Aromatic and Amine Substituent Effects on the Apparent Lipophilicities of *N*-[(2-Pyrrolidinyl)methyl]-Substituted Benzamides

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Abstract □ Lipophilic properties of 92 dopamine D-2 receptor antagonists belonging to the substituted benzamide class of compounds (orthopramides and methoxysalicylamides) were determined by octadecylsilane reversed-phase HPLC. The apparent lipophilicity at pH 7.5 (log k_w) was obtained from the chromatographic capacity factors in 0.02 M 3-(morpholino)propanesulfonic acid (MOPS) buffer at various concentrations of methanol. The experimental log k_w values were validated by comparison with the apparent octanol–water partitioning (log P_{app}) of 15 compounds of low to medium lipophilicity. The global lipophilicity of the neutral molecule (log k_w^o) was obtained by correcting for ionization of the amine and the phenol, using known relationships for the effects on the pK_a (where K_a is the dissociation constant) of aromatic and aliphatic substituents. Multiple regression analysis showed that log k_w^o can be expressed as the sum of π contributions and a cross correlation term ($\Sigma\rho\sigma$) for interactions between the aromatic substituents. Comparison between the methoxysalicylamide (raclopride) series and the orthopramide (sulpiride) series demonstrated that an aromatic 6-hydroxy group increased log k_w by 0.4 in the 5-halogen series and by 0.8 in the 5-alkyl series, and that a 6-methoxy group decreased log k_w by 0.5. These paradoxical effects can be explained by the masking of the polarity of the amide caused by the 6-hydroxy group forming an intramolecular hydrogen bond with the amide carbonyl group. Introduction of an additional *ortho*-methoxy substituent had the opposite effect because the resulting steric hindrance prevents the amide moiety from adopting a coplanar conformation with the benzene ring. The presence of a substituent in the aromatic 3-position lowered log k_w by 0.3 via a combination of steric and electronic influences on the adjacent 2-methoxy group, causing a weakening of the hydrogen bond between the amide and the oxygen atom of the 2-methoxy group. As a result, halogen and alkyl substituents in the 3-position increase the apparent lipophilicity only half that of similar substituents in the 5-position. Substitution with ω-fluoroalkoxyl groups in the aromatic 2- and 3-positions and with ω-fluoroalkyl groups in the 5-position reduced lipophilicities by 0.5 as compared with the corresponding desfluoro derivatives, thereby making them equivalent to an alkyl derivative with one less carbon atom in the chain. In contrast, substitution on the pyrrolidine nitrogen atom with a 2-fluoroethyl or a 3-fluoropropyl group produced compounds with apparent lipophilicities ~1.5 and ~0.5 higher, respectively, than those of the corresponding *N*-ethyl derivatives. These effects result from a fluorine-induced decrease of the basicity of the amine. With these relationships, the apparent lipophilicities at pH 7.4 were predicted for a series of recently developed benzamide radioligands to evaluate their utility as single photon emission computed topography and positron emission topography imaging agents of the dopamine D-2 receptor in the human brain.

Lipophilicity is an important parameter in drug design because it allows empirical correlation with biological data and provides information about hydrogen bonding and cross interactions between functional groups in the molecule.[1] A useful descriptor of global lipophilicity of therapeutic agents has been the octanol–water partition coefficient (log P_{oct}), traditionally obtained by the shake-flask method. The most frequently used technique to obtain log P_{oct} is to distribute the organic compound between aqueous buffer and 1-*n*-octanol and measure the concentration in the least concentrated phase.[2,3] Recently, alternative descriptors of lipophilicity, particularly capacity factors (log k_w) from reversed-phase HPLC have been employed.[3] These determinations usually correlate well with log P_{oct} values and expand the measurable range of lipophilicities beyond those obtainable by the shake-flask method.

The global lipophilicity of polysubstituted aromatic molecules can be predicted by a combination of substituent contributions (π) and the mutual cross interaction between the substituents.[4] The cross-interaction terms reflect the effects of hydrogen bonding and are a function of the electronic properties (σ) and the susceptibility for hydrogen bonding (ρ) between each pair of substituents.[5] Calculation of the global lipophilicity as the sum of its substituent contributions provides an estimate of the lipophilicity of the molecule in its neutral state and not of its ionization state *in vivo*. The partitioning of an amine at physiological pH (7.4) is a more relevant measure of its behavior *in vivo*. For this reason, HPLC capacity factors in a buffer of pH 7.5 (log k_w) were used to measure the apparent lipophilicity.

We have participated in the development of [^{123}I]iodine[6]- and [^{18}F]fluorine[7]-substituted benzamides as potential imaging agents of cerebral dopamine D-2 receptors (Figure 1). We have found that the image contrast (i.e., the ratio of uptake of radioligand in high receptor density brain tissue to nonspecific uptake) is dependent on the apparent lipophilicity of the radioligand at pH 7.5.[6] Uptake in receptor-rich areas depends on the affinity of the ligand for the dopamine D-2 receptor, whereas low specific binding requires relatively low apparent lipophilicity.[6] The contribution of the substituent in the aromatic 5-position (R_5 in Figure 1) plays a major role in determining the receptor affinity of these compounds.[8]

At pH 7.5, the pyrrolidine base of *N*-[(1-ethyl-2-pyrrolidinyl)-methyl]benzamides exists largely as a protonated cation, the degree of ionization being dependent on the pK_a (where K_a is the dissociation constant) of the amine. Therefore, the apparent lipophilicity at pH 7.5 (log P_{app}) is less than that of the neutral molecule, the difference being dependent on the pK_a. Reported values for a series of metoclopramide (4-amino-5-chloro-*N*-[2-(*N*,*N*-diethylamino)ethyl]-2-methoxybenzamide) derivatives have demonstrated that there exists a measurable effect on pK_a of the tertiary amine [pK_a(NH)] that is induced by the aromatic substituents in relation to their position to the 1-carboxamide group.[9] In the substituted 6-methoxysalicylamide series, the dissociation constant of the phenol [pK_a(OH)] is strongly influenced by the electronegativity of the aromatic substituents,[10] in particular the 3- and 5-positions of the benzamides which are *para* and *ortho* relative to the phenol, respectively (see Figure 1). Ionization constants for polysubstituted phenols can be accurately predicted from the Hammett σ constant of their substituents.[11] In some halogen-substituted methoxysalicylamides (i.e., raclopride), in which the phenol becomes quite acidic (i.e., pK_a < 7.0), the formation of an intramolecular zwitterion must also be considered.[12] Therefore, correlation of log P_{app} with substituent effects assume a multivariate relationship.

Initial attempts to quantify the substituent effect on the antidopaminergic activity of substituted benzamides have indicated that the aromatic 3- and 5-*meta*-positions are unequal,[13]

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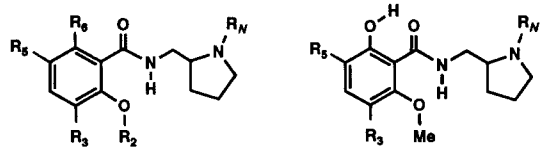

A B

Figure 1—Chemical structures of the 2-alkoxybenzamide (orthopramide) series (**A**) and 6-hydroxybenzamide (methoxysalicylamide) series (**B**).

and also have suggested that the influence of the hydroxy group on the potency of the methoxysalicylamide series might be negligible. To further elucidate the role of lipophilicity in the structure–activity relationships of these compounds and to determine the suitability of other fluoro- and iodobenzamides and 6-methoxysalicylamides as potential radioligands for positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging of the dopamine D-2 receptor, we have prepared a large number of *N*-[(1-alkyl-2-pyrrolodinyl)methyl]-2-alkoxy-3,5-substituted benzamides and some corresponding 6-hydroxy or 6-methoxy analogues and determined their log k_w at pH 7.5. After correcting for ionization to reflect their lipophilicies as neutral molecules (log k_w^o) quantitative structure–lipophilicity relationships were established in terms of the classical structural parameters ρ, σ, and π. Using these relationships, log k_w^o was calculated, corrected back to pH 7.5, and compared with the experimentally determined values. By quantifying the effects on log P_{app} of various aromatic and aliphatic substituents in terms of their physicochemical properties, we present a quantitative structure–activity relationship (QSAR) basis for designing new substituted benzamide radioligands with highly predictable log P_{app} values as potential imaging agents of the dopamine D-2 receptor.

Experimental Section

New compounds—Previously reported substituted benzamides were synthesized by the described methods (Tables 1–6).[6–31] New compounds were synthesized in analogy with their corresponding structurally related halogen- or alkyl-substituted benzamides by reacting the corresponding substituted benzoyl chloride with the appropriate 1-alkyl-2-(aminomethyl)pyrrolidine as described in the preparation of iodopride (*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2-methoxybenzamide).[14] The ^{1}H NMR spectra of the aromatic substituents (given below) and mass spectra were consistent with the proposed structures. *N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2,5-dimethoxybenzamide (**5**) was synthesized from 2,5-dimethoxybenzoic acid (Aldrich): viscous oil; ^{1}H NMR: δ 8.47 (b, 1H), 7.78 (d, *J* 3.2 Hz, 1H), 6.99 (dd, *J* 8.9 and 3.2 Hz, 1H), 6.91 (d, *J* 8.9 Hz, 1H), 3.90 (s, 3H), 3.82 (s, 3H) ppm. *N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2,3-dimethoxy-5-(1-propenyl)benzamide (**14**) was synthesized from 1,2-dimethoxypropenylbenzene (Aldrich): viscous oil; ^{1}H NMR: δ 7.92 (b, 1H), 7.59 (d, 1H), 6.98 (d, 1H), 3.88 (ds, 6H) ppm. 5-Bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-3-iodo-2-methoxybenzamide (**55**) was synthesized from norremoxipride (5-bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxybenzamide; **35**) in analogy with the preparation of itopride (5-ethyl-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-3-iodo-2-methoxybenzamide; **56**).[6] Compound **55** was an unstable oil at room temperature, slowly losing its iodine substituent, and was examined by HPLC as a mixture: ^{1}H NMR: δ 8.92 (b, 1H), 7.99 (s, 1H), 3.83 (s, 3H) ppm. The *N*-allyl derivative (**70**) of epidepride (*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide **12**),[18] was prepared from the corresponding secondary amine in analogy with the *N*-allyl derivative of the 5-bromo-6-hydroxybenzamide (**46**).[22] *N*-[(1-Allyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide (**70**) was obtained as an oil. ^{1}H NMR: δ 8.28 (b, 1H), 7.76 (d, *J* 2.4 Hz, 1H), 7.09 (d, *J* 2.4 Hz, 1H), 5.89 (m, 1H), 5.19 (dt, *J* 16.8 Hz, 1H), 5.19 (d, *J* 10.0 Hz, 1H), and 3.88 ppm (ds, 6H). The *N*-alkyl and *N*-(ω-fluoroalkyl) derivatives[28] (**76–80**) of raclopride (3,5-dichloro-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxybenzamide; **40**)[23] were gifts from Dr. Steve Moerlein, St. Louis, MO. The 5-(ω-fluoroalkyl) derivatives[15] (**7**, **8**, and **19**) of iodopride[14] (**4**) were gifts from Dr. Jogeshwar Mukherjee, Chicago, IL. Iodobenzofuran (5-iodo-7-[*N*-[(1-ethyl-2-pyrrolidinyl)-methyl]carboxamido-2,3-dihydrobenzofuran; IBF; **90**),[32] was a gift from Dr. Hank Kung, Philadelphia, PA. The tropapride (*N*-[8-benzyl)-8-azabicyclo[3.2.1]octan-3β-yl]-2,3-dimethoxybenzamide) analogues, methoxypiperidinylbenzamide [4-(2,3-dimethoxybenzamido)-1-(4-fluorobenzyl)piperidine; (MPB; **91**)[33] and the azabicyclononyl derivative (N-[9-(4-fluorobenzyl)-9-azabicyclo[3.3.1]nonan-3β-yl]-2,3-dimethoxybenzamide; MABN; **92**),[33] were gifts from Dr. Robert Mach, Philadelphia, PA.

Lipophilicity as Determined by HPLC—The Biagi method as described by El Tayar[34] was utilized for measuring the lipophilicity of substituted benzamides. For comparison with their previous data, the same pH (7.5) was used instead of physiological pH (7.4). The compounds were analyzed by C-18 reversed-phase chromatography with a 3-*N*-(morpholino)propanesulfonic acid (MOPS) buffer (20 mM, pH 7.50) containing *n*-decylamine at 2.0 mL/L and methanol in concentrations between 25 and 65%. The capacity factor (k_x) at each methanol concentration (x) was calculated with eq 1a, where t_x is the retention time of the compound and t_0 is the retention time of the void volume (2.73 mL).

$$k_x = (t_x - t_o)/t_o \quad (1a)$$

$$\log k_x = ax + \log k_w \quad (1b)$$

The logarithms of the capacity factors (log k_x) were plotted against methanol concentration and log k_w was obtained by linear extrapolation to 0% methanol concentration according to eq 1b. Multiple determinations of log k_w at different times and with different HPLC columns and batches of buffers were averaged. The HPLC system consisted of a Kontron 420 pump, a Rheodyne 4125 injection valve, a 25 cm × 4.6 mm Lichrosorb RP-18 10-μm HPLC column (Alltech) protected by a Waters Resolve C-18 Guard-Pak column, and a Kontron 430 scanning UV detector operating at 235 nm. The column was operated at ambient temperature (21 °C) and a flow rate of 1.5 mL/min. Once a week, the column was back-flushed with 100% methanol in the attempt to maintain the original partitioning conditions. However, whereas the capacity factors obtained with new columns were reproducible, the retention times showed a progressive increase over several weeks of use. To compare data recorded at different times, the retention times of all compounds were normalized for each concentration of methanol to match the capacity factors of epidepride[18] [e.g., k_{50} = 5.19] or raclopride[23] (e.g., k_{50} = 9.28). The columns were replaced when this correction factor exceeded 10%.

Partitioning by Shake-Flask Method—The method of Rauls and Baker was used.[2] Standard solutions (10 mM) of the compounds in 50% aqueous ethanol (0.05 mL) were added to a mixture of 5.0 mL of 1-*n*-octanol and 5.0 mL of MOPS or disodium phosphate buffer (20 mM, pH 7.5, 21 °C). The mixture was shaken for 5 min on a vortex apparatus and then centrifuged at 1000 rpm for 5 min. A sample of the aqueous layer (0.100 mL) was analyzed on the RP-18 column (Alltech), and the amount before (n_o) and after shaking (n_{aq}) was determined by comparison of its UV absorption at 235 nm (peak urea) with that of 2–100 nmol of the stock solution. The octanol–water distribution (P_{app}) of the compounds was taken as the ratio between the difference in concentration before and after shaking ($n_o - n_{aq}$) and the aqueous concentration (n_{aq}). The log P_{app} values were not corrected for partial molar volumes of water in octanol (3.8%) and octanol in water (0.11%),[9] nor corrected for the partial ionization of the benzamide molecule (see below).

Amine and Phenol Ionization—The ionization constants of the tertiary amine of the 1-alkyl-2-(aminomethyl)pyrrolidinyl group were obtained by correlation of substituent effects with reported values taken from the works of Van Damme et al.[9] and Tsai et al.[10] They determined the pK_a for a series of 3- and 5-substituted orthopramides and methoxysalicylamides, respectively. The pK_a for iodopride (**4**; 8.89)[14] and isoremoxipride (5-bromo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dimethoxybenzamide; **11**; 8.79),[21] were taken from our previous work. Correlation with the sum of the Hammet σ constant for the 3- and 5-*meta* and 4- and 6-*para* positions gave the following relationship (eq 2a), where I_{SAL} is 1 for the methoxysalicylamides and 0 otherwise.

$$pK_a(NH) = 9.02(\pm 0.21) - 0.42(\pm 0.05)\Sigma\sigma_{3,5m+4,6p} + 0.35(\pm 0.08)I_{SAL} \quad (2a)$$

$$r = 0.92;\ n = 17;\ s = 0.07;\ F = 83.4$$

In eq 2a, r is the correlation coefficient, n is the number of compounds, s is the standard error, and F is the Fisher ratio. Effects on the pK_a of compounds with amine substituents other than ethyl were taken from

Table 1—Structures and Physical Properties of *N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-Substituted 2-Methoxybenzamides

Compound	Structure (R_2 = Me, R_6 = H, R_N = Et)[a]		Experimental[b]			Calculated		
	R_3	R_5	Log k_w	Slope	n	Log k_w[c]	pK_a[d]	Ref.[e]
1	H	H	0.986	−0.011	5	1.04	9.02	13
2	H	Cl	1.852	−0.017	6	1.82	8.87	13
3	H	Br	1.979	−0.018	5	1.94	8.86	13
4	H	I	2.167	−0.019	5	2.09	8.87	14
5	H	OMe	1.124	−0.013	6	1.06	8.97	—[f]
6	H	Et	1.832	−0.018	5	1.74	9.05	13
7	H	2-FEt	1.210	−0.015	3	1.32	9.05	15
8	H	3-FPr	1.702	−0.018	4	1.74	9.05	15
9	OMe	H	0.936	−0.014	6	0.95	8.97	16
10	OMe	Cl	1.697	−0.017	4	1.78	8.81	16
11	OMe	Br	1.818	−0.019	4	1.89	8.81	17
12	OMe	I	2.036	−0.020	6	2.05	8.82	18
13	OMe	Et	1.608	−0.019	7	1.60	9.05	16
14	OMe	Pr	2.102	−0.020	5	2.01	9.00	13
15	OMe	Δ^1-Pr	2.077	−0.020	3	2.05	8.95	—
16	OMe	Δ^2-Pr	1.848	−0.019	3	1.89	8.97	19
17	OMe	3-HOPr	0.649	−0.014	4	0.82	9.00	19
18	OMe	3-TosOPr	2.718	−0.033	2	2.70	9.00	20
19	OMe	3-FPr	1.636	−0.017	6	1.63	9.00	20

[a] Aromatic substituents according to Figure 1. [b] Linearly extrapolated to 0% methanol from n different concentrations as shown in Figure 2 (eq 1c). [c] Calculated with eq 2a. [d] Calculated with eq 5b. [e] Original synthesis and characterization; unreported compounds are described in the methods. [f] —, see Experimental Section.

Table 2—Structures and Physical Properties of *N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2,3-dialkoxybenzamides[a]

Compound	Structure (R_6 = H, R_N = Et)			Experimental			Calculated		
	R_2	R_3	R_5	Log k_w	Slope	n	Log k_w	pK_a	Ref.
20	Et	OMe	H	1.211	−0.020	3	1.21	8.97	21
21	Et	OMe	3-HOPr	1.025	−0.024	3	1.11	9.00	21
22	Et	OMe	3-TosOPr	2.964	−0.033	2	3.02	9.00	21
23	Et	OMe	3-FPr	1.832	−0.023	3	1.91	9.00	21
24	Et	OMe	Br	2.031	−0.024	3	2.20	8.81	21
25	Et	OMe	I	2.182	−0.024	3	2.35	8.82	21
26	2-EtF	OMe	Br	1.736	−0.024	3	1.81	8.81	7
27	3-PrF	OMe	Br	2.031	−0.024	3	2.19	8.81	21
28	2-EtF	OMe	I	1.967	−0.027	3	1.95	8.83	7
29	Me	OH	Br	1.923	−0.024	3	1.77	8.81	17
30	Me	OEt	Br	2.395	−0.025	3	2.15	8.81	21
31	Me	2-OEtF	Br	1.801	−0.025	3	1.70	8.81	21
32	Me	OEt	I	2.339	−0.026	3	2.30	8.83	21
33	Et	OEt	Br	2.467	−0.026	3	2.44	8.81	21

[a] See Table 1 for explanations of all columns.

the sulpiride (5-aminosulfonyl-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide) series[9,35] (i.e., ΔpK_a for methyl, propyl, allyl, and 4-fluorobenzyl groups are −0.29, −0.05, −0.79, and −1.26, respectively).

Dissociation constants for the phenol of the methoxysalicylamides, pK_a(OH), were obtained by correlation of data of a series of alkyl and halogen polysubstituted phenols by Blackman et al.[11] and of methoxysalicylamides by Tsai et al.[10] The Taft steric parameter (*Es*) was used for the two *ortho* positions in the phenols and for the *para* position to the phenol of the methoxysalicylamides as described by Tsai et al.[10] Combination of the two series gave pK_a values of the substituted methoxysalicylamides according to eq 2b. Table 7 shows a comparison of observed and calculated pK_a values that were determined with eqs 2a and 2b.

$$pK_a(OH) = 9.45(\pm 0.09) - 2.38(\pm 0.22)\Sigma\sigma_{3,5p} - 1.13(\pm 0.15)Es - 1.69(\pm 0.13)I_{SAL} \quad (2b)$$

$$r = 0.98;\ n = 22;\ s = 0.29;\ F = 239$$

The difference in lipophilicity of neutral and ionized substituted benzamides depends on the degree of ionization (i.e., their pK_a). Given log k_w(pH), the neutral lipophilicity is determined from both the protonation of the amine and the dissociation of the phenol by a Collander-type relationship shown in equation 2c[1].

$$\log k_w^{\circ} = \log k_w(pH) + \log[1 + 10^{(pK_a(NH) - pH)} + 10^{(pH - pK_a(OH))}] \quad (2c)$$

When pK_a(OH) < pK_a(NH), the molecule forms a zwitterion in which the observed macroscopic dissociation constants are not identical to the true microscopic constants.[36] However, even in cases where a large fraction of the molecules in aqueous solution exist as zwitterions, the distribution in an organic phase represent that of the neutral species.[10] For raclopride, the compound with the largest difference in pK_a between acid and base (Δ pK_a, 3.39), 96% of the molecules exist as zwitterions in aqueous solution.[10] Because zwitterions are incapable of partitioning into the octanol layer, Tsai et al.[10] concluded that the apparent octanol–water distribution of raclopride (log P_{app}, 1.33) and its congeners reflect that of the neutral molecules and not that of the zwitterions.

Substituent Effects—The effect of the substituents on log k_w° was expressed in terms of the Hansch aromatic lipophilicity constant (π),[37] Hammett aromatic electronic constant (σ),[38] and Fujita susceptibility constant (ρ).[5,37] The cross interaction term (T_{ij}) between the i^{th} and j^{th}

Table 3—Structures and Physical Properties of *N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-Substituted Methoxysalicylamides

Compound	Structure (R_2 = Et, R_6 = OH, R_N = Et)[a]		Experimental			Calculated			Ref.
	R_3	R_5	Log k_w	Slope	n	Log k_w[b]	pK_a(NH)[c]	pK_a(OH)[d]	
34	H	H	1.629	−0.022	6	1.69	9.52	8.12	8
35	H	Br	2.496	−0.024	4	2.47	9.37	7.57	8
36	H	I	2.783	−0.028	4	2.79	9.37	7.68	8
37	H	OMe	1.253	−0.020	4	1.12	9.54	8.66	22
38	H	Et	2.805	−0.026	5	2.80	9.55	8.48	8
39	Cl	H	2.044	−0.025	4	2.17	9.37	6.48	8
40	Cl	Cl	2.681	−0.031	4	2.62	9.21	5.93	23
41	Cl	Br	2.786	−0.032	5	2.75	9.20	5.93	23
42	Cl	I	3.202	−0.037	4	3.12	9.22	6.05	14
43	Cl	Et	3.323	−0.031	4	3.31	9.40	6.84	23
44	OMe	H	1.460	−0.019	6	1.47	9.47	8.14	22
45	OMe	Cl	1.920	−0.025	4	2.05	9.32	7.60	22
46	OMe	Br	2.151	−0.026	5	2.22	9.31	7.60	22
47	OMe	I	2.484	−0.028	3	2.51	9.33	7.71	24
48	OMe	Et	2.507	−0.028	5	2.54	9.50	8.50	22
49	OMe	2-FEt	2.074	−0.025	3	2.02	9.50	8.50	19
50	OMe	Pr	3.083	−0.033	4	3.16	9.50	8.55	21
51	OMe	Allyl	2.873	−0.032	2	2.92	9.48	8.50	19
52	OMe	3-HOPr	1.436	−0.019	2	1.32	9.50	8.55	19
53	OMe	3-FPr	2.521	−0.029	3	2.61	9.50	8.55	19
54	Br	Br	2.741	−0.031	5	2.74	9.20	5.72	23
55	I	Br	2.931	−0.032	5	3.83	9.21	5.56	—[e]
56	I	Et	3.585	−0.032	4	3.54	9.41	6.47	6
57	Br	OMe	1.760	−0.023	5	1.78	9.31	6.91	22
58	I	OMe	1.910	−0.024	4	1.95	9.33	6.75	25
59	Et	OMe	1.718	−0.024	5	2.02	9.50	8.33	22

[a] Substituent numbering is that of the 2-methoxybenzamides (Figure 1A). [b] Calculated with eq 6 and corrected for ionization at pH 7.5. [c] Calculated basicity of the amine with eq 2a. [d] Calculated acidity of the phenol with eq 2b. [e] See Experimental Section.

Table 4—Structures and Physical Properties of *N*-[(1-Alkyl-2-pyrrolidinyl)methyl]-2,3-dimethoxybenzamides

Compound	Structure (R_2 = Me, R_3 = OMe, R_6 = H)[a]		Experimental			Calculated		Ref
	R_5	R_N	Log k_w	Slope	n	Log k_w	pK_a[b]	
60	Br	Me	1.826	−0.023	2	1.99	8.52	21
61	Br	Pr	2.164	−0.025	3	2.18	8.76	21
62	Br	2-FEt	3.197	−0.041	4	3.19	6.98	7
63	Br	3-FPr	2.690	−0.034	4	2.72	7.83[c]	7
64	Br	Allyl	2.601	−0.029	4	2.74	8.02	16
65	Br	4-FBz	4.093	−0.045	3	4.06	7.55[d]	26
66	I	Me	2.068	−0.023	2	2.13	8.53[e]	21
67	I	Pr	2.324	−0.026	4	2.36	8.77	21
68	I	2-FEt	3.369	−0.042	4	3.35	6.99[f]	7, 27
69	I	3-FPr	2.898	−0.037	4	2.89	7.85	7
70	I	Allyl	2.881	−0.032	3	2.91	8.03[g]	21
71	I	4-FBz	4.428	−0.050	3	4.22	7.56	27
72	3-FPr	Pr	1.898	−0.024	4	1.90	8.95	28
73	3-FPr	Allyl	2.481	−0.025	3	2.48	8.21	28
74	3-HOPr	Pr	1.101	−0.019	3	1.02	9.05	28
75	3-HOPr	Allyl	1.700	−0.023	3	1.66	8.21	28

[a] See Table 1 for general explanations. [b] Calculated from the Bjerrum formula[43] (eq 7) or with the corresponding values from the sulpiride series.[8] [c] Measured value, 7.66. [d] Measured value, 6.41. [e] Measured value, 8.40. [g] Measured value, 7.82. [f] Measured value, 7.19.

aromatic positions was calculated as the sum of the mutual electronegativity (σ) and susceptibility (ρ) according to eq 3a.[5] The calculated log k_w^o was obtained by multiple regresssion fit with eq 3b. To account for conformational effects, steric parameters (Es_j) for certain positions, such as *ortho* to the common methoxy or carboxamido substituents, were used. Because the precise effects of fluorine substitution (π_F) in various positions are unknown, the regression of eq 3b was first established with all fluorine-containing compounds excluded. Then, π_F was determined by fitting the experimental log k_w values while keeping the coefficients of eq 3b at the desfluoro values.

$$T_{ij} = \rho_i\sigma_j + \rho_j\sigma_i \quad (3a)$$

$$\log k_w^o = b\,\Sigma\pi_i + c\,\Sigma T_{ij} + d_i Es_i + e \quad (3b)$$

Regression Analysis—Retention times (t_r) for each compound were recorded and the capacity factors (k_x) were calculated with eq 1a. Linear regression of the log k_x values gave log k_w (7.5) according to eq 1b. Correction for ionization with eq 2c gave log k_w^o. Multiple regression analysis of the log k_w^o values was performed with standard statistical software (Statworks, Cricket Software, Philadelphia, PA). Regression coefficients are given with their 95% confidence intervals.

Table 5—Structures and Physical Properties of *N*-[(1-Alkyl-2-pyrrolidinyl)methyl]-5-chloro-6-methoxysalicylamides

Compound	Structure (R_2 = Me, R_5 = Cl, R_6 = OH)[a]		Experimental			Calculated			
	R_3	R_N	Log k_w	Slope	n	Log k_w[b]	R, Å[c]	pK_a(NH)[d]	Ref.
76	Cl	Pr	2.926	−0.038	2	2.98	—[e]	9.21	29
77	Cl	Bu	3.070	−0.031	2	3.12	—	9.21	29
78	Cl	2-FEt	4.018	−0.041	2	3.61	3.58	7.39	29, 30
79	Cl	3-FPr	3.319	−0.029	2	3.32	4.81	8.24	29, 30
80	Cl	4-FBu	—	—		3.19	6.04	8.76	29
81	Et	2-FEt	4.354	−0.041	4	4.47	3.58	7.57	30

[a] See Table 3 for general explanations. [b] Calculated from log k_w^0 with eq 2c. [c] Distance between the nitrogen and fluorine atoms adopted from the corresponding aminoalkylamines according to Tanford.[44] [d] Calculated from the Bjerrum formula[43] with eq 7. [e] —, Not applicable.

Table 6—Structures and Physical Properties of *N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamides

Compound	Structure (R_2 = Me, R_6 = OMe, R_N = Et)[a]		Experimental			Calculated		
	R_3	R_5	Log k_w	Slope	n	Log k_w[b]	pK_a[c]	Ref.
82	H	H	0.465	−0.012	2	0.33	9.02	31
83	H	Br	1.238	−0.022	5	1.29	8.86	31
84	H	I	1.489	−0.025	3	1.47	8.87	8
85	H	Et	1.138	−0.019	2	1.07	9.05	8
86	OMe	H	0.239	−0.010	4	0.35	8.97	22
87	OMe	Br	1.183	−0.020	5	1.37	8.81	22
88	OMe	I	1.614	−0.028	3	1.54	8.82	25
89	OMe	Et	1.068	−0.019	6	1.08	9.00	22

[a] See Table 1 for general explanations. [b] Calculated with eq 2a. [c] Calculated with eq 5b.

Table 7—Comparison Between Calculated and Measured pK_a Values

Compound[a]	Structure[b]					pK_a(NH)		pK_a(OH)		
	R_2	R_3	R_5	R_6	R_N	Observed[c]	Calculated[d]	Observed[c]	Calculated[e]	Ref.
4	Me	H	I	H	Et	8.89	8.87	—[f]	—	14
11	Me	OMe	Br	H	Et	8.79	8.81	—	—	12
24	Et	OMe	Br	H	Et	8.82	8.81	—	—	21
30	Me	OEt	Br	H	Et	8.64	8.81	—	—	21
34	Me	H	H	OH	Et	9.56	9.52	8.76	8.12	10
38	Me	H	Et	OH	Et	9.57	9.55	8.86	8.48	10
40	Me	Cl	Cl	OH	Et	9.21	9.21	5.82	5.93	10
43	Me	Cl	Et	OH	Et	9.69	9.40	6.94	6.84	10
54	Me	Br	Br	OH	Et	9.18	9.20	5.95	5.72	10

[a] Compound numbers are consistent for all tables. [b] See Figure 1 for chemical structures. [c] Data taken from the reference. [d] Calculated with eq 2a. [e] Calculated with eq 2b. [f] —, Not applicable.

Results

Apparent Lipophilicities—Octanol-buffer partitioning of representative compounds is shown in Table 8. Comparison of the log P_{app} for these selected compounds with log k_w showed the following relationship (eq 4), which is in fair agreement with the results of El Tayar et al.[34] and Hinderling et al.[39].

$$\log P_{app} = 1.174(\pm 0.093) \log k_w - 0.688(\pm 0.191) \quad (4)$$

$$r = 0.97;\ n = 15;\ s = 0.21;\ F = 221$$

No differences in lipophilicity values were seen when samples were partitioned in MOPS buffer compared with that in sodium phosphate. No significant difference in the partitioning of raclopride (**40**) was detected when the stock solution was prepared in water instead of 50% ethanol.

6-H Series (Tables 1 and 2)—The slope of the regression line (a in eq 1b) can also be correlated with lipophilicity.[40] The slope of the regression line and the number of methanol concentrations are given in Tables 1 and 2. A typical graph is illustrated in Figure 2, which shows log k_x, slope, and intercept (log k_w) of some compounds in the 3-OMe, 6-H (epidepride, **12**) series. Because the regression for all these compounds was highly linear with correlation coefficients (r) greater than 0.996 (not shown), we have also included results with some compounds for which only two methanol concentrations were used. Comparison of log k_w values with that of the unsubstituted **1** shows that alkyl groups in the 5-position increased log k_w by 0.7 of their π values, whereas the effect of halogen atoms was normal.

After correcting for ionization (eq 2c), correlation of log k_w^o with the sum of π-values and the sum of *ortho*, *meta*, and *para* cross interactions gave the following eq 5a.

$$\log k_w^o = 0.72(\pm 0.06)\Sigma\pi_{N,2,3,5} + 0.59(\pm 0.16)\Sigma T_{(o,m,p)} + 1.72(\pm 0.11) \quad (5a)$$

$$r = 0.94;\ n = 32;\ s = 0.16;\ F = 64$$

Compounds with fluoroalkyl substituents were excluded. Improved statistics were obtained when steric effects of the aromatic substituent were included. This method allowed the 2,6-dimethoxy series (R_6 = OMe) to be included. Separating the type of cross interactions in eq 5a showed that the *meta* interaction (i.e., $T_{13} + T_{15} + T_{35}$) has a predominant effect. Using

Table 8—Octanol–Water Partitioning of Substituted Benzamides at pH 7.5

Compound[a]	Aqueous Concentration, nmol/0.10 mL[b] Pre-partitioning (n_0)[b]	Post-partitioning (n_{aq})	Lipophilicity (Log P_{app})[c]
1	1.82 ± 0.20	0.63 ± 0.09	0.28 (0.19–0.35)
5	9.93 ± 0.06	2.13 ± 0.09	0.56 (0.54–0.58)
6	9.45 ± 0.26	0.21 ± 0.01	1.64 (1.62–1.67)
12	9.98 ± 0.03	0.36 ± 0.05	1.43 (1.36–1.48)
13	4.83 ± 0.16	0.38 ± 0.02	1.07 (1.04–1.09)
32	10.4 ± 0.04	0.05 ± 0.01	2.32 (2.22–2.40)
36	96.0 ± 3.6	0.14 ± 0.02	2.84 (2.77–2.90)
40	92.6 ± 0.5	0.16 ± 0.02	2.76 (2.70–2.81)
44	47.6 ± 2.5	4.86 ± 0.08	0.94 (0.92–0.97)
48	37.4 ± 2.3	0.27 ± 0.01	2.14 (2.11–2.17)
53	37.2 ± 2.9	0.56 ± 0.03	1.82 (1.77–1.86)
63	51.3 ± 3.1	0.17 ± 0.04	2.48 (2.36–2.57)
83	1.99 ± 0.01	0.33 ± 0.01	0.70 (0.69–0.71)
89	11.4 ± 0.1	2.66 ± 0.14	0.52 (0.49–0.54)
90	10.4 ± 0.1	0.087 ± 0.004	2.07 (2.05–2.09)

[a] Compound numbers are consistent for all tables. See Tables 1–6 for chemical structures. [b] Concentration in the aqueous phase (0.02 M buffer at pH 7.5) before (n_0) and after (n_{aq}) partitioning with an equal volume of 1-octanol. Errors are SDs about the mean of three determinations. [c] Logarithm of the partition coefficient according to $P_{app} = (n_0 - n_{aq})/n_{aq}$; the interval indicates the lower and upper limit of plus and minus one SD.

the steric parameters to express the *ortho* interaction (i.e., Es_3 and Es_6 instead of $T_{23} + T_{56}$) gave a better correlation (r = 0.99). Fitting log k_w for π(F) of the fluoroalkyl- and fluoroalkoxy-substituted benzamides (Table 1 and 2), but excluding the *N*-fluoroalkyl compounds (Table 4) gave an aliphatic π(F) = –0.53. Subsequent regression of all compounds in Tables 1 and 2 gave the parameter coefficients shown in eq 5b.

$$\log k_w^o(\text{6-H,OMe}) = 0.72(\pm 0.03)\Sigma\pi_{N,2-6} + 0.52(\pm 0.07)\Sigma T_{m,p} - 0.35(\pm 0.08)Es_3 - 0.99(\pm 0.09)Es_6 + 1.71(\pm 0.05) \quad (5b)$$

$$r = 0.97;\ n = 57;\ s = 0.12;\ F = 229$$

6-OH (Methoxysalicylamide) Series (Tables 3 and 5)—The intercept, log k_w, slope, and number of methanol concentrations (n) of the substituted 6-methoxybenzamides are shown in Table 3. For clarity, the same numbering of the aromatic ring positions were used as in the 2-methoxybenzamides. Comparison of log k_w values with that of the unsubstituted **34** shows that, in contrast to the results of the 6-H series, ethyl and *n*-propyl groups in this position increased log k_w by 1.2 of their π values. As in the 6-H series, substituents in the aromatic 3-position increased log k_w only by 0.5 of their π values.

Correlation of log k_w^o according to eq 3b using the sum of all cross interactions (T_{ij}) had large errors in the calculated values (r = 0.72, s = 0.54). However, correlation with separate cross-interaction terms for each pair of substituents produced a useful equation (eq 6).

$$\log k_w^o(\text{6-OH}) = 1.12(\pm 0.06)\Sigma\pi_{N,2-6} - 2.7(\pm 0.36)T_{1,5} + 0.51(\pm 0.16)T_{5,6} - 0.45(\pm 0.07)Es_3 + 3.68(\pm 0.06) \quad (6)$$

$$r = 0.99,\ n = 26,\ s = 0.14;\ F = 128$$

Other combinations of T_{ij} gave lower F and higher s values for the same number of terms. As in the orthopramide series, there was a need for a descriptor of the bulk properties (Es_3) of the substituent in the 3-position.[10] The coefficient was twice that in eq 5b, suggesting that the susceptibility for steric perturbation

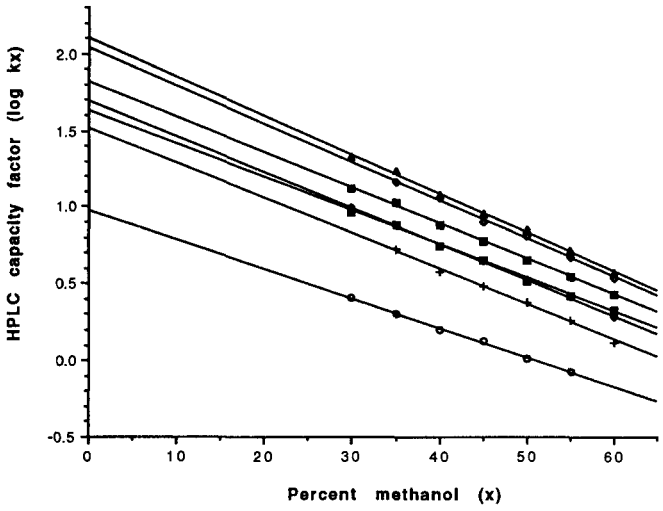

Figure 2—Typical graph of the logarithm of capacity factors (log k_x) at different methanol concentrations for the 5-substituted 2,3-dimethoxybenzamides **9–14** and **19**. Both the slope and intercepts (log k_w) of the linear regression lines are descriptors of the apparent lipophilicity, log P_{app}. Key: (○) H (9); (◆) Cl (10); (□) Br (11); (●) I (12); (■); Et (13); (▲) Pr (14); (+) F-Pr (19).

of the amide hydrogen bond is increased in the methoxysalicylamide series.

***N*-Alkyl Series**—Intercept, slope, and the number of methanol concentrations of the *N*-alkyl-substituted methoxybenzamides and salicylamides are shown in Tables 4 and 5, respectively. Increases in chain length of the alkyl substituent on the tertiary amine nitrogen atom of the pyrrolidine ring increased the apparent lipophilicity on the average by 0.32 per carbon atom (Table 4). Introduction of a fluorine atom in the terminal position of the alkyl chain caused an increase in log k_w, the magnitude being dependent on the number of carbon atoms between the fluorine atom and the tertiary amine. At a distance of two carbon atoms, the fluorine atom increased log k_w by 1.3 compared with the corresponding *N*-ethyl compounds in both the 2,3-dimethoxybenzmide (epidepride) series (Table 4) and the 6-methoxysalicylamide (raclopride) series (Table 5). At a distance of three carbon atoms, the increases in the two series were 0.6 and 0.4, respectively. Thus, the effect of fluorine substitution in the *N*-alkyl group was opposite that found in the aromatic 5-alkyl and 3-alkoxy group. Because the lipophilicity in the desfluoro series increased with chain length by ~0.3 and an aliphatic fluorine atom decreases the lipophilicity by the same amount, the observed effect of fluorine substitution on the apparent lipophilicity was attributed to a decrease in basicity of the amine. The shift in equilibrium (ΔpK_a) by an electrostatic interaction between a charge (q) and a dipole vector ($\mu \cos \varphi$) at a distance (R) is given by the Bjerrum formula[43] (eq 7), where k is the Boltzman constant, T is the temperature, D_E is the effective dielectric constant of the solvent and can assume values between that of water (78) and a pure hydrocarbon (2.0),[43] and R is the distance between the fluorine and nitrogen atoms assuming that the chain adopts an extended *trans* conformation due to the steric hindrance of the pyrrolidine ring.

$$\Delta pK_a = q\mu \cos \varphi / D_E \ln(10)\, kTR^2 \quad (7)$$

This equation gave similar values as those reported[44] for a series of aliphatic diamines, after adjusting for the length of the fluorine—carbon bond (i.e., R = 3.58, 4.82, and 6.04 Å for the ethyl, propyl, and butyl chains, respectively; Table 5). The dipole is the sum of nitrogen-carbon and carbon—fluorine bond moments, 0.22 and –1.39 D, respectively. Correlation of the observed log k values in the isoremoxipride (**62, 63**), epidepride

(68, 69), and eticlopride (43, 80) series with the calculated decreases in pK_a(NH) from eq 7 (D_E = 38, q = 1, μ = 1.17, and φ = 36) gave ΔpK_a = values of 1.83, 0.97, and 0.44 for the ω-fluoroalkyl derivatives. As a consequence, the 4-fluorobutyl homologue was predicted to have the lowest apparent lipophilicity at pH 7.4, similar to that of the propyl derivative. However, for the 2-fluoroethyl derivatives, the observed increases in log k_w were larger than that explained from a decrease in pK_a. For this distance, an additional correction of +0.3 was needed to match the calculated and experimental values. The reason for this discrepancy is unknown.

Conformational Effects (Table 6)—The apparent lipophilicities of 2,6-dimethoxybenzamides are shown in Table 6. The presence of two *ortho* substituents in these benzamides reduces log k_w by 0.5. In contrast to the results of the previous series, an additional 3-methoxy group caused a further decrease of only 0.1 rather than a decrease of 0.3, supporting the view that conformational effects on the 2-methoxybenzamide moiety play a major role in explaining the observed influences of the 3-substituent. In all series, correlation for each position revealed that the *N*- and 2-*O*-substituent increase log k_w by 0.6 of their π values.

Discussion

The objective of this study was to measure the apparent lipophilicity at pH 7.5 of a series of structurally related substituted benzamides and thereby determine the substituents effects. In particular, we wanted to develop a method for calculating the effects of iodine and fluorine substitution in various positions in the aromatic and aliphatic rings on log k_w to aid in the design of substituted benzamides for optimal use as noninvasive SPECT or PET imaging agents of cerebral dopamine D-2 receptors in humans. The partition coefficient P is defined as a constant relating the concentration of a solute in two immiscible liquid phases at equilibrium.[1] At a specific pH, this gives the distribution coefficient, which is related to the neutral partition coefficient by adding a linear Collander-type correction term for the ionization. The same relation is found between log P values obtained from different solvent systems, such as octadecylsilyl polymers and aqueous buffers.[3] The reason for log P being accurately determined by reversed-phase HPLC is that the dominant mode of retention in the stationary phase is that of partitioning, not adsorption.[45] One can therefore consider log k_w a linearly scaled function of log P. It is irrelevant for the QSAR study whether log k_w^0 or log P_{oct} for the neutral molecule is used as long as the pH correction is the same. Under the conditions of our study, the log k_w scale was 85% of that of log P_{app} and an offset of +0.7. This means that for values between 2.5 and 3.5, log k_w can be used interchangeable with log P_{app}. The ionization correction term which relates log P_{app} with log P_{oct} and log k_w with log k_w^o is given in eq 2c. If the dissociation constant is well above 7.5 (e.g., >8.5), this correction term becomes virtually identical to with the pK_a(NH). Because pK_a is correlated to Hammett's $\Sigma\sigma_i$, the substituent effects on log k_w can be expressed with this parameter. This explains why previous correlations of the biological activity of substituted benzamides using standard QSAR parameters have given good results in spite of the fact that in these studies the effects of partial ionization were not considered.[13,35]

Only *N*-[(1-alkyl-2-pyrrolidinyl)methyl]benzamides, having the same two-carbon distance between the two nitrogen atoms as metoclopramide, were used in this study. Therefore, similar substituent effects relative to the carboxamide (1) position were expected, justifying the use of pK_a(NH) constants from the metoclopramide series. In the 6-hydroxy (raclopride) series, the possibility of dissociation of the phenol at pH 7.5 had to be considered. This effect is also correlated with Hammett's $\Sigma\sigma_j$, but with the parameter values of the substituents defined in relation to the phenolic (6) position. Blackman et al.[11] determined the dissociation constants for di-, tri-, and tetrasubstituted chloromethylphenols, which gave a reaction constant of −2.80 that is similar to the value of −2.38 used in this study. This is also in good agreement with the results obtained by Biggs and Robinson from the pK_a of a series of 13 mono-substituted phenols (−2.23).[46] Addition of the effects of 1-carboxamide (σ_p, 0.36) and 2-methoxy (σ_m, 0.12) substituents to the relationship for the phenols gave a decrease in pK_a for the 6-methoxysalicylamides that match the coefficient of the indicator I_{SAL}. Thus, by combining the relationships for pK_a(NH) found in metoclopramide analogues by Van Damme et al.,[9] the relationships for pK_a(OH) found in chloromethylphenols by Blackman et al.,[11] and the relationships for pK_a(NH) and pK_a(OH) found in methoxysalicylamides by Tsai et al.,[10] we were able to calculate the pH correlation term and establish quantitative expressions of the lipophilicity (eqs 5b and 6).

Lipophilicity can be factored into separate terms representing steric (bulk) properties and electronic (polar) properties. The bulk term can be described by the molar volume or the Hansch π parameters. The bulk portion of log P_{oct} is well represented by $\Sigma\pi$ of the aromatic substituents because its coefficient in eq 5b is close to unity when converted to the log P scale with eq 4. The polar term is usually more difficult to parameterize and is considered a function of the dipole–dipole polarizability in the molecule and the presence of hydrogen bonds.[3] We have chosen to express the polar cross-interaction term with the Hammett σ parameter by the method of Fujita[5] as applied by Tsantili-Kakoulidou et al.[4] It implies that the overall effect on the hydrogen binding capacity of the molecule by a particular substituent is the sum of all cross interactions between the substituent and each of the other substituents.[5] For substituents in the *ortho* position, the values of the *para* position were used. It was recently demonstrated in a series of trisubstituted benzene derivatives that the so-called *ortho* effect (i.e., an anomalous behavior of *ortho* substituents compared with *meta* and *para* substituents), is a consequence of cross-interaction effects and can be described by standard parameters.[47]

Changes in log k_w with different aromatic substituents in the 2-methoxybenzamide series (Table 1) followed the hansch π values with two exceptions. Whereas the contribution of halogen atoms in the aromatic 5-position to the apparent lipophilicity was close to their Hansch π values, ethyl and *n*-propyl groups contributed only ~0.7 of their corresponding π values (Figure 2). This is explained by the positive cross-interaction term, particularly between the 1 and 5 positions. The other exception was the fact that throughout this series, an additional methoxy substituent in the aromatic 3-position caused an average decrease in log k_w of 0.23. This is a result of steric interaction of the 3-substituent on the adjacent 2-methoxy group that weakens the intramolecular amide hydrogen bond.

Increased chain length of the 2- and 3-alkoxy derivatives resulted in increases in lipophilicity in concordance with the results of Collin et al. in the tropapride series.[41] The effect of homologization of the 2-methoxy group increased the apparent lipophilicity by 0.25, whereas homologization in the 3-position caused an increase of 0.42 (Table 2).

Effect of 6-Hydroxy Substitution—A phenolic OH group would be expected to decrease the apparent lipophilicity, both as a consequence of its negative π value (−0.67) and by its increasing effect on the basicity of the amine (σ_p, −0.37; eq 2a) resulting in a larger negative correction for ionization. Paradoxically, comparison with the deshydroxy series gives an average increase of 0.56 in log k_w resulting from the 6-hydroxy group. Closer examination revealed that for 5-halogen-substituted benzamides, the increase was 0.42, whereas for the 5-alkyl series, the increase was 0.80. The larger contribution from 5-alkyl groups compared with halogen atoms is the result of a negative

coefficient for the 1–5 cross-interaction term in eq 6. Further, in the methoxysalicylamide series, the acidity of the phenolic hydrogen atom causes the pyrrolidine ring to adopt a unique conformation in relation to the benzamide moiety as a result of the formation of an intramolecular zwitterion.[12] As a consequence of this and of the presence of strong intramolecular hydrogen bonds, the cross-interaction effects of the substituents in the methoxysalicylamide series are distorted and could be only partly described by selective interaction terms. Principal component analysis of the ^{13}C NMR chemical shifts of the aromatic carbon atoms have demonstrated that substituted benzamides with a 6-hydroxy group (methoxysalicylamides) are markedly different from those that either lack a substituent or have a methoxy group in this position,[13] even though their recognition site on the dopamine D-2 receptor is considered the same.[10] Attempts to combine the substituent effects on lipophilicity for the 6-H and 6-OH series gave poor statistics with large correction factors for cross interaction (not shown). Substituted 2-methoxybenzamides (orthopramides) have a planar conformation due to the hydrogen bond between the amide hydrogen atom and the oxygen atom of the methoxy group.[8,12] The introduction of a hydroxy group in the aromatic 6-position would not be expected to change the amide conformation because coplanarity with the benzene ring is already accomplished by the amide-to-methoxy hydrogen bond. The observed increase in apparent lipophilicity induced by the 6-OH group is possibly due to intramolecular hydrogen bonds masking the polarity of the amide making it less susceptible to intermolecular interactions.

Introduction of a 6-methoxy group forces the amide moiety out of plane with respect to the aromatic ring due to steric interaction with the carbonyl group.[8] This would prevent the forming of an amide—methoxy hydrogen bond, thereby exposing the polar amide to intermolecular forces and causing a decrease in the apparent lipophilicity. Indeed, an average decrease in log k_w of 0.5 in comparison with the corresponding 6-hydrogen derivatives was observed (Table 6). Interestingly, the corresponding des-3-*O*-methyl analogue (**29**) of isoremoxipride (**11**) displayed a log k_w of 1.92, very similar to that of isoremoxipride despite the fact that the phenol of **29** lacks the ability to form an intramolecular hydrogen bond with the amide carbonyl, as in the methoxysalicylamide series. An explanation for this result can be found in comparing the corresponding calculated $\Sigma\rho\sigma$ values for **11** (+0.49) and **29** (+0.93); these values suggest that in this position, the combined cross interaction between the aromatic substituents is able to disguise the phenolic character of the 3-hydroxy group. The excellent correlation between experimentally determined and calculated log k_w with the two different eqs 5b and 6 is shown in Figure 3.

Effect of Iodine Substitution—Halogen atoms such as bromine and iodine were similar in their substituent contribution to the apparent lipophilicity with iodine, creating the most lipophilic compounds, as expected. This is reflected in the close similarity in pharmacological properties between iodine- and bromine-substituted benzamides. For example, isoremoxipride (**11**) has the highest potency known in the rat [50% effective dose (ED_{50}), 0.002 μmol/kg, intraperitoneally] for antagonizing apomorphine-induced behavior in vivo.[17] Whereas the corresponding iodo analogue, epidepride (**12**) is the most potent benzamide radioligand known (K_D, 0.024 nM) for blocking the dopamine D-2 receptor.[6,48] Thus, high potency compounds are found at relatively low log P_{app} values (i.e., log P_{app} of 1.72 and 1.85, respectively). The effect of low apparent lipophilicity is lack of nonspecific binding to nondopaminergic tissue.[6]

The log P_{app} of iodine-substituted benzamides with potential use as SPECT imaging agents, including that of IBF[32] are summarized in Table 9. Their relative apparent lipophilicity in comparison with that of epidepride (**12**) demonstrates that epidepride is the receptor ligand that has the lowest log P_{app} of

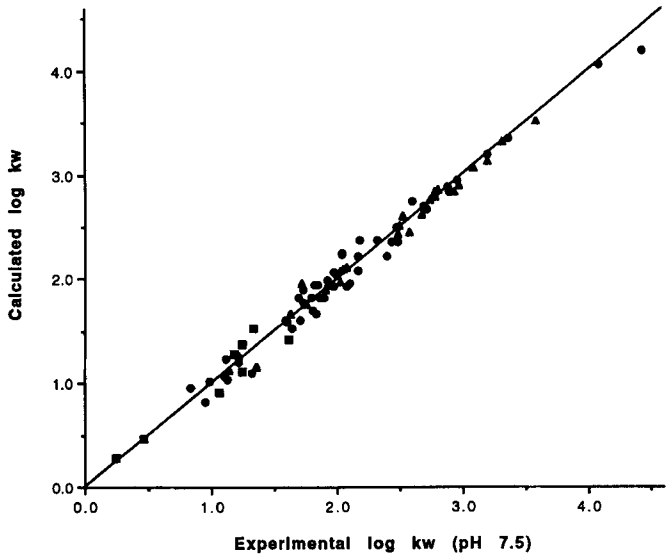

Figure 3—Correlation between calculated and measured log k_w, calculated with eq 5b for the (◆) 6-H (orthopramide) and (■) 6-OMe (remoxipride) series and eq 6 for the (▲) 6-OH (methoxysalicylamide) series. The line represents equal values.

all SPECT agents, a fact that has been attributed to producing the exceptionally high regional contrast seen with radiolabeled epidepride.[6] Of the iodine-substituted benzamides, epidepride (**12**),[18,48] its corresponding 2-fluoroethoxy analogue FIDA-1 (**28**),[7,27] the epidepride homologues **25**, **67**, and **32**,[21] IBF (**90**),[33] and ioxipride (**47**)[6,49] stand out as examples of substituted benzamides that combined a moderate apparent lipophilicity (log P_{app} 1.6–2.6) with a lipophilic substituent in the aromatic 5-position (π, 1.12), a prerequisite for potent antidopaminergic activity.[8,13]

Effects of Fluorine Substitution—Compounds with 5-fluoroalkyl and 2-fluoroalkoxyl substituents in the aromatic ring were considerably less lipophilic than the corresponding desfluoro derivatives. The decreasing effect on log k_w of an aliphatic fluorine atom required $\pi_F = -0.53$, in close agreement with those values used by Brändström (−0.65)[42] and Rekker (−0.51),[50] but different from that of Hansch (−0.17),[38] thereby making the lipophilicity of the 2-fluoroethoxy group equivalent to that of a methoxy group. This effect was also seen in the aromatic 3-position. For example, substitution with a 2-fluoroethoxy group in **31** (Table 2) resulted in a log k_w of 1.80, which is equivalent to having a methoxy group in this position (1.82 for **11**, Table 1).

The *N*-(ω-fluoroalkyl)benzamides **61**, **62**, **68**, **69**, **78**, **79**, and **80** were considerably more lipophilic than would be predicted from the combined influence of a fluorine atom and an alkyl group, with *N*-(2-fluoroethyl) derivatives being the most lipophilic. A possible explanation is that fluoroalkylation reduces the basicity of the tertiary amine. Reifenrath et al.[51] found that *N*-fluoroethyl substitution of the narcotic analgesics meperidine and metacozine lowered their pK_a values by 1.6 and 1.1, respectively. This decrease in pK_a increases log k_w, as described by eq 2c. Generally, increased lipophilicity of a receptor ligand results in increased potency, but in this case, fluorine-induced increases in apparent lipophilicity cause a 10-fold loss in binding affinities. Fukumura et al.[52] found that *N*-fluoroethyl-eticlopride (**81**) had lost 90% of its potency compared with that of eticlopride (**43**). Lannoye et al.[29] explained the reverse rank order of affinity and brain uptake with increased chain length as the result of a parabolic relationship. However, by plotting the receptor affinity [log(1/K_i)] versus the apparent lipophilicity (log P_{app}) as revealed by HPLC and corrected to pH 7.4, it is evident that the *N*-(4-fluorobutyl) derivative **80** might be the most potent fluorinated

Table 9—Apparent Lipophilicity of Iodobenzamides for Potential SPECT Imaging of the Dopamine D-2 Receptor

Ligand[a]	Compound[b]	Log k_w	Log k_w^0 [c]	Log P_{app}[d]	Ref.
FIDA-1 (TDP 758)	**28**	1.967	3.31	1.75 ± 0.04	7, 27
Epidepride	**12**	2.036	3.38	1.85 ± 0.03	6, 18
Iodopride	**4**	2.163	3.56	1.91 ± 0.03	6, 14
Iodobenzofuran (IBF)	**90**	2.378	—[e]	2.00 ± 0.12	32
HOMEP-3 (TDP 768)	**32**	2.392	3.79	2.14 ± 0.12	21
HOMEP-2 (TDP 649)	**67**	2.324	3.62	2.17 ± 0.08	21
HOMEP-1 (TDP 755)	**25**	2.182	3.52	2.19 ± 0.06	21
Ioxipride (NCQ 298)	**47**	2.484	4.33	2.52 ± 0.07	6, 25
FIDA-3 (TDP 631)	**69**	2.898	3.41	2.69 ± 0.05	7, 21
Nalepride (TDP 872)	**70**	2.891	3.53	2.72 ± 0.05	21
Iodobenzamide (IBZM)	**36**	2.783	4.68	2.78 ± 0.05	8, 57
Iclopride (TDP 450)	**42**	3.202	5.12	3.19 ± 0.11	6
Itopride (TDP 602)	**56**	3.585	5.55	3.73 ± 0.18	6
FIDA-2 (TDP 516)	**71**	4.428	4.76	4.22 ± 0.22	27

[a] Lab code or acronym used by authors in the references. [b] The structure of each receptor ligand is shown in the appropriate Tables (1–6) or given in the methods. [c] Lipophilicity of the neutral molecule calculated from eqs 5b or 6. [d] Calculated with eq 4 and corrected for physiological pH (7.4); errors are transformed SEM estimates from multiple determinations of log k_w. [e] Not determined.

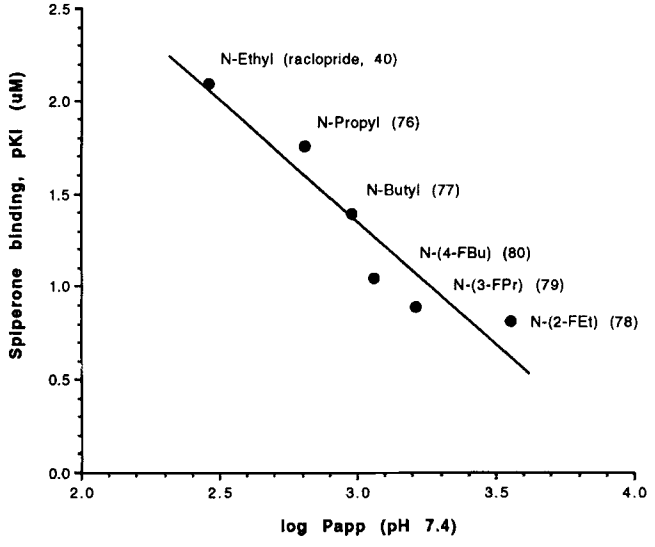

Figure 4—Activities (-log K_i) for blocking [^{3}H]spiperone binding of *N*-alkyl (**76** and **77**) and *N*-(ω-fluoroalkyl) derivatives (**77–80**) of raclopride (**40**) plotted against their apparent lipophilicity at pH 7.4 (log P_{app}). The line is a linear fit to the binding data taken from Lannoye et al.[29]

ligand in this series because it is least lipophilic (Figure 4). This combination of low affinity and high lipophilicity might explain why the *N*-[^{18}F]fluoroalkyl derivatives **78–81** of raclopride and eticlopride are such poor imaging agents.[30] Kiesewetter et al.[53] reported a striatum-to-cerebellum uptake ratio in the rat after 1 h with the fluorine-18-labeled derivatives **78** and **81** to be 1.1 and 1.6, respectively. These results have been independently confirmed by Halldin et al.[30] Molecular orbital calculations of the electron density of fluoroethylraclopride (**78**) have not been able to explain the loss of receptor selectivity.[54] The paradoxical increase in lipophilicity and loss in potency on fluoroalkyl substitution on the pyrrolidine ring can not be explained by conformational implications as a result of salicylamide zwitterion formation because the same effects are seen in the isoremoxipride series (Table 4). Our results have demonstrated that it is the absence of ionization at pH 7.5 that causes the *apparent* lipophilicity to become detrimentally high with *N*-fluoroethyl substitution and to a lesser degree with *N*-fluoropropyl substitution. This also provides an explanation for the poor contrast seen in biodistribution studies with *N*-fluoroalkylated radioligands because of the inverse relationship found between uptake ratios and the product of potency and lipophilicity.[6] In contrast,

Table 10—Apparent Lipophilicity of Fluoroalkylbenzamides for PET Imaging

Ligand[a]	Compound	log k_w	log k_w^0	log P_{app}	Ref.
FPMB (FPB)	**19**	1.634	3.15	1.38	19, 20
TDP 775	**31**	1.801	3.14	1.44	7, 21
NCQ 616 (TDP 748)	**26**	1.736	3.06	1.57	20, 56
ZYY-102	**72**	1.898	3.36	1.67	28
TDP 727	**23**	1.832	3.30	1.72	7, 21
FIDA-1 (TDP 758)	**28**	2.043	3.31	1.75	7, 27
FES (JEB-21)	**49**	2.074	4.12	1.94	19
ZYY-104	**73**	2.481	3.27	2.25	28
MPB	**91**	2.712	—[b]	2.39	33
MABN	**92**	2.857	—[b]	2.57	33
TDP 630	**63**	2.690	3.19	2.60	7, 21
FPS (JEB-26)	**53**	2.521	4.57	2.62	19
TDP 629	**62**	3.197	3.32	3.22	7, 21
GSL-4	**79**	3.319	4.38	3.33	29
NCQ 258	**78**	4.018	4.82	3.66	30
NCQ 115	**65**	4.093	4.42	4.04	26
FIDA-2	**71**	4.427	4.76	4.22	27
NCQ 134	**81**	4.354	5.25	4.71	30, 51

[a] See Table 9 for explanations of parameters. [b] Note added in proof: lit. value 2.86. (*J. Med. Chem.* **1993**, *36*, 3707–3720.)

all the 5-substituted (ω-fluoroalkyl)benzamides (**7**, **8**, **19**, **23**, **49**, and **53**) as well as the 2- and 3-substituted (ω-fluoroalkoxy)-benzamides (**26**, **27**, **28**, and **31**), were considerably less lipophilic than their corresponding desfluoro derivatives and required an aliphatic fluorine atom to decrease log k_w by 0.5 units.

Table 10 shows a summary of fluorine-substituted potential PET agents, including MPB (**91**) and MABN (**92**),[33] listed in order of increased log P_{app}. The 5-fluoropropyl derivative of epidepride, FPMB (**19**),[20] has the lowest apparent lipophilicity and the *N*-fluorobenzyl derivative FIDA-2 (**70**)[27] has the highest. Optimal brain uptake and high imaging contrast is found in the narrow log P_{app} range 1.4–2.4. Mathis et al.[19] reported the log k_w of 1.2 for the corresponding fluoroethyl analogue of FPMB, JET-10 (i.e., the 3-methoxy derivative of **7**). The log P_{app} value for this compound, calculated with eqs 4 and 5b, was 0.57, suggesting that it is too hydrophilic for the transport across the blood–brain barrier and consequently a poor imaging agent. Furthermore, QSAR studies of these compounds have shown that high affinity for the dopamine D-2 receptor requires a quite lipophilic substituent ($\pi > 0.9$) in the aromatic 5-position.[8,13] The 5-(3-fluoropropyl) derivative of epidepride, FPMB (**19**), has

an apparent substituent value (π) of 0.4 (i.e., less than that of a chloro atom or a methyl group). This suggests that FPMB (log P_{app} 1.38) is not the ultimate PET ligand in spite of the promising results obtained with this compound.[19,20] By the same rationale, high apparent lipophilicity for the *N*-(4-fluorobenzyl) derivative of isoremoxipride, NCQ 115 (**65**)[26]; log P_{app} 4.04), suggests that it would not be a good candidate ligand contrary to what has been stated.[54] Compounds in Table 10 that meet the above criteria of low log P_{app} and a lipophilic 5-substituent are NCQ 616 (**26**)[56]; log P_{app} 1.57); FIDA-1 (**28**)[7,27]; log P_{app} 1.75); XYY-104 (**73**)[28]; log P_{app}, 2.35); MPB (**91**)[33]; log P_{app} 2.39); and JEB-26 (**53**)[19]; log P_{app} 2.64). Preliminary evaluations with iodine-125-radiolabeled **28**[27] and F-18-radiolabeled **53**,[19,57] **73** (Kessler, unpublished result), and **91**[33] in visualizing the dopamine D-2 receptor have confirmed their excellent potential.

Conclusions—Determination of the apparent lipophilicity (log k_w) at pH 7.5 of a large series of 3- and 5-alkyl-, alkoxy-, and halogen-substituted 2-alkoxy-*N*-[(1-alkyl-2-pyrrolidinyl)methyl]benzamides and their corresponding 6-hydroxy analogues has established a statistically significant data base for equantitative understanding of the substituent effects on the global octanol–water partition, log P_{oct}, and in particular the behavior of this descriptor at pH 7.5, log P_{app}. The basicity of the amine and the aromatic substituent effects on intramolecular amide hydrogen bonds both play major roles in determining the apparent lipophilicity. Therefore, in quantifying these effects, the hydroxy (methoxysalicylamide) and deshydroxy (orthopramide) series must be considered separately. The results have demonstrated that once these specific benzamide effects were accounted for, multisubstituent interactions can be described by the Fujita method as perturbations of the hydrogen bonding capacity on the sum of the individual substituent contributions (i.e., log $P = \Sigma\pi + \Sigma\rho\sigma$). An aromatic 6-hydroxy group increased the lipophilicity, in particular in the 5-alkyl series (0.8), whereas a 6-methoxy group decreased lipophilicity by 0.5. Replacement of a bromo substituent with an iodine atom or of the methoxy groups with a fluoroethoxy group caused little or no change in lipophilicity, whereas introducing a fluorine atom in the 5-alkyl group decreased lipophilicity by 0.5. Substitution of fluoroalkyl groups in the nitrogen 1-pyrrolidine position caused an increased in lipophilicity as an consequence of the lowering of the basicity of the amine by the electrostatic influence from the fluorine atom. Because radioligands with potential utility as imaging agents of the dopamine D-2 receptor seem to require relatively low log P_{app} to display high contrast and low nonspecific binding, this study has identified possible candidate compounds for SPECT and PET imaging and provided a rationale for developing other potential radioligands with high image contrast.

References and Notes

1. van de Waterbeemd, H.; Testa, B. In *Advances in Drug Research*, Testa, B., Ed.; Academic: London, 1987; Vol. 16, pp 85–225.
2. Rauls, D. O.; Baker, J. K. *J. Med. Chem.* **1979**, *22*, 81–86.
3. Altomare, C.; Tsai, R-S.; El Tayar, N.; Testa, B.; Carrotti, A.; Cellamare, S.; De Benedetti, P. G. *J. Pharm. Pharmacol.* **1991**, *43*, 191–197.
4. Tsantili-Kakoulidou, A.; El Tayar, N.; van de Waterbeemd, H.; Testa, B. *J. Chromatogr.* **1987**, *389*, 33–45.
5. Fujita, T. *J. Pharm. Sci.* **1983**, *72*, 285–289.
6. Kessler, R. M.; Ansari, M. S.; de Paulis, T.; Schmidt, D. E.; Clanton, J. A.; Ebert, M.; Smith, H. E.; Manning, R. G.; Gillespie, D. *J. Nucl. Med.* **1991**, *32*, 1593–1600.
7. de Paulis, T.; Bingham, D. R.; Manning, R. G.; Mason, N. S.; Ansari, M. S.; Schmidt, D. E.; Kessler, R. M. *New Trends in Radiopharmaceutical Synthesis*; Emran, Ali, M., Ed.; Plenum: Washington, D.C., 1992; pp 97–107
8. de Paulis, T.; Kumar, Y.; Johansson, L. G.; Ramsby, S.; Florvall, L. G.; Hall, H.; Angeby-Moller, K.; Ogren, S-O. *J. Med. Chem.* **1985**, *28*, 1263–1269.
9. Van Damme, M.; Hanocq, M.; Molle, L. *Pharm. Acta Helv.* **1984**, *59*, 235–240.
10. Tsai, R-S.; Carrupt, P-A.; Testa, B.; Gaillard, P.; El Tayar, N.; Hogberg, T. *J. Med. Chem.* **1993**, *36*, 196–204.
11. Blackman, G. E.; Parke, M. H.; Garton, G. *Arch. Biochem. Biophys.* **1955**, *55*, 54–71.
12. Carrupt, P-A.; Tsai, R-S.; El Tayar, N.; Testa, B.; de Paulis, T.; Hogberg, T. *Helv. Chim. Acta* **1991**, *74*, 956–967.
13. de Paulis, T.; El Tayar, N.; Carrupt, P-A.; Testa, B.; van de Waterbeemd, H. *Helv. Chim. Acta* **1991**, *74*, 241–254.
14. de Paulis, T.; Janowsky, A.; Kessler, R. M.; Clanton, J. A.; Smith, H. E. *J. Med. Chem.* **1988**, *31*, 2027–2033.
15. Mukherjee, J.; Perry, B. D.; Cooper, M. *J. Label. Comp. Radiopharm.* **1990**, *28*, 609–616.
16. Hogberg, T.; Strom, P.; Hall, H.; Orgren, S-O. *Helv. Chim. Acta* **1990**, *73*, 417–2425.
17. Hogberg, T.; de Paulis, T.; Johnansson, L. G.; Kumar, Y.; Hall, H.; Ogren, S.-O. *J. Med. Chem.* **1990**, *33*, 2305–2309.
18. (a) Clanton, J.; A.; de Paulis, T.; Schmidt, D. E.; Ansari, M. S.; Manning, R.; G.; Baldwin, R. M.; Kessler, R. M. *J. Label. Comp. Radiopharm.* **1991**, *29*, 745–751; (b) Yue, E. W.; Gerdes, J. M.; Mathis, C. A. *J. Org. Chem.* **1991**, *56*, 5451–5456.
19. (a) Bishop, J. E.; Mathis, C. A.; Gerdes, J. M.; Whitney, J. M.; Eaton, A. M.; Mailman, R. B. *J. Med. Chem.* **1991**, *34*, 1612–1624; (b) Mathis, C. A.; Bishop, J. E.; Gerdes, J. M.; Whitney, J. M.; Brennan, K. M.; Jagust, W. J. *Nucl. Med. Biol.* **1992**, *19*, 571–588.
20. (a) Mukherjee, I. *Appl. Radiat. Isot.* **1991**, *42*, 713–721; (b) Mukherjee, J.; Luh, K. E.; Yasillo, N.; Perry, B. D.; Levy, D.; Chen, C.; Ortega, C.; Beck, R. N.; Cooper, M. *Eur. J. Pharmacol.* **1990**, *175*, 363–369.
21. de Paulis, T.; Clanton, J. A.; Schmidt, D. E.; Ansari, M. S.; Smith, H. E.; Votaw, J. R.; Manning, R. G.; Kessler, R. M., unpublished results.
22. Hogberg, T.; Bengtsson, S.; de Paulis, T.; Johansson, L. G.; Strom, P.; Hall, H.; Ogren, S-O. *J. Med. Chem.* **1990**, *33*, 1155–1163.
23. de Paulis, T.; Kumar, Y.; Johansson, L. G.; Ramsby, S.; Hall, H.; Sallemark, M.; Angeby-Moller, K.; Ogren, S-O. *J. Med. Chem.* **1986**, *29*, 61–69.
24. de Paulis, T.; Hall, H.; Ogren, S-O.; Wagner, A.; Stensland, B.; Csoregh, I. *Eur. J. Med. Chem.* **1985**, *20*, 273–276.
25. Hogberg, T.; Strom, P.; Hall, H.; Kohler, C.; Halldin, C.; Farde, L. *Acta Pharm. Nord.* **1990**, *2*, 53–60.
26. Hogberg, T.; Strom, P.; de Paulis, T.; Stensland, B.; Csoregh, I.; Lundin, K.; Hall, H.; Ogren, S-O. *J. Med. Chem.* **1991**, *34*, 948–955.
27. Chrumpradit, S.; Kung, M-P.; Billings, J.; Mach, R.; Kung, H. F. *J. Med. Chem.* **1993**, *36*, 221–228.
28. Mukherjee, J.; Yang, Z.-Y.; Das, M. K.; Kronmal, S.; Brown, T.; Cooper, M. *J. Nucl. Med.* **1993**, *34*, 243p.
29. Lannoye, G. S.; Moerlein, S. M.; Parkinson, D.; Welch, M. J. *J. Med. Chem.* **1990**, *33*, 2430–2437.
30. Halldin, C.; Farde, L.; Högberg, T.; Hall, H.; Ström, P.; Ohlberger, A.; Solin, O. *Nucl. Med. Biol.* **1991**, *18*, 871–881.
31. Florvall, L.; Ögren, S-O. *J. Med. Chem.* **1982**, *25*, 1280–1286.
32. Murphy, R. A.; Kung, H. F.; Kung, M-P.; Billings, J. *J. Med. Chem.* **1990**, *33*, 171–178.
33. Mach, R. H.; Scripko, J. G.; Ehrenkaufer, R. L.; Nowak, P. A.; Luedtke, R. R.; Molinoff, P. B.; Reivich, M. *J. Nucl. Med.* **1991**, *32*, 1842 (abstr.).
34. (a) El Tayar, N.; van de Waterbeemd, H.; Testa, B. *J. Chromatogr.* **1985**, *320*, 293–304; (b) El Tayar, N.; van de Waterbeemd, H.; Testa, B. *J. Chromatogr.* **1985**, *320*, 305–312.
35. El Tayar, N.; Kilpatrick, G. J.; van de Waterbeemd, H.; Testa, B.; Jenner, P.; Marsden, C. D. *Eur. J. Med. Chem.* **1988**, *23*, 173–182.
36. Taylor, P. J.; Cruickshank, J. M. *J. Pharm. Pharmacol.* **1985**, *37*, 143–144.
37. Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175–5180.
38. Craig, P. N. *J. Med. Chem.* **1971**, *14*, 680–684.
39. Hinderling, P. H.; Schmidlin, O.; Seydel, J. K. *J. Pharmacokinet. Biopharm.* **1984**, *12*, 263–287.
40. Miyake, K.; Terada, H. *J. Chromatogr.* **1982**, *240*, 345–351.
41. Collin, S.; El Tayar, N.; van de Waterbeemd, H.; Moureau, F.; Vercauteren, D. P.; Durant, F.; Langlois, M.; Testa, B. *Eur. J. Med. Chem.* **1989**, *24*, 163–169.
42. Brändström, A. *Acta Pharm. Suec.* **1982**, *19*, 175–198.
43. Westheimer, F. H.; Kirkwood, J. G. *J. Chem. Phys.* **1938**, *6*, 513–517.
44. Tanford, C. *J. Am. Chem. Soc.* **1957**, *79*, 5348–5352.
45. Sentell, K. B.; Dorsey, J. G. *J. Chromatogr.* **1989**, *461*, 193–207.
46. Biggs, A. I.; Robinson, R. A. *J. Chem. Soc.* **1961**, 388–393.
47. Kawaki, H.; Sasaki, Y.; Takagi, T.; Fujii, S.; Masuda, F. *Chem. Pharm. Bull.* **1989**, *37*, 3268–3271.
48. Kessler, R. M.; Ansari, M. S.; Schmidt, D. E.; de Paulis, T.; Clanton, J. A.; Innis, R.; Al-Tikriti, M.; Manning, R. G.; Gillespie, D. *Life Sci.* **1991**, *49*, 617–628.
49. Hall, H.; Högberg, T.; Halldin, C.; Köhler, C.; Ström, P.; Ross, S. B.; Larsson, S. A.; Farde, L. *Psychopharmacology* **1991**, *103*, 6–18.

50. Rekker, R. F.; De Kort, H. M. *Eur. J. Med. Chem.* **1979**, *14*, 479–488.
51. Reifenrath, W. G.; Roche, E. B.; Al-Turk, W. A. *J. Med. Chem.* **1980**, *23*, 958–990.
52. Fukumura, T.; Dohmoto, H.; Maeda, M.; Fukuzawa, E.; Kojima, M. *Chem. Pharm. Bull.* **1991**, *38*, 1740–1742.
53. Kiesewetter, D. O.; Kawai, R.; Chelliah, M.; Owens, E.; McLellan, C.; Blasberg, R. G. *Nucl. Med. Biol.* **1990**, *17*, 347–356.
54. Boudreau, R. J.; Efange, S. M. N. *J. Nucl. Med.* **1990**, *31*, 727 (abstr.).
55. Hall, H.; Hogberg, T.; Halldin, C.; Bengtsson, S.; Wedel, I. *Eur. J. Pharmacol.* **1991**, *201*, 1–10.
56. Halldin, C.; Hogberg, T.; Hall, H.; Karlsson, P.; Hagberg, C-E.; Strom, P.; Farde, L. *J. Nucl. Med.* **1992**, *33*, 860 (abstr).
57. Kung, H. F.; Alavi, A.; Chang, W.; Kung, M-P.; Keyes, J. W.; Velchik, M. G.; Billings, J.; Pan, S.; Noto, R.; Rausch, A.; Reilley, J. *J. Nucl. Med.* **1990**, *31*, 573–579.
58. Kessler, R. M.; Votaw, J. R.; de Paulis, T.; Clanton, J. A.; Ansari, M. S.; Schmidt, D. E.; Mason, N. S.; Manning, R. G. *J. Nucl. Med.* **1992**, *33*, 847 (abstr.). AASCOLLECT