Synthesis and Pharmacological Examination of 1-(3-Methoxy-4-methylphenyl)-2-aminopropane and 5-Methoxy-6-methyl-2-aminoindan: Similarities to 3,4-(Methylenedioxy)methamphetamine (MDMA)

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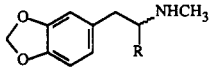
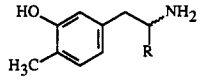
The racemate and the enantiomers of 1-(3-methoxy-4-methylphenyl)-2-aminopropane (6) and racemic 5-methoxy-6-methyl-2-aminoindan (11) were tested for stimulus generalization in the two-lever drug-discrimination paradigm. Both 6 and 11 were found to substitute with high potency in 3,4-(methylenedioxy)methamphetamine (1) and (*S*)-1-(1,3-benzodioxol-5-yl)-2-(methylamino)butane (2) trained rats. In the latter assay, both enantiomers of 6 had identical potencies, but their dose–response curves were not parallel. Racemic 6, but not 11, partially substituted for LSD. Racemic 6 and 11 did not substitute in (*S*)-amphetamine-trained rats. All of the test compounds were potent inhibitors of [^{3}H]-5-HT uptake into synaptosomes in vitro, with the *S* enantiomer of 6 being most active. Rat brain monoamine levels were unaltered 1 week following a single high dose (10 or 20 mg/kg, sc) of 6 or 11, or two weeks following a subacute dosing regimen (20 mg/kg, sc, twice a day for 4 days). In addition, radioligand-binding parameters in rat brain homogenate with the 5-HT uptake inhibitor [^{3}H]paroxetine were unchanged after subacute dosing with either racemic 6 or 11. The results indicate that compounds 6 and 11 have animal behavioral pharmacology similar to the methylenedioxy compounds 1 and 2, but that they do not induce the serotonin neurotoxicity that has been observed for the latter two drugs.

In ongoing study of the mechanism of action of 3,4-(methylenedioxy)methamphetamine (1; MDMA;



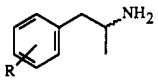
1 R = CH_3
2 R = CH_2CH_3



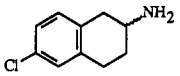
3 R = CH_3
4 R = CH_2CH_3

"ecstasy"), a popular recreational drug, and 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butane (2; MBDB), a substance with similar pharmacology, our efforts have been directed toward a variety of substituted phenethylamine derivatives that produce 1-like behavioral effects in rats. Our attention was recently drawn to a series of *m*-hydroxyamphetamine derivatives first studied by Carlsson et al.[1] That study and others (e.g. refs 2–5) had shown that 4-methyl-*m*-tyramine derivatives with either an α-methyl (3; H77/77) or an α-ethyl (4; H75/12) group attached to the side chain have the ability to acutely deplete central stores of dopamine, norepinephrine, and serotonin. We were particularly intrigued by these compounds, since 1 possesses pharmacology which could be interpreted as having certain parallels to that of 3. Furthermore, the α-ethyl homologue of 1 (2) appears to have a selective effect on central serotonergic processes similar to what has been reported regarding the α-ethyl-substituted 4.

Although a number of studies of the behavioral pharmacology of 3 and 4 have appeared,[6,7] these compounds have not been examined for 1-like effects. In addition, the demonstrated long-term serotonin neurotoxicity of 1[8,9] and *p*-chloroamphetamine (5)[10,11] suggested that 3 and 4 might also produce similar central toxicity. By drawing parallels to the known structure–activity relationships of hallucinogen amphetamines, it seemed likely that the 3-*O*-methyl derivatives of these compounds might possess interesting central activity. One report that 6 had been identified as an illicit drug in Italy[12] seemed to provide further support for these ideas.



5 R = 4-Cl
6 R = 3-OCH_3-4-CH_3
7 R = 2,5-$(OCH_3)_2$-4-CH_3
8 R = 3,4-OCH_2O-



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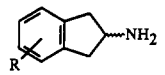

10 R = 5,6-OCH_2O-
11 R = 5-OCH_3-6-CH_3

In particular, 6 is an analogue of 3 and has a structure similar to that of 1-(2,5-dimethoxy-4-methyl)-2-aminopropane (7; DOM), a potent hallucinogenic amphetamine. Indeed, compound 6 had been shown to be nearly as potent as 7 for disruption of behavior in the mouse swim maze test.[13] In preliminary experiments using the drug-discrimination paradigm with a limited number of rats, it appeared that racemic 6 would substitute both in LSD- and 1-trained rats, actions similar to those of 3,4-(methylenedioxy)amphetamine (8). Therefore, we undertook the preparation of the enantiomers of 6.

Previous reports have indicated that rigid analogues of certain substituted amphetamines, such as 9 and 10, lack the hallucinogenic and neurotoxic actions of the corresponding amphetamines 5 and 8.[14-16] Therefore, the 2-

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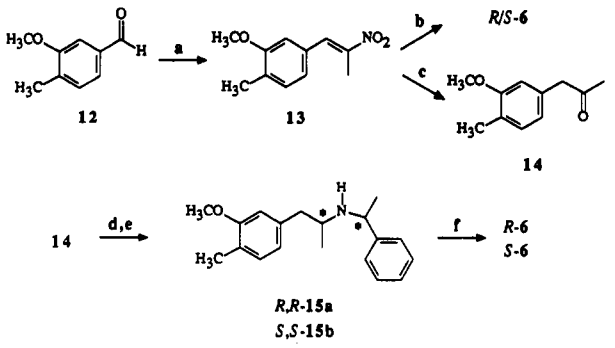
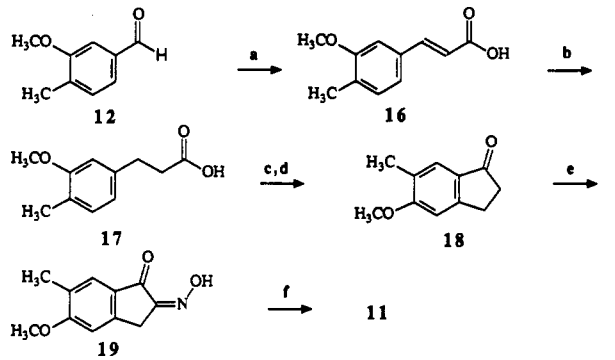
Scheme I[a]



[a] (a) $CH_3CH_2NO_2$, NH_4OAc, reflux; (b) $LiAlH_4$, $(CH_3CH_2)_2O$; (c) W-2 Raney Ni, NaH_2PO_2, CH_3CH_2OH, Δ; (d) (*R*)- or (*S*)-α-phenethylamine, benzene, reflux, $-H_2O$; (e) H_2, W-2 Raney Ni, CH_3CH_2OH; (f) H_2; 10% Pd–C, CH_3OH.

Scheme II[a]



[a] (a) Malonic acid, piperdine, pyridine, reflux; H^+; (b) H_2, 10% Pd–C, CH_3CH_2OH; (c) oxalyl chloride, benzene, reflux; (d) $SnCl_4$; CH_2Cl_2; (e) isoamyl nitrite, HCl, CH_3OH; (f) H_2, 10% Pd–C, CH_3CO_2H, H_2SO_4.

aminoindan analogue 5-methoxy-6-methyl-2-aminoindan (**11**) of **6** was also synthesized. These compounds were evaluated for their discriminative stimulus effects in rats trained to discriminate saline from various training drugs: LSD, **1**, *S*-(+)-**2**, and (*S*)-(+)-amphetamine. In addition, the ability of the test compounds to inhibit synaptosomal [^{3}H]serotonin uptake and to induce long-term serotonin neurotoxicity was examined.

Chemistry

Condensation of 3-methoxy-4-tolualdehyde[17,18] (**12**) with nitroethane readily afforded the corresponding nitropropene (Scheme I). The 1-phenyl-2-propanone derivative **14** was then prepared by employing a modification of the method of Monti et al.,[19] using Raney nickel and sodium hypophosphite. The phenylpropanone was converted into the diastereomeric *N*-α-phenethyl derivatives **15a** and **15b**, which were then catalytically N-debenzylated to yield the enantiomers of **6**.[20,21]

Table I. Drug-Discrimination Data for 1-Trained Rats

	dose					ED_{50} (95% CI)	
compd	mg/kg	μmol/kg	n^a	D^b	% SDL[c]	mg/kg	μmol/kg
saline			8	0	0		
1						0.64[d]	2.79
6	0.25	1.16	10	1	11	0.59	2.74
	0.50	2.32	8	0	50	(0.35–0.99)	(1.63–4.60)
	1.00	4.64	8	0	63		
	2.00	9.28	8	0	100		
11	0.50	2.34	8	0	13	0.81	3.77
	0.75	3.51	8	0	25	(0.63–1.02)	(2.96–4.79)
	1.00	4.68	8	0	75		
	2.00	9.36	8	0	100		

[a] Number of animals tested. [b] Number of animals disrupted. [c] Percentage of nondisrupted animals that selected the drug lever. [d] Value included for comparison, taken from Nichols et al.[16]

Table II. Drug-Discrimination Data for *S*-2-Trained Rats

	dose				%	ED_{50} (95% CI)	
compd	mg/kg	μmol/kg	n^a	D^b	SDL[c]	mg/kg	μmol/kg
saline			8	0	0		
S-2						0.79[d]	3.25
6	0.25	1.16	10	3	13	0.47	2.16
	0.50	2.32	10	2	38	(0.32–0.67)	(1.51–3.09)
	0.75	3.48	8	1	100		
11	0.25	1.17	8	0	13	0.56	2.63
	0.50	2.34	8	0	38	(0.36–0.88)	(1.67–4.13)
	1.00	4.68	8	0	75		
	2.00	9.36	8	0	100		
S-(+)-6	0.25	1.16	11	5	17	0.46	2.14
	0.50	2.32	11	5	33	(0.29–0.72)	(1.36–3.36)
	0.75	3.48	7	1	100		
R-(−)-6	0.25	1.16	10	2	33	0.46	2.12
	0.50	2.32	11	3	38	(0.36–0.88)	(1.67–4.13)
	1.00	4.64	11	4	75		
	1.50	6.95	6	2	100		

[a] Number of animals tested. [b] Number of animals disrupted. [c] Percentage of nondisrupted animals that selected the drug lever. [d] Value included for comparison, taken from Nichols and Oberlender.[27]

2-Aminoindan **11** was prepared by a synthetic route previously employed to make substituted 2-aminoindans.[16] This procedure utilized the intramolecular cyclization of 3-(3-methoxy-4-methylphenyl)propionyl chloride, followed by treatment of indanone **18** with amyl nitrite in methanol and HCl to yield hydroxyimino ketone **19**.[22] The catalytic reduction of **19** afforded **11**, which was isolated as the HCl salt.

Pharmacology

The racemate and enantiomers of **6** and racemic **11** were evaluated for their ability to substitute for **2** in a two-lever drug-discrimination assay. In addition, racemic **6** and **11** were examined in 1- and LSD-trained animals. Racemic **6** and **11** were also tested in animals trained to discriminate (*S*)-amphetamine from saline. The ability of the test compounds to inhibit [^{3}H]-5-HT uptake in vitro was examined by using the procedure of Steele et al.[23] The neurotoxic potential of racemic **6** and **11** was evaluated by examining rat brain monoamines and their metabolite levels and [^{3}H]paroxetine binding 1 week following an acute dose (10 or 20 mg/kg, sc) or 2 weeks after subacute dosing (20 mg/kg, sc, twice a day for 4 days). Changes in serotonin, its metabolite 5-hydroxyindoleacetic acid (5-HIAA), and [^{3}H]paroxetine binding have been found to

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Table III. Drug-Discrimination Data for LSD-Trained Rats

compd	dose mg/kg	dose μmol/kg	n^a	D^b	% SDL[c]	ED_{50} (95% CI) mg/kg	ED_{50} (95% CI) μmol/kg
saline			8	0	0		
LSD						0.025[d]	0.058
6	0.50	2.32	8	1	0	PS[e]	
	1.00	4.64	8	0	38		
	2.00	9.28	15	7	75		
	3.00	13.9	6	5	-		
11	0.50	2.34	9	0	11	NS[f]	
	1.00	4.68	10	2	13		
	2.00	9.36	10	2	38		
	2.50	11.7	12	4	50		
	3.00	14.0	7	6	-		

[a] Number of animals tested. [b] Number of animals disrupted. [c] Percentage of nondisrupted animals that selected the drug lever. [d] Value included for comparison, taken from Nichols et al.[26] [e] Indicates partial substitution occurred. [f] Indicates no substitution occurred.

Table IV. Drug-Discrimination Data for *S*-Amphetamine-Trained Rats

compd	dose mg/kg	dose μmol/kg	n^a	D^b	% SDL[c]	ED_{50} (95% CI) mg/kg	ED_{50} (95% CI) μmol/kg
saline			8	0	0		
S-AMP						0.31[d]	1.69
6	0.25	1.16	8	0	0	NS[e]	
	0.50	2.32	9	1	0		
	1.00	4.64	13	5	0		
	2.00	9.28	6	5	-		
11	0.50	2.34	10	4	17	NS[e]	
	1.00	4.68	10	7	33		
	1.50	7.02	7	5	50		
	2.00	9.36	7	7	-		

[a] Number of animals tested. [b] Number of animals disrupted. [c] Percentage of nondisrupted animals that selected the drug lever. [d] Value included for comparison, taken from Oberlender and Nichols.[28] [e] Indicates no substitution occurred.

correlate with selective serotonin neurotoxicity following high doses of certain substituted amphetamines.[24,25]

Results and Discussion

The results of the drug-discrimination studies in rats are summarized in Tables I–IV. Racemic **6** and **11** were found to fully substitute in 1- and 2-trained animals. In animals trained to discriminate saline from (*S*)-amphetamine, racemic **6** did not substitute, while in LSD-trained rats **6** gave partial substitution. In contrast, **11** did not substitute in LSD-trained animals. This is similar to what is seen with the 3,4-(methylenedioxy)-substituted amphetamine **8**. For instance, **8** substitutes in rats trained to discriminate saline from LSD, consistent with its hallucinogenic actions.[26] In contrast, the methylenedioxy substituted aminoindan **10** did not substitute in LSD-trained rats,[16] suggesting that it probably would not have LSD-like actions in man. Therefore, as with methylenedioxy-substituted analogues, there appears to be greater similarity between the drug-discrimination properties of LSD and **6** than between those of LSD and **11**.

Another parallel between the pharmacology of the methylenedioxy- and 3-methoxy-4-methyl-substituted amphetamines is stereoselectivity. It has been previously found that the more potent enantiomer of **1**, **2**, and **8**, in producing 1-like effects and in inhibiting the neuronal uptake of [^{3}H]-5-HT, has the *S* absolute configuration.[23,26-28] Similarly, the *S* enantiomer of **6** is the more potent inhibitor of synaptosomal [^{3}H]-5-HT uptake (Table V). In contrast, it can be seen in Table II that both the *S* and the *R* enantiomers had equal potencies for substitution in *S*-2-trained rats. However, examination of the dose–response curves of *S*-, *R*-, and *rac*-**6** indicated that the slope of the curve for *S*- and *rac*-**6** was very similar, while the slope for *R*-**6** was much more shallow (Figure 1). This would suggest that the two enantiomers of **6** are working by different mechanisms of substitution in *S*-2-trained rats. Therefore, the in vitro, but not the in vivo, results parallel the stereoselectivity for the methylenedioxy-substituted amphetamine.

Table V. Uptake Inhibition of [^{3}H]-5-HT

compd	IC_{50} (nM)[a]	compd	IC_{50} (nM)[a]
R/S-**6**	136 ● 22	**7**	10655 ± 549[b]
S-**6**	99 ± 7	**11**	212 ● 20
R-**6**	200 ± 15		

[a] Values are mean ± SEM. [b] Significantly higher than that of *R/S*-**6**. ($p < 0.05$, ANOVA followed by post hoc comparison). [c] Significantly lower than that of *R*-**6**. ($p < 0.05$, ANOVA followed by post hoc comparison).

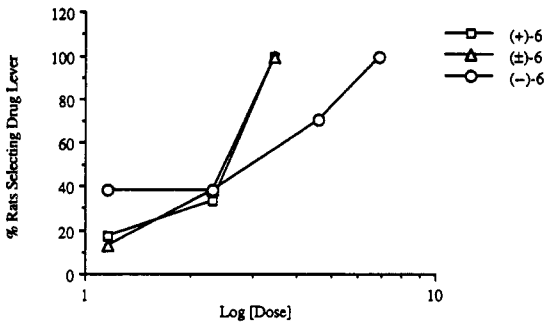

Figure 1. Dose–response curves for *S*-, *R*- and *S/R*-**6** in *S*-2-trained rats. The percent of nondisrupted rats responding on the drug lever was plotted versus the log dose (μmol/kg). The slopes of the linear portion of the dose response curves are as follows: *S*-**6**, 389; *S/R*-**6**, 360; *R*-**6**, 129.

The short-term monoamine changes measured after high doses of **6** and **11** are similar to those occurring in response to **1**, **2**, **8**, and **10**.[29-31] As seen in Table VI, the levels of 5-HT and 5-HIAA in the hippocampus and frontal cortex are significantly decreased at 3 h after a 20 mg/kg (sc) dose of either **6** or **11**. In addition, cortical levels of DA are significantly increased. This is similar to previous findings for methylenedioxy-substituted amphetamines (**1** and **8**), and the 2-aminoindan **10**.[29,30] It should be noted that both **6** and **11** increased levels of the DA metabolite HVA. In contrast, **1** does not increase HVA, but causes a significant decrease in the DA metabolite DOPAC.[29]

Despite similarities, one distinct difference between these two series of substituted amphetamines (ie **1** vs **6**)

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Table VI. Monoamine and Metabolite Levels at 3 h Postdrug

compd (dose, mg/kg)	NE[a]	DA	DOPAC	HVA	5-HT	5-HIAA
			Frontal Cortex			
saline	543 ± 23	63 ± 5	36 ± 5	61 ± 5	459 ± 35	223 ± 13
6 (20)	585 ± 23	120 ± 17[b]	46 ± 3	138 ± 10[b]	212 ± 40[b]	139 ± 9[b]
11 (20)	558 ± 22	113 ± 16[b]	40 ± 2	112 ± 6[b]	148 ± 22[b]	146 ± 7[b]
			Hippocampus			
saline	649 ± 25	c	c	c	496 ± 28	487 ± 25
6 (20)	641 ± 26	c	c	c	238 ± 12[b]	260 ± 24[b]
11 (20)	646 ± 40	c	c	c	256 ± 14[b]	272 ± 21[b]

[a] Values are mean ± SEM (ng/g wet weight). [b] Significantly different than that of saline. ($p < 0.05$, ANOVA followed by post hoc comparison). [c] Below the levels of detection.

Table VII. Monoamine and Metabolite Levels 1 Week following Acute Dosing

compd (dose, mg/kg)	NE[a]	DA	DOPAC	HVA	5-HT	5-HIAA
			Frontal Cortex			
saline	403 ± 14	76 ± 5	46 ± 8	70 ± 4	300 ± 15	231 ± 11
6 (10)	394 ± 16	88 ± 2	39 ± 5	70 ± 6	281 ± 13	233 ± 18
11 (10)	414 ± 15	88 ± 10	36 ± 4	74 ± 4	312 ± 15	210 ± 16
			Hippocampus			
saline	628 ± 42	b	b	b	311 ± 38	496 ± 36
6 (10)	620 ± 33	b	b	b	255 ± 30	460 ± 31
11 (10)	633 ± 41	b	b	b	312 ± 15	434 ± 22
			Frontal Cortex			
saline	490 ± 10	70 ± 3	45 ± 4	75 ± 8	300 ± 6	236 ± 16
6 (20)	487 ± 12	86 ± 10	45 ± 4	85 ± 4	316 ± 10	229 ± 6
11 (20)	509 ± 18	81 ± 13	35 ± 4	65 ± 2	316 ± 4	240 ± 14
			Hippocampus			
saline	565 ± 19	b	b	b	435 ± 15	428 ± 17
6 (20)	578 ± 22	b	b	b	431 ± 24	430 ± 28
11 (20)	583 ± 22	b	b	b	462 ± 18	476 ± 12

[a] Values are mean ± SEM (ng/g wet weight). [b] Below the level of detection.

Table VIII. Monoamine and Metabolite Levels 2 Weeks following Subacute Dosing[b]

compd	NE[a]	DA	DOPAC	HVA	5-HT	5-HIAA
			Frontal Cortex			
saline	595 ± 21	80 ± 8	61 ± 7	52 ± 5	440 ± 12	270 ± 8
6	573 ± 6	83 ± 8	74 ± 6	62 ± 7	411 ± 10	253 ± 12
11	568 ± 12	92 ± 5	54 ± 7	59 ± 4	415 ± 20	256 ± 13
			Hippocampus			
saline	741 ± 53	c	c	c	409 ± 38	541 ± 43
6	724 ± 62	c	c	c	392 ± 21	503 ± 28
11	716 ± 33	c	c	c	434 ± 18	518 ± 39

[a] Values are mean ± SEM (ng/g wet weight). [b] Animals were sacrificed 2 weeks after multiple dosing (20 mg/kg, 2×/day for 4 days). [c] Below the level of detection.

Table IX. [^{3}H]Paroxetine Receptor Binding after Subacute Dosing[b]

compd	no. of uptake sites[a]	
	frontal cortex	hippocampus
saline	13.4 ± 0.8	16.2 ± 0.7
6	12.3 ± 1.0	14.9 ± 0.6
11	13.6 ± 0.8	15.6 ± 0.7

[a] Values are mean ± SEM (fmol/mg wet weight). [b] Animals were sacrificied 2 weeks after subacute dosing (20 mg/kg, 2×/day for 4 days).

is their relative serotonin neurotoxicity. It has been well-established that acute or subacute high doses of **1**, **2**, **5**, or **8** result in a selective long-term decrease in brain 5-HT and 5-HIAA.[8-11,31] Recent studies suggest that this is due to a selective degeneration of the fine axons originating from the dorsal raphe nucleus.[32,33] In contrast, this work indicates that neither **6** nor **11** cause a long-term depletion of 5-HT or 5-HIAA after single or multiple high doses. As seen in Table VII, the monoamines and their metabolite levels were unchanged 1 week after a single 10 or 20 mg/kg (sc) dose. Higher doses of **6** were lethal; all rats administered 40 mg/kg died within 45 min. In addition, ca. 12% of the animals given 20 mg/kg of **6** died, suggesting this dose to be at the bottom of the dose–lethality curve. Therefore, multiple dosing (twice a day for 4 days) of 20 mg/kg **6** or **11** was performed and the rats were sacrificed 2 weeks after the last dose. As seen in Table VIII, even after multiple dosing, neither the substituted amphetamine **6** nor aminoindan **11** resulted in decreased monoamine levels in the frontal cortex or hippocampus. In addition, [^{3}H]paroxetine-binding parameters to tissue homogenates were unchanged (Table IX). Therefore, the present report indicates that unlike some substituted amphetamines (such as **1**, **2**, **5**, and **8**), the test compounds **6** and **11** do not result in selective degeneration of serotonin axons.

One other important point should be made regarding structure–activity relationships. It is apparent from the uptake inhibition data (Table V) that *o*-methoxy substitution can dramatically alter the mechanism of action. Addition of an *o*-methoxy to **6** to give **7** results in a nearly 2 order of magnitude decrease in the ability to inhibit [^{3}H]-5-HT uptake. Cheng et al.[34] have proposed that hallucinogenic amphetamine derivatives may be classified either as direct-acting or indirectly-acting. The latter category includes compounds which induce the release of neuronal stores of transmitter. The failure of **7** and similar 2,5-dimethoxy-substituted derivatives to effect release[34] and the observation that **1**, **2**, **6**, and **8** interact potently with the 5-HT uptake carrier suggest that an *o*-methoxy on a phenethylamine is a structural feature that dramatically attenuates affinity for the uptake site. Given the neurotoxicity of certain *o*-hydroxy-substituted phenethylamines such as 6-hydroxydopamine,[35] it is tempting to speculate that this may be an evolutionary development to protect serotonin neurons against such neurotoxic compounds.

To summarize, the present study indicates that there are a number of pharmacological similarities between the 3,4-(methylenedioxy)- and 3-methoxy-4-methyl-substituted compounds. This includes their behavioral effects, as measured in the drug-discrimination paradigm, their ability to inhibit the uptake of [^{3}H]-5-HT, their effects on

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brain monoamines at short time periods, and the stereoselectivity of action for the S enantiomer. As with 1 and 2,[27] the behavioral effects of 6 and 11 in animals do not appear to be typical of either stimulants such as amphetamine or hallucinogens such as LSD or 7. It should be noted that the primary amines 6 and 8, in both series, possess some LSD-like animal behavioral pharmacology. In contrast, 2-aminoindans 10 and 11 in both series lack this effect. Similarly, neither of the 2-aminoindans produce serotonin neurotoxicity. However, the methylenedioxy-substituted amphetamines result in long-term deficits in serotonergic markers. In contrast, this action is not observed in the methoxymethyl-substituted series studied here.

Thus, these results suggest that the primary pharmacological effects of compounds 6 and 11 probably occur through the release of neuronal serotonin. Previously, the only compounds known to have this action, such as 2 or 5, also induce serotonin neuron degeneration. Therefore, 6 and 11 may prove to be useful tools in elucidating the role of central serotonergic processes.

Experimental Section

Uncorrected melting points were taken on a Mel-Temp apparatus. ^{1}H NMR spectra were recorded on a Varian VXR-500S spectrometer. Chemical shifts are reported in δ values (parts per million). Microanalysis was performed at the Purdue Microanalysis Laboratory (West Lafayette, IN) or Galbraith Laboratories (Knoxville, TN), and all values were within 0.4% of those calculated.

1-(3-Methoxy-4-methylphenyl)-2-nitropropene (13). Nitroethane (300 mL), 7.7 g (100 mmol) of ammonium acetate, and 15.0 g (100 mmol) of 3-methoxy-4-methylbenzaldehyde (12)[17,18] were combined and heated at reflux in an oil bath for 2.5 h. The excess nitroethane was removed by rotary evaporation and the residue was partioned between CH_2Cl_2 (2 × 75 mL) and H_2O. The organic extracts were combined and dried (Na_2SO_4), and the CH_2Cl_2 was evaporated. Crystallization was induced by dilution of the residual oil in MeOH. The product was recrystallized from MeOH–H_2O to yield 17.3 g (83.5%) of a yellow solid; mp 51 °C (lit.[1] mp 52 °C).

1-(3-Methoxy-4-methylphenyl)-2-propanone (14). W-2 Raney Ni (8.0 g), as a slurry in EtOH, was added to a mechanically stirred solution of 12.0 g (58 mmol) of nitroolefin 13 in 300 mL of 95% EtOH and 150 mL of 0.2 M aqueous acetate buffer (pH 5) contained in a 1-L round-bottom flask.[19] The flask was suspended in a Branson B-220H ultrasonic bath maintained at 40 °C. While sonication was maintained, 54.0 g (0.61 mol) of NaH_2PO_4 in 120 mL of H_2O was added at a rate sufficient to maintain moderate H_2 evolution. The reaction was stirred with sonication at 40 °C for 1.5 h. The initial mixture had a green tint, which disappeared on completion of the reaction. The catalyst was removed by vacuum filtration through Celite, brine (200 mL) was added to the filtrate, and the solution was extracted with Et_2O (3 × 200 mL). The organic layer was washed with 5% $NaHCO_3$ and brine and was concentrated under vacuum. The residue was taken up into Et_2O, dried ($MgSO_4$), and filtered and the Et_2O removed under vacuum. The crude ketone was distilled under vacuum to yield 5.5 g (53.4 %) of 14 as a colorless oil; bp 90 °C (0.4 mmHg) [lit.[18] bp 101 °C (1 mmHg)].

(R,S)-1-(3-Methoxy-4-methylphenyl)-2-aminopropane Hydrochloride (R/S-6·HCl). A solution of 7.0 g (33.8 mmol) of nitroolefin 13 in 20 mL of Et_2O was added dropwise to a stirring suspension of 3.6 g (94.9 mmol) of $LiAlH_4$ in 150 mL of dry Et_2O. After the addition, the mixture was heated at reflux on a steam bath for 2.5 h. The excess $LiAlH_4$ was decomposed by slow addition of 10 mL of H_2O and the mixture was filtered through Celite. The ethereal filtrate was extracted with 2 N HCl (3 × 75 mL), and the combined aqueous extracts were basified with excess NaOH. The free base was extracted with CH_2Cl_2 (3 × 75 mL), dried (Na_2SO_4), filtered, and concentrated by rotary evaporation. The residual oil was dissolved in absolute ethanol, acidified with concentrated HCl, diluted with Et_2O, and cooled to yield 4.9 (88.1%) of white crystalline R/S-6·HCl: mp 181 °C (lit.[18] mp 181.5–183.5 °C); ^{1}H NMR ($CDCl_3$) δ 8.41 (s, 3, NH_3^+), 7.06 (d, 1, ArH, J = 7.5 Hz), 6.67 (d, 1, ArH, J = 7.5 Hz), 6.71 (s, 1, ArH), 3.82 (s, 3, OCH_3), 3.57 (m, 1, CHN), 3.20 (dd, 1, $ArCH_2$, J = 5.3, 13.4 Hz), 2.81 (dd, 1, $ArCH_2$, J = 9.3, 13.4 Hz), 2.17 (s, 3, $ArCH_3$), 1.39 (d, 3, CH_3, J = 6.5 Hz).

(R,R)-(+)-N-(1-Phenethyl)-1-(3-methoxy-4-methylphenyl)-2-aminopropane Hydrochloride (R,R-15a·HCl). A solution of 9 g (50 mmol) of ketone 14 and 6.06 g (50 mmol) of (R)-(+)-α-phenethylamine (Aldrich, Milwaukee, WI) in 50 mL of benzene was heated at reflux under N_2 for 2 h with continuous H_2O removal with a Dean–Stark trap.[20,21] The benzene was removed under vacuum, and the residual oil was taken up into 30 mL of absolute ethanol and shaken for 24 h with W-2 Raney Ni (2.5 g as a slurry in EtOH) at 50 psig of H_2. The catalyst was removed by filtration through Celite and the filtrate was acidified with concentrated HCl. The solvents were removed under vacuum, and the crude salt was recrystallized from acetone to yield 10.23 g (64.6%) of white crystalline R,R-15a·HCl: mp 216–217 °C; $[\alpha]_D$ +17.23° (c = 2, EtOH); ^{1}H NMR ($CDCl_3$) δ 7.67 (d, 2, ArH, J = 7.6 Hz), 7.47 (t, 2, ArH, J = 7.5 Hz), 7.41 (t, 1, ArH, J = 7.4 Hz), 6.99 (d, 1, ArH, J = 7.5 Hz), 6.51 (d, 1, ArH, J = 7.5 Hz), 6.42 (s, 1, ArH), 4.37 (m, 1, ArCHN), 3.72 (s, 3, OCH_3), 3.37 (dd, 1, $ArCH_2$, J = 4.4, 13.1 Hz), 3.30 (m, 1, CHN), 2.80 (dd, 1, $ArCH_2$, J = 9.9, 13.1 Hz), 2.15 (s, 3, $ArCH_3$), 1.93 (d, 3, CH_3, J = 6.8 Hz), 1.42 (d, 3, CCH_3, J = 6.6 Hz). Anal. ($C_{19}H_{23}ClNO$) C, H, N.

(S,S)-(-)-N-(1-Phenethyl)-1-(3-methoxy-4-methylphenyl)-2-aminopropane Hydrochloride (S,S-15a·HCl). Following the above procedure but using 15.1 g (84.7 mmol) of ketone 14 and 10.17 g (84.7 mmol) of (S)-(-)-α-phenethylamine yielded 15.01 g (55.9%) of white crystalline S,S-15a·HCl; mp 215–216 °C; $[\alpha]_D$ -17.04° (c = 2, EtOH). Anal. ($C_{19}H_{23}ClNO$) C, H, N.

(R)-(-)-1-(3-Methoxy-4-methylphenyl)-2-aminopropane Hydrochloride (R-6·HCl). A solution of 9.60 g (30.3 mmol) of R,R-15a·HCl dissolved in 100 mL of MeOH was added to 0.9 g of 10% Pd–C (as a slurry in 10 mL of H_2O) contained in a 500-mL Parr pressure bottle. The mixture was shaken at 50 psig of H_2 for 48 h. The catalyst was removed by filtration through Celite, the solvent was removed by rotary evaporation, and the crude salt was recrystallized from EtOH–Et_2O to yield 5.97 g (91.3%) of white crystalline R-6·HCl; mp 163 °C; $[\alpha]_D$ -23.42° (c = 2, H_2O). Anal. ($C_{11}H_{18}ClNO$) C, H, N.

(S)-(+)-1-(3-Methoxy-4-methylphenyl)-2-aminopropane Hydrochloride (S-6·HCl). An exact replication of the above procedure using 15.01 g (47.4 mmol) of S,S-15b·HCl yielded 6.73 g (65.4%) of white crystalline S-6·HCl; mp 163 °C; $[\alpha]_D$ +23.30° (c = 2, H_2O). Anal. ($C_{11}H_{18}ClNO$) C, H, N.

3-Methoxy-4-methylcinnamic Acid (16). A mixture of 25 g (167 mmol) of 12, malonic acid (31.7 g, 310 mmol), and 1.3 mL of piperidine in 70 mL of pyridine was stirred on a steam bath for 5 h.[36] The mixture was poured into 400 mL of ice water containing 100 mL of concentrated HCl. The resulting precipitate was collected by filtration and washed by resuspension in 300 mL of water. The precipitate was then collected, dried, and recrystallized from MeOH-H_2O to yield 32.0 g (99.7%) of a white crystalline product; mp 181–182 °C; ^{1}H NMR ($DMSO_4$-d_6) δ 7.57 (d, 1, =CH, J = 16.0 Hz), 7.25 (s, 1, ArH), 7.15 (m, 2, ArH), 6.52 (d, 1, =CH, J = 16.0 Hz), 3.82 (s, 3, CH_3O), 3.34 (s, 1, OH), 2.15 (s, 3, $ArCH_3$). Anal. ($C_{11}H_{12}O_3$) C, H.

3-(3-Methoxy-4-methylphenyl)propanoic Acid (17). Cinnamic acid 16 (29 g, 151 mmol) was dissolved in 500 mL of 95% ethanol and shaken for 6 h in a Parr apparatus at 60 psig of H_2 over 3.0 g of 10% Pd–C. The mixture was then filtered through a Celite pad and concentrated under vacuum. The resulting solid material was recrystallized from MeOH-H_2O to give 29.2 g (99.7%) of a white crytalline solid: mp 73–74 °C; ^{1}H NMR ($CDCl_3$) δ 7.05 (d, 1, ArH, J = 7.4 Hz), 6.71 (d, 1, ArH, J = 7.5 Hz), 6.69 (s, 1, ArH), 3.82 (s, 3, CH_3O), 2.94 (t, 2, CH_2, J = 7.8 Hz), 2.69 (t, 2, CH_2, J = 7.8 Hz), 2.18 (s, 3, $ArCH_3$). Anal. ($C_{11}H_{14}O_3$) C, H.

5-Methoxy-6-methyl-1-indanone (18). Propanoic acid 17 (20 g, 103 mmol) was dissolved in 300 mL of dry benzene containing a few drops of N,N-dimethylformamide, and 27 mL (209 mmol)

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of oxalyl chloride was added dropwise.[37] The mixture was stirred for 4 h at room temperature and the benzene was removed by rotary evaporation. The crude acid chloride was redissolved in 400 mL of dry dichloromethane and placed in an ice bath. A solution of $SnCl_4$ (14.8 mL, 129 mmol) in 50 mL of CH_2Cl_2 was added dropwise over 10 min. Stirring was continued for 10 min, the ice bath was removed, and the reaction was allowed to stir for 1 h. The reaction mixture was then poured over 50 g of crushed ice and the organic layer was washed with 3 N HCl (2 × 100 mL) and water (200 mL). The solution was dried with $MgSO_4$, filtered, and passed through a short silica gel pad. After solvent removal the residue was recrystallized from benzene–hexanes to yield 13.2 g (72.5%): mp 114–115 °C; ^1H NMR ($CDCl_3$) δ 7.53 (s, 1, ArH), 6.84 (s, 1, ArH), 3.92 (s, 3, CH_3O), 3.07 (t, 2, CH_2, J = 5.7 Hz), 2.66 (t, 2, CH_2, J = 5.7 Hz), 2.23 (s, 3, $ArCH_3$). Anal. ($C_{11}H_{12}O_2$) C, H.

2-(Hydroxyimino)-5-methoxy-6-methyl-1-indanone (19). Indanone 18 (12 g, 68 mmol) was dissolved in 375 mL of MeOH and heated to 45 °C. Isoamyl nitrite (10 mL, 75 mmol) was added, and then 7 mL of concentrated HCl was added dropwise over 5 min. After 20 min of stirring an off-white precipitate began to form. Excess isoamyl nitrite and HCl (1 mL of each) were added after 45 min of stirring. The precipitated product was collected by filtration and washed with cold methanol and then ether (100 mL). The filtrate was concentrated by rotary evaporation and the residue was recrystallized from MeOH. The two crops were combined to give 13.6 g (96.9%): mp 212–213 °C; ^1H NMR ($DMSO_4$-d_6) δ 7.53 (s, 1, ArH), 7.13 (s, 1 ArH), 3.91 (s, 3, CH_3O), 3.68 (s, 2, CH_2), 2.18 (s, 3, $ArCH_3$). Anal. ($C_{11}H_{11}NO_3$) C, H, N.

5-Methoxy-6-methyl-2-aminoindan Hydrochloride (11). Hydroxyimino ketone 19 (10 g, 49 mmol) was dissolved by warming in 1 L of acetic acid. Concentrated H_2SO_4 (6 mL, 98 mmol) and 2.5 g of 10% Pd–C were added to this solution. The mixture was shaken at 60 psig of H_2 for 18 h.[38] The catalyst was then removed by filtration and the mixture was concentrated by rotary evaporation. The residue was dissolved in 400 mL of water and the acidic solution was washed with 3 × 200 mL of ethyl acetate and 200 mL of ether. The aqueous layer was basified with NaOH and the amine was extracted into 3 × 200 mL of dichloromethane. The organic layer was dried ($MgSO_4$) and filtered, and the solvent was removed by rotary evaporation. The resulting oil was dissolved in EtOH and acidified with concentrated HCl. The resulting HCl salt was crystallized from EtOH–ether to yield 8.0 g (77.0%): mp 316–317 °C; ^1H NMR ($DMSO_4$-d_6) δ 8.27 (s, 3, NH_3^+), 7.01 (s 1, ArH), 6.85 (s, 1, ArH), 3.93 (m, 1, CH), 3.73 (s, 3, CH_3O), 3.19 (dd, 1, CH, J = 5.3, 16.0 Hz), 3.13 (dd, 1, CH, J = 5.3, 16.4 Hz), 2.92 (dd, 1, CH, J = 7.6, 16.0 Hz), 2.82 (dd, 1, CH, J = 7.6, 16.4 Hz), 2.09 (s, 3, $ArCH_3$). Anal. ($C_{11}H_{16}ClNO$) C, H, N.

Pharmacological Methods. Animals. Male Sprague–Dawley rats (175–199 g, Harlan Industries, Indianapolis, IN) were either group housed 6 per cage or individually caged in a temperature-controlled room with a 12/12-h light cycle. In drug-discrimination experiments, animals were provided with water ad lib, and sufficient supplemental food to maintain 80% of their free-feeding weight. In all other pharmacological experiments rats were given food and water ad lib. Animal dissections were done over ice and the brain areas removed according to the methods of Glowinski and Iverson.[39] The frontal cortex and hippocampal regions from each hemisphere were separated, frozen with liquid nitrogen, and stored at −70 °C until assayed.

Drug-Discrimination Studies. Six standard operant chambers (Coulbourn Instruments) equipped with two response levers, a food pellet delivery system, masking white noise, and a house light were utilized. The chambers were enclosed in ventilated, sound-attenuated cubicles and were controlled by solid-state logic interfaced through a Coulbourn Instruments Dynaport to an IBM-PC. Food pellets (Bioserve, 45 mg, dustless) were used as positive reinforcement during maintenance sessions.

The complete training procedure has been previously described.[26] Briefly, rats were trained to discriminate the drug treatment from saline on an FR-50 schedule with 15-min maintenance sessions. The training drug and doses (mg/kg) were as follows: 1, 1.75; *S*-2, 1.75; LSD tartrate, 0.08; and (*S*)-amphetamine sulfate, 1.0. All injections were administered ip 30 min prior to testing. To avoid positional preferences, half the animals were trained to press DRUG-L and SAL-R while the other half were trained on DRUG-R and SAL-L.

Test sessions were run once or twice a week with at least two maintenance sessions between each test session. Test sessions were terminated when either 5 min passed or 50 responses were made on a lever, whichever came first. It the rat did not press either lever 50 times he was scored as disrupted and was not included in the calculations. On test days, responding was drug positive if the rats pressed the drug lever 50 times. A rat was only given a test drug if he responded correctly (85% responding on the correct lever) on the previous two maintenance sessions. Following the criteria of Colpaert et al.[40] test data were discarded and the test condition later retested if the rat responded incorrectly in either of the two subsequent maintenance sessions.

In Vitro [^{3}H]-5-HT Uptake Inhibition. The procedure of Steele et al.[23] was employed with minor modifications. Briefly, the cortical region of the rat brain was homogenized in 15 vol of ice-cold 0.32 M sucrose with a glass mortar with a motor-driven pestle. The homogenate was centrifuged at 1086*g* at 4 °C for 10 min. The resulting supernatant was centrifuged at 17800*g* for 10 min and the pellet was then resuspended in the same volume of sucrose. Incubations were carried out in a shaking incubator under a 95% O_2/5% CO_2 atmosphere at 37 or 0 °C to measure total tissue uptake and nonspecific uptake, respectively. A 5-min preincubation was begun by adding 0.2 mL of the synaptosomal preparation to test tubes containing 1.65 mL of O_2-saturated Krebs–Henseleit buffer (118 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 10 mM glucose, 0.6 mM ascorbic acid, and 0.03 mM Na_2EDTA), 50 μL of drug or buffer, and 50 μL of pargyline (final concentration of 1 μM). Then [^{3}H]-5-HT was added in a 50-μL aliquot to give a final concentration of 10 nM, and incubations were continued for another 5 min. Incubations were terminated by cooling in ice and then rapidly filtering through Whatman GF/B filters with a Brandel cell harvester (Gaithersburg, MD). The filters were washed twice with ice-cold buffer and allowed to air-dry before placing them in plastic scintillation vials. Scintillation cocktail (10 mL of Budget-Solve, Research Products International, Mount Prospect, IL) was added, and the vials were sealed and allowed to set for 8 h before counting at an efficiency of 54%.

[^{3}H]Paroxetine Binding to the Serotonin Uptake Carrier. The method of Habert et al.[41] was utilized with minor modifications. Brain regions from one hemisphere in neurotoxicity studies were thawed, weighed, and homogenized in 5 mL of 50 mM Tris HCl with 120 mM NaCl and 5 mM KCl (pH 7.4) with a Brinkman polytron (2 × 20 s, setting 6). The homogenates were centrifuged twice at 30000*g* for 10 min, and the resulting pellet was resuspended in the same volume of buffer. As adopted by Battaglia et al.,[25] the ability of a single saturating concentration (1 nM) of [^{3}H]paroxetine to bind to tissue homogenates was examined. Specific binding was defined as that displaceable with 1 μM fluoxetine. Incubations were commenced by the addition of tissue to the buffer to give a total volume of 2 mL. The tubes were allowed to equilibrate for 1 h at 24 °C before filtering through Whatman GF/C filters presoaked with 0.05% PEI, using a cell harvester. The tubes were then washed twice with ice-cold buffer, and the filters were allowed to air-dry before placing them in scintillation vials. Scintillation cocktail was then added, and the vials were allowed to set overnight before counting at an efficiency of 54%.

HPLC with Electrochemical Detection. The brain regions from animals sacrificed 3 h or 1 or 2 weeks following dosing were weighed and homogenized in 0.5 mL of 0.4 N $HClO_4$ containing

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0.05% Na_2EDTA and 0.1% $Na_2S_2O_5$, with a motor-driven Teflon pestle and Eppendorf 1.5-mL centrifuge tubes. An internal standard of 100 ng/mL of 3-(3,4-dihydroxyphenyl)propionic acid (DHPPA) was used. The samples were centrifuged at 14000g for 4 min with a tabletop centrifuge. The supernatant was assayed for catecholamines, 5-HT, and their metabolites by injection of 50 μL onto a Brownlee C18 analytical column (Anspec, Ann Arbor, MI). The HPLC-EC system consisted of a refrigerated autosample (TosoHaas, Philadelphia, PA) and a Model 400 EG&G Princeton electrochemical detector (Princeton, NJ) with a dual electrode potential set at E_1 = -200 mV and E_2 = 850 mV versus the Ag/AgCl reference electrode. The mobile phase containing 0.05 M NaH_2PO_4, 0.03 M citric acid, 0.1 mM Na_2EDTA, 0.034% sodium octyl sulfate, and 25% methanol was delivered at a flow rate of 1.0 mL/min. The concentrations of NE, DA, HVA, DOPAC, 5-HT, and 5-HIAA were determined with the Dynamax Method Manager software (Rainin, Woburn, MA) implemented on an Apple Macintosh SE computer.

Statistical Analysis. In drug-discrimination experiments a compound was defined as fully substituting for the training drug if at one or more doses 80% of the animals responded on the drug lever. The ED_{50} and 95% confidence intervals values were determined from quantal dose-response curves according to the procedure of Litchfield and Wilcoxon.[42] Drugs were said to partially substitute if at the highest dose at least 60-79% of the animals responded on the drug lever; no substitution was said to occur if 59% or less of the animals responded at the highest dose. Percent uptake inhibition was defined as the difference between specific [^{3}H]-5-HT uptake in control and drug tubes divided by control × 100%. The IC_{50} values for uptake inhibition were determined from graded dose-response curves according to the procedure of Tallarida and Murray.[43] All comparisons utilized an analysis of variance followed by a post hoc comparison as embodied in the computer program EPISTAT (EPISTAT Services, Richardson, TX).

Materials. Pargyline hydrochloride and the HPLC standards were purchased from Sigma Chemical Co. (St. Louis, MO). [^{3}H]Paroxetine and [^{3}H]-5-HT were obtained at a specific activity of 28.8 and 15.1 Ci/mmol from New England Nuclear (Boston, MA). LSD tartrate was obtained from the National Institute on Drug Abuse. Fluoxetine hydrochloride was graciously provided by Eli Lilly Laboratories (Indianapolis, IN). MDMA·HCl, 2·HCl and (*S*)-amphetamine sulfate were synthesized in our laboratory by using standard procedures.

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Using Theoretical Descriptors in Quantitative Structure–Activity Relationships: Some Toxicological Indices

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The application of computational techniques to medicinal chemistry is growing at a tremendous rate. Quantitative structure–activity relationships (QSAR), which relate biological and toxicological activities to structural features, have been employed widely to correlate structure to activity. A difficulty of this approach has been nonuniformity of parameter sets and the inability to examine contributions across properties and data sets. Linear solvation energy relationships (LSER) developed by Kamlet and Taft circumvent many of the difficulties and successfully utilize a single set of parameters for a wide range of physical, chemical, and biological properties. We have replaced the LSER solvato-chromatic parameters with theoretically determined parameters to permit better a priori prediction of properties. Comparison of the two parameter sets for five biological activities is presented, showing the excellent fit of the theoretically determined parameters.

1. Introduction

Quantitative structure–activity relationships (QSAR) have been used extensively in correlating molecular structural features of compounds to their biological, chemical, and physical properties. The basic tenet of QSAR is that there is a quantitative connection between the microscopic (molecular structure) and the macroscopic (empirical) properties (particularly biological activity) of a compound. Furthermore, this connection can be used to predict empirical properties of a compound given its molecular structure. One such connection is the *Linear free energy relationship* (LFER). In 1935 Burkhardt[1] and Hammet[2] reviewed the existence of LFER's and in 1937 Hammet[3] proposed the equation that bears his name.

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2. Linear Solvation Energy Relationships

Based on solvent effect LFER's proposed by earlier workers,[4] Kamlet and Taft[5-10] developed a method for writing linear free energy relationships involving solute/

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