oxide (0.72 g, 5 mmol) were heated to reflux in dioxan (30 mL) under nitrogen for 16 h. The resulting dark reaction mixture was allowed to cool, and 4 M HCl (30 mL) was added. A precipitate resulted which was collected and purified by repeated recrystallization from ethanol. This gave (2-[(2,5-dichlorophenyl)amino]benzoic acid as a white solid: 360 mg, 13% yield; mp 240–241 °C; IR (KBr, cm^{-1}) 3440, 3028, 1680, 1587, 1440, 1260; ^{1}H NMR (DMSO-d_6) δ 9.97 (1 H, br s), 8.01 (1 H, dd, J = 7.7, 1.1 Hz), 7.56 (3 H, m), 7.37 (1 H, d, J = 8.3 Hz), 7.14 (1 H, dd, J = 8.5, 2.4 Hz), 7.01 (1 H, t, J = 7.4 Hz); MS *m/e* 281 (57, $M^{\cdot+}$), 263 (39), 282 (100).

Method B: Carboxyphenylation with Diphenyliodonium Carboxylate. 2-[(3,5-Dibromophenyl)amino]benzoic Acid (13). 3,5-Dibromoaniline (700 mg, 2.79 mmol), (2-carboxylatophenyl)phenyliodonium monohydrate (1.2 g, 3.5 mmol) and cupric acetate (3 mg, 0.05 mmol) suspended in isopropyl alcohol (20 mL) were heated at reflux under nitrogen for 6 h. The reaction mixture was allowed to cool, and the precipitated product was collected by filtration. The crude material was recrystallized from ethanol to give a pale brown solid: 354 mg, 34% yield; mp 261–263 °C; IR (KBr, cm^{-1}) 3328, 1656, 1569, 1413, 1242; ^{1}H NMR (DMSO-d_6) δ 9.58 (1 H, br s), 7.91 (1 H, d, J = 6.6 Hz), 7.42 (1 H, t, J = 7.6 Hz), 7.38 (3 H, m), 7.34 (1 H, s), 6.95 (1 H, t, J = 6.9 Hz); MS *m/e* 373 (59, $M^{\cdot+}$), 371 (100, $M^{\cdot+}$), 369 (59 $M^{\cdot+}$), 274 (95), 272 (94). Anal. ($C_{13}H_9Br_2NO_2$) C, H, N.

Supplementary Material Available: IR, NMR, and MS data for the phenylanthranilic acids synthesized (4 pages). Ordering information is given on any current masthead page.

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