or mechanistic inferences from the regression coefficients, pattern-recognition techniques are mainly predictive in nature. Their utility lies in the screening of proposed or existing agents for activity. In this respect, the tendency that was consistently observed in this study, to correctly predict a greater proportion of active compounds than inactive ones, could be a benefit. It is clearly better to misclassify in favor of active derivatives, to avoid missing potentially beneficial agents. This tendency was seen to extend to the prediction sets as well.

Recent studies with *m*-AMSA in phase II clinical trials have given both encouraging[42] and disappointing[43] results. Thus, the search for a clinically effective agent in this series is not yet complete. Based on the results that have been presented here, it is reasonable to expect that pattern-recognition techniques could play a useful role in the identification of active members in the future.

Acknowledgment. The PRIME-750 and MODCOMP-II computers, on which this research was performed, were purchased with partial financial support of the National Science Foundation. The work was also partially funded by the U.S. Environmental Protection Agency.

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Structure–Activity Relationships in Potentially Hallucinogenic *N,N*-Dialkyltryptamines Substituted in the Benzene Moiety

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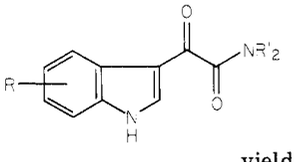
A series of *N,N*-dialkyltryptamines with methylthio or methylenedioxy substituents in the 4, 5, and 6 positions and methyl or isopropyl on the side-chain nitrogen has been synthesized. The behavioral pharmacology of these compounds showed them to possess Bovet-Gatti profiles characteristic of hallucinogens, and the 5-methylthio congener was the most potent. Binding studies at [^{3}H]LSD and [^{3}H]5-HT sites demonstrated that no single structural feature correlated with binding or behavioral changes and suggest a complex mode of action for these potential hallucinogenic agents.

Reports that tryptamines other than 5-hydroxytryptamine (5-HT) may be endogenous neuroregulatory agents,[1] complete with all appropriate biosynthetic and metabolic transformations,[2] have led us to prepare a series of *N,N*-dialkyltryptamines with novel substituents in the benzene moiety to be evaluated as hallucinogens. Recently reported methodologies in competitive binding studies[3] and measurement of behavior-disrupting activity in the rat make possible clearer distinction between behavioral and serotonergic effects of the compounds examined in this study.

Substituents, e.g., methoxy or hydroxy, in the 4, 5, or 6 positions of *N,N*-dimethyltryptamine induce significant changes in the neuropharmacological properties of these indolealkylamines.[4] In order to constrain *o*-methoxy groups into planar conformation, 4,5-(methylenedioxy)-*N,N*-dimethyltryptamine (**1**) and 5,6-(methylenedioxy)-*N,N*-dimethyltryptamine (**2**) were synthesized, and the pharmacological properties of **1** and **2** were compared with those of the known 4-methoxy- (**3**), 5-methoxy- (**4**), and 6-methoxy- (**5**) congeners.[5]

Significant changes in the potencies of substituted 2-phenylisopropylamines occurred when methylthio was substituted for methoxy.[6] Thioanisole partially exists in a rotated conformation in which the π system of the aromatic ring overlaps with the d orbitals rather than with the lone-pair p lobe, which has a rotational energy barrier of 2.05 kcal/mol,[7] slightly lower than most biological weak forces. It was therefore decided to synthesize the 4-, 5-, and 6-(methylthio)-*N,N*-dimethyltrytamines (**6–8**) in order to examine the differences between isosteres.

Table I. Ring-Substituted *N,N*-Dialkylindole-3-glyoxalamides



no.	R	R′	mp, °C	yield, %	formula[a]
22a	4,5-OCH_2O	CH_3	240–241	77	$C_{13}H_{12}N_2O_4$
22b	4,5-OCH_2O	i-C_3H_7	260 dec	56	$C_{17}H_{20}N_2O_4$
22c	5,6-OCH_2O	CH_3	217–220	79	$C_{13}H_{12}N_2O_4$
22d	5,6-OCH_2O	i-C_3H_7	278–280	81	$C_{17}H_{20}N_2O_4$
22e	4-SCH_3	CH_3	163–164	43	$C_{13}H_{14}N_2O_2S$
22f	4-SCH_3	i-C_3H_7	190–192	27	$C_{17}H_{22}N_2O_2S$

[a] IR and NMR spectra were consistent with structures given; the dried products were not analyzed but were reduced without further purification.

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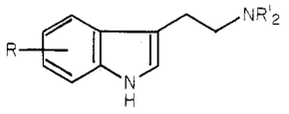
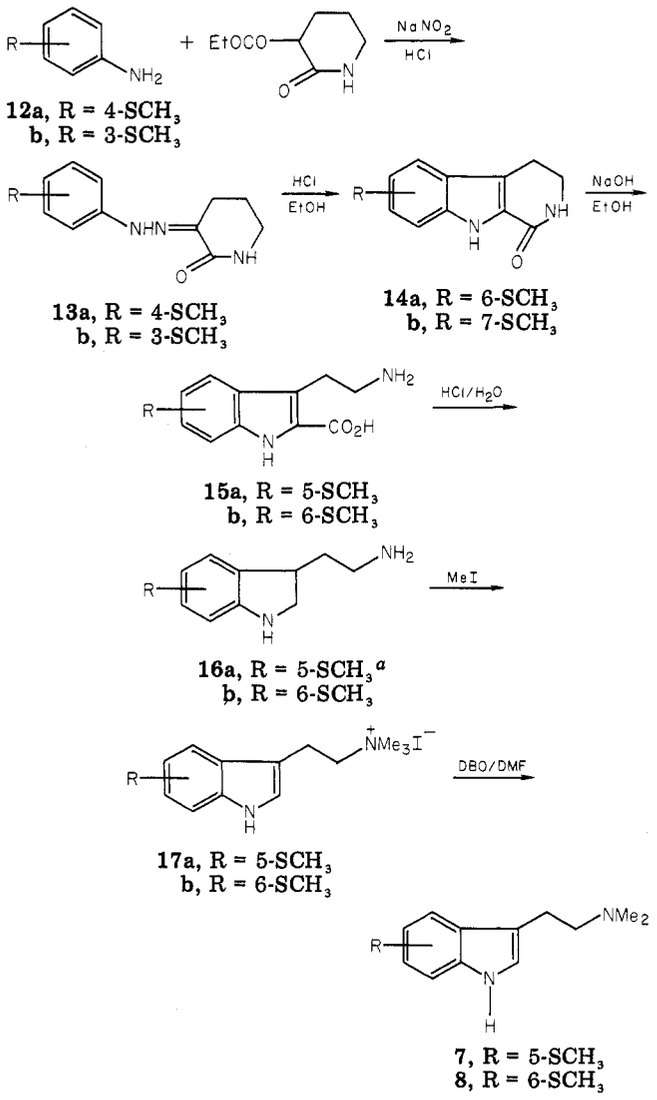
Table II. Ring-Substituted *N,N*-Dialkyltryptamines



no.	R	R'	mp, °C	synth method	purification method[c]	yield, %	formula	anal.[d]
1	4,5-OCH_2O	CH_3	93–95	B	I	8	$C_{12}H_{16}N_2O_2$	C, H, N
2	5,6-OCH_2O	CH_3	109–110	B	I	48	$C_{12}H_{16}N_2O_2$	C, H, N
3	4-OCH_3	CH_3	*b*					
4	5-OCH_3	CH_3	*b*					
5	6-OCH_3	CH_3	*b*					
6	4-SCH_3	CH_3	110	B	III	68	$C_{13}H_{18}N_2S$	C, H, N, S
7	5-SCH_3	CH_3	97–100	A	I	76	$C_{13}H_{18}N_2S$	C, H, N, S
8	6-SCH_3	CH_3	57–58	A	I	33	$C_{13}H_{18}N_2S$	C, H, N, S
9	4,5-OCH_2O	i-C_3H_7	109–113	B	IV	31	$C_{17}H_{24}N_2O_2$	C, H, N
10	5,6-OCH_2O	i-C_3H_7	85–86	B	II	16	$C_{17}H_{24}N_2O_2$	C, H, N
11	4-SCH_3	i-C_3H_7	92–94	B	I	61	$C_{17}H_{26}N_2S$	C, H, N, S

[a] 5-(Methylthio)tryptamine was obtained from 4-(methylthio)aniline as described by Andrelova et al.[27] [b] Samples obtained from Dr. A. Manion, NIMH. [c] I, benzene/petroleum ether; II, EtOH/H_2O; III, Kugelrohr distillation; IV, benzene/*n*-hexane. [d] NMR and IR spectra measured on all compounds are consistent with structures given.

Scheme I



12a, R = 4-SCH_3
b, R = 3-SCH_3

13a, R = 4-SCH_3
b, R = 3-SCH_3

14a, R = 6-SCH_3
b, R = 7-SCH_3

15a, R = 5-SCH_3
b, R = 6-SCH_3

16a, R = 5-SCH_3[a]
b, R = 6-SCH_3

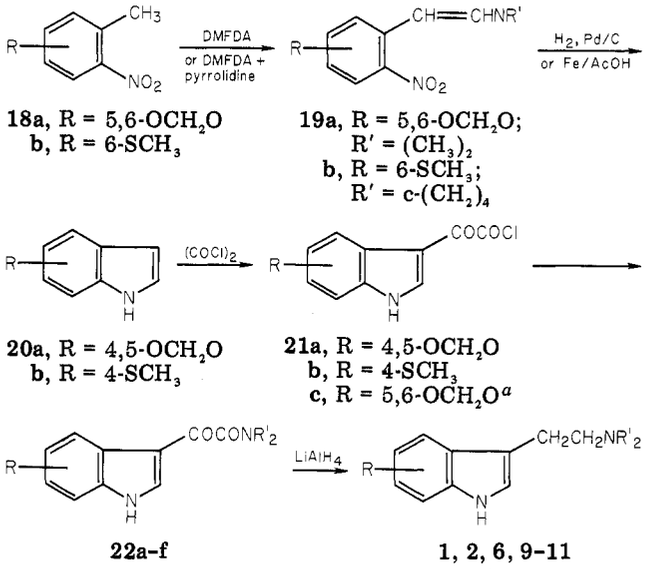
17a, R = 5-SCH_3
b, R = 6-SCH_3

7, R = 5-SCH_3
8, R = 6-SCH_3

[a] Reported by Andelerova et al.[27]

In order to compare the relative activities of *N,N*-diisopropyltryptamines vs. their *N,N*-dimethyl homologues,

Scheme II



18a, R = 5,6-OCH_2O
b, R = 6-SCH_3

19a, R = 5,6-OCH_2O; R' = $(CH_3)_2$
b, R = 6-SCH_3; R' = c-$(CH_2)_4$

20a, R = 4,5-OCH_2O
b, R = 4-SCH_3

21a, R = 4,5-OCH_2O
b, R = 4-SCH_3
c, R = 5,6-OCH_2O[a]

22a-f

1, 2, 6, 9–11

[a] Obtained from commercially available 5,6-(methylenedioxy)indole.

in selected cases both *N,N*-dimethyl- and *N,N*-diisopropyltrypamines (**9–11**) were synthesized and evaluated.

Chemistry. Two general methods were used to obtain the desired ring-substituted *N,N*-dialkyltryptamines. Method A (Scheme I) utilized the procedure of Abramovich[8] to synthesize a substituted tryptamine, which was then quaternized with methyl iodide and demethylated to

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Table III. Behavior Disruption Data

no.	dose, mg/kg	changes in response from drug vs. saline,[a] % B/P	E	L
1	0.25	3↑	3↓	5↑
	0.50	0	5↓	5↑
	1.00	5↑	10↓	5↑
	2.00	11↑	31↓	20↑
2	5.00	9↑	11↓	2↑
	10.00	19↑	26↓	9↑
	15.00	22↑	41↓	19↑
3	0.50	0	2↓	2↑
	1.00	9↑	25↓	6↑
	2.00	10↑	27↓	7↑
4	1.00	6↑	11↓	5↑
	2.00	19↑	26↓	9↑
5	1.25	25↑	21↓	5↑
	2.50	26↑	34↓	8↑
	5.00	29↑	50↓	21↑
6	1.00	0	1↓	1↑
	2.00	8↑	11↓	3↑
	4.00	20↑	27↓	6↑
	8.00	36↑	52↓	15↑
7	0.50	13↑	26↓	7↑
	1.00	16↑	65↓	49↑
8	0.50	4↑	8↓	4↑
	1.00	8↑	13↓	4↑
	2.00	20↑	30↓	19↑
	4.00	34↑	48↓	14↑
9	1.25	24↑	28↓	74↑
	2.50	38↑	49↓	11↑
10	1.25	1↓	0	1↑
	2.50	0	2↑	2↓
	5.00	2↑	4↓	2↑
	10.00	29↑	45↓	16↓
11	5.00	1↑	5↓	4↑
	10.00	23↑	32↓	9↑

[a] B = burst; P = premature; E = efficient; L = late.

the tertiary amine.[9] The Fischer-type cyclizations proceeded regioselectively to afford 6- or 7-substituted β-oxocarbolines. Although **13b** presumably could have undergone cyclization to either a 5- or 7-(methylthio)-β-carboline intermediate, this closure occurred regioselectively to give the latter derivative as was evident from the NMR spectra of **14b**, **15b**, **16b**, and **17b**. Similar regioselectivity has been reported by Manske[10] for the cyclization of the 3-methoxy analogue of **13b** to 7-methoxy-1-oxo-1,2,3,4-tetrahydro-β-carboline. Both cases involve the same activation by an electron-donating group. In method B (Scheme II), the appropriate substituted indole was synthesized by the procedure of Batcho and Leimgruber[11] or a modification of this method, and the 3-(dialkylamino)-ethyl side chain was introduced by the route reported by Speeter and Anthony.[12]

2-Nitro-5,6-(methylenedioxy)toluene (**18a**) was prepared from 3-methylcatechol via methylenation to give 2,3-(methylenedioxy)toluene[13] and nitration.[14] Although a mixture of 2- and 3-nitro derivatives was formed, separation was not required, since only the 2-nitro isomer participated in the Batcho–Leimgruber reaction.

2-Methyl-3-nitrothioanisole was prepared by methylation of 2-methyl-3-nitrothiophenol with methyl iodide and KOH;[6] the thiophenol was obtained from 3-nitro-*o*-toluidine via diazotization, treatment of the diazonium salt with potassium ethyl xanthate, followed by hydrolysis.[15]

Pharmacology. Table III shows the results of the behavior-disruption studies. The 4-, 5-, and 6-methoxy-*N*,*N*-dimethyltryptamines (**3–5**) were examined in the same behavioral protocol as experimental compounds **1**, **2**, and **6–11**, and the overall order of potency in conditioned avoidance response disruption was determined to be **7** > **4** > **9** > **3** > **1** = **8** = **5** > **6** = **10** > **11** > **2**. Thus, the previously reported trend among the methoxy compounds **3–5**, in which maximum activity was associated with 5-substitution followed by substitution in the 6 and 4 positions, respectively, was similar in the experimental compounds **6–8**. However, in the compounds bearing two aromatic substituents (**1**, **2**, **9**, and **10**), 4,5-disubstitution conferred greater activity than did 5,6-disubstitution. In examining the differences between *N*,*N*-dimethyl and *N*,*N*-diisopropyl homologues, the methylenedioxy compounds showed opposing trends from the methylthio compounds. The diisopropyl compounds **9** and **10** were more active than the dimethyl compounds **1** and **2**, whereas with the methylthio compounds, **11** was less active than **6**. These data suggested that although all compounds tested could be classified behaviorally as hallucinogens, the methylenedioxy and the methylthio series could be operating via separate modes of action.

In order to compare the behavior disruption of the various compounds with the extent of receptor site binding, a unit of behavior-disrupting activity (*A*) was defined based on a comparison with the reference hallucinogen *N*,*N*-dimethyltryptamine (DMT). Thus, "DMT" units were obtained by taking the ratio of the dose of the experimental compounds in milligrams per kilogram to that dose of DMT (10 mg/kg) required to produce equivalent pharmacological effects, here defined as a 50% decrease in the efficient responses of the Bovet–Gatti profile. The DMT scale is analogous in derivation and use to the currently accepted mescaline unit (MU) scale as defined by Shulgin.[16]

Regression analyses were performed on the $-\log IC_{50}$ values at the [^{3}H]5-HT and [^{3}H]LSD sites and behavior-disrupting activity as shown in Table V. Although the correlation at the 5-HT site is only at the threshold of statistical significance, the relationship of activity to ligand affinity merits some comment. Correlations between activity and binding affinities, which generally have given the best linear results, are restricted to a measure of 5-HT agonism as the defined "activity".[17-19] It is to be expected that hallucinogenic activity as a more complex pharmacological phenomenon would depend on a series of events beyond binding. If merely binding to a 5-HT site accounts for 69% ($r^2 = 0.69$) of the observed effects, there is some evidence for the implication of this receptor in the overall mechanism of action. These data imply, however, that

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Table IV. Affinity Values at Binding Sites

	[^{3}H]LSD site			[^{3}H]5-HT site		
no.	IC_{50}, mol/L	Hill coefficient	K_d	IC_{50}, mol/L	Hill coefficient	K_d
2	1.78×10^{-6}	0.95	1.9×10^{-6}	4.07×10^{-5}	1.01	8.4×10^{-6}
6	1.25×10^{-5}	0.75		1.51×10^{-4}	1.01	8.4×10^{-6}
7	1.44×10^{-7}	0.91	1.79×10^{-7}	1×10^{-6}	0.995	4.3×10^{-8}
8	7.59×10^{-6}	0.97	3.4×10^{-6}	4.08×10^{-5}	0.57	
9	2.88×10^{-6}			2.24×10^{-4}	0.343	
10	1×10^{-5}			9.33×10^{-4}	0.642	
11		0.70				

Table V. Regression Analyses of Drug Activity (A) and Specific Binding at Serotonergic and LSD Receptor Sites (IC_{50})

correlation equation[a]	n	r^2	p
$A = 4.25(-\log IC_{50}) - 18.77$	6	0.69	0.045
$A = 5.88(-\log IC_{50}) - 29.1$	7	0.85	0.003

[a] n = number of compounds in correlation; r^2 = variance; p = probability that relationship is statistically significant.

serotonergic effects are not identical with nor absolute predictors of hallucinogenic effects.

If there exists some mechanism common to all hallucinogenic agents, it might be expected that the receptor which binds LSD in high specificity would also bind other hallucinogens with affinities proportional to the potencies of such substances, as shown in the second equation in Table V. This relationship supports the existence of at least one site at which all of the compounds examined might exert their psychopharmacological effects.

Conclusions

A single site at which all of these compounds bind does not imply that all hallucinogens bind to the same neurotransmitter sites and exert their effects through identical mechanisms. The difference in correlations between [^{3}H]5-HT and [^{3}H]LSD site affinities and activity could reflect the different mechanisms of action of the experimental compounds. The structure–activity trends among the methylenedioxy congeners were distinctly different from those of the methylthiodialkyltryptamines. The finding that no single structural feature (e.g., an unsubstituted 4-position) correlated exclusively with the biological events of binding or behavioral changes is significant and suggestive of heterogeneity in the mode of action of these agents. The evidence from these studies suggests a complex overall mechanism in which more than one discreet binding site is involved but in which binding at the [^{3}H]LSD site is a requisite for all compounds which have hallucinogenic activity.

Experimental Section

Behavioral Pharmacology. Four adult, male, hooded rats weighing between 300 and 350 g at the beginning of the study served as subjects. The paradigm used is based on a modified discriminated Sidman avoidance schedule.[20] Using this test, Smythies et al.[21] classified certain drugs on the basis of their disruptive effects, finding that hallucinogenic compounds consistently induced effects different from those induced by stimulant compounds. Substances that exhibit hallucinogenic activity in man possess a unique Bovet–Gatti profile that differs from all other psychotropic drugs. The subjects were trained until they emitted a minimum of 80% efficient responses with very little variation in response distribution (±3%) between five successive sessions. This stability required approximately 75 daily training sessions. All injections were given after a 15-min warm-up period. The data were than collected for the following 100 min. All compounds were dissolved, as their HCl salts, in saline and injected subcutaneously at a volume of 0.1 mL/100 g of body weight at the indicated doses.

Binding Studies. The affinities of the drugs for LSD sites, i.e., sites labeled by [^{3}H]LSD with 1 μM 5-HT as masking ligand, and for 5-HT sites, i.e., sites labeled by [^{3}H]5-HT with 1 μM LSD as masking ligand, were measured by S. Maayani and H. Weinstein in competition binding studies as described.[3] To obtain a range of 20–80% inhibition of the binding of 3–6 nM [^{3}H]LSD (Amersham, 20 Ci/mmol) or [^{3}H]5-HT (New England Nuclear, 20–25 Ci/mmol), 8–10 different concentrations of the drug were chosen. The competition studies were carried out at 25 °C with membranes from the P_2 fraction of guinea pig cortex, 0.3 mg of protein per assay. Incubations of 90 min were carried out at each concentration, and then the filtration was done on Whatman GF/C filters. Data were analyzed by Dixon and Hill plots as described[22] with the specific amount bound in the absence of competing drug taken as B_{max}. Only when Hill slopes were 1.2 ± 0.2 (see Table IV) were dissociation constants (K_d) values calculated from the IC_{50} values (Table IV).

Chemistry. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Infrared spectra were recorded on a Beckman Acculab 2 as KBr pellets unless otherwise indicated. All IR frequencies are reported in reciprocal centimeters. Nuclear magnetic resonance spectra were recorded on a Varian EM 390 100-MHz instrument and are reported as δ values in parts per million downfield from the internal standard tetramethylsilane. The solvent for NMR samples was $CDCl_3$ unless otherwise indicated. Elemental analyses were performed by Midwest Microlabs, Indianapolis, IN.

All commercially available starting materials were of the highest grade of purity obtainable. Solvents that were required to be moisture free were distilled from the appropriate dessicating agent prior to use.

Method A. 2,3-Piperidinedione 3-[3-(Methylthio)-phenyl]hydrazone (13b). To a well-stirred, ice-cold solution of the diazonium salt prepared from 18.0 g (0.1 mol) of **12b**,[23] 9.0 g (0.13 mol) of $NaNO_2$, 45 mL of hydrochloric acid, and 380 mL of H_2O was added a solution of 17.1 g (0.1 mol) of 3-carbethoxy-2-piperidinone in 250 mL of H_2O containing 6.2 g (0.11 mol) of KOH. The workup procedure of Abramovich[8] resulted in an overall yield of 31% of **13b**: mp 218–220 °C; NMR ($CDCl_3$ + Me_2SO-d_6) δ 9.05 (s, 1, Ar NH), 7.4 (s, 1, CONH), 6.6–7.3 (m, 4, Ar H), 3.2 (m, 2, CH_2CH_2N), 2.5 (m, 2, CH_2CH_2N), 2.4 (s, 3, SCH_3), 1.9 (t, 2, $CH_2CH_2C{=}NH$). Anal. ($C_{12}H_{15}N_3OS$) C, H, N, S.

7-(Methylthio)-1-oxo-1,2,3,4-tetrahydro-β-carboline (14b). The phenylhydrazone **13b** (10 g, 0.04 mol) was added to 400 mL of dry EtOH, and the mixture was saturated with HCl gas (cooling). The reaction mixture was stirred overnight at room temperature and cooled in an ice-bath, and the resulting green solid was collected. Recrystallization from hot EtOH gave 3.3 g (36%) of **14b**: mp 160–168 °C; TLC (silica gel plate; benz-

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(23) Tridom Chemical Inc.

ene/$CHCl_3$/EtOH, 45:45:10, UV visualization) showed a single spot, R_f 0.52; NMR [$(CD_3)_2CO$] δ 11.5 (br, s, 1 NH), 6.8–7.5 (m, 4, Ar and CONH), 3.6 (t, 1, CH_2CH_2NH), 3.2 (t, 2, CH_2CHN_2), 3.0 (t, 2, CH_2CHN_2), 2.5 (s, 3, SCH_3).

2-Carboxy-6-(methylthio)tryptamine (15b). A solution of the β-carboline **14b** (3.3 g, 0.014 mol) in 70 mL of 2 N NaOH in 60% aqueous EtOH was refluxed overnight. After the solvent was removed in vacuo and the pH was adjusted to 6 with AcOH, the precipitated amino acid **15b** was collected. Recrystallization from hot EtOH gave 2.5 g (71%) of pure **15b**: mp 185–188 °C; IR (KBr) 3000–3600 (OH and NH), 2100 (NH_3^+), 1580 (CO_2^-), 1200 (SCH_3 wag); NMR (D_2O + Me_2SO-d_6) δ 6.8–7.5 (m, 4, Ar H), 3.0–3.2 (br, dd, 4, CH_2CH_2), 2.5 (s, CH_3S overlapping CH_3SO).

6-(Methylthio)tryptamine (16b). The amino acid **15b** (2.5 g, 0.01 mol) was refluxed in 50 mL of 10% aqueous HCl for 20 h. After cooling, the solution was adjusted to pH 12 with 5 N NaOH and then extracted with EtOAc. The organic layer was dried ($MgSO_4$), filtered, and evaporated to give 1.75 g (85%) of a yellow oil. On the basis of spectral data, this material was used without further purification in the subsequent step: IR (neat) 2950–3200 (NH and indole NH), 1200 (SCH_3 wag); NMR δ 8.9 (br s, 1, indole NH), 6.7–7.6 (m, 4, Ar H), 2.6–3.2 (m, 4, CH_2CH_2), 2.5 (s, 3, SCH_3), 1.6 (br s, 2, NH_2).

6-(Methylthio)-*N*,*N*-dimethyltryptamine Methiodide (17b). Crude tryptamine **16b** (4.95 g, 0.025 mol) was dissolved in 300 mL of MeOH containing 10.6 g (0.08 mol) of CH_3I and 6.3 g (0.08 mol) of $NaHCO_3$. This mixture was refluxed for 72 h, and 2 g of CH_3I was added at 24 and 48 h, respectively. Removal of the solvent in vacuo gave a colorless solid, which was recrystallized from boiling EtOH (Norite) to give 3.3 g (36%) of **17b** as colorless needles: mp 215–217 °C; IR 3250 (indole NH), 1620 (Ar C=C), 1470 (NCH_3), 1330 (SCH_3); NMR (Me_2SO-d_6) δ 7.5–7.6 (d, 1, C-2 H), 7.2 (dd, 2, C-4 H, C-5 H), 6.9–7.0 (dd, 1, C-7 H), 3.6 (m, 2, CH_2N), 3.2 (s, 9, CH_3), 3.0–3.2 (m, overlap NCH_3, 2, CH_2CH_2N), 2.5 (s, SCH_3 overlap CH_3 of Me_2SO).

6-(Methylthio)-*N*,*N*-dimethyltryptamine (8). Methiodide **17b** (2.2 g, 6 mmol) was refluxed in 50 mL of DMF containing 1.3 g (11.5 mmol) of 1,4-diazabicyclo[2.2.2]octane (DBO) for 3 h. The reaction mixture was diluted with 300 mL of H_2O and then extracted with 600 mL of a 1:2 mixture of EtOAc/benzene, and the organic layer was back-extracted with 10% HCl. Basification of the aqueous layer with 5 N NaOH liberated the crude amine, which was extracted with EtOAc. After drying ($MgSO_4$), the solvent was evaporated to yield a dark-yellow oil, which was subjected to Kugelrohr distillation (140 °C, 0.01 mm). Recrystallization of the viscous distillate from benzene/petroleum ether yielded 0.543 g (33%) of pure **17b**: mp 62–63 °C; NMR δ 8.1 (br s, 1, NH), 6.8–7.5 (m, 4, Ar H), 2.7–3.0 (td, 2, CH_2CH_2N), 2.5–2.7 (m, 2, CH_2CH_2), 2.5 (s, 3, SCH_3), 2.2 (s, 6, NCH_3). Anal. ($C_{13}H_{18}N_2S$) C, H, N, S.

Method B. 2-Nitro-5,6-(methylenedioxy)toluene (18a). To a stirred mixture of 3.7 g of Adogen 464,[24] 138 mL of CH_2Br_2, and 160 mL of H_2O was added a solution of 100 g (0.8 mol) of 3-methylcatechol in 400 mL of H_2O and 80 g (2 mol) of NaOH during 2 h under N_2. After stirring for an additional hour, the mixture was steam distilled to give 85 g (78%) of 2,3-(methylenedioxy)toluene[13] as a colorless oil. To a stirred mixture of 21 g (0.19 mol) of this oil, 59 g of AcOH, and 1 g of $Hg(OAc)_2$ heated to 80 °C was added dropwise 12.8 g (0.2 mol) of nitric acid, and the mixture was heated and stirred at 80 °C for an additional 2 h. The mixture was poured into ice–H_2O, extracted with Et_2O, dried ($MgSO_4$), and filtered, and the solvent was evaporated. The orange solid residue was recrystallized from EtOH to yield 20 g (58%) of the mixed ortho- and meta-nitration products, mp 65–67 °C. TLC on silica gel plates in benzene/$CHCl_3$ (both at 9:1 and 1:9) gave two spots: IR 1535 (NO_2), 1360 (NO_2), 1290 (OCO) cm^{-1}; NMR δ 7.7 (d, J = 3 Hz, 1, over d, J = 10 Hz, C-3, C-4), 7.4 (d, J = 3 Hz, C-2), 6.2 (s, 4, OCH_2), 2.4 (s, 3, CH_3), 2.3 (s, 3, CH_3). Anal. ($C_8H_7NO_4$) C, H, N.

(*E*)-β-(Dimethylamino)-5,6-(methylenedioxy)-2-nitrostyrene (19a). Under an atmosphere of N_2, a mixture of 15 g (0.08 mol) of the mixed nitromethylenedioxytoluenes, 100 mL of freshly distilled DMF (H_2O pump pressure), and 12.8 g (0.104 mol) of *N*,*N*-dimethylformamide dimethyl acetal was distilled slowly through a glass helices packed column equipped with a total reflux-variable take off head to permit continuous removal of MeOH as formed while maintaining a head temperature of 50 to 70 °C. After 4 h, 5.9 mL of MeOH (theory, 6.4 mL) had been collected. DMF was removed from the pot residue by evaporation in vacuo, the dark solid residue was dissolved in benzene, washed with H_2O, dried ($MgSO_4$), and filtered, and the benzene was removed by evaporation in vacuo to give the crude aminostyrene **19a**: yield 4.8 g (50%) of red needles after recrystallization from hexane–benzene: mp 126–128 °C; NMR δ 7.5 (dd, 2, C-3 H), 6.5 (d, 1, $ArCH$=CH), 6.1 (s, 2, OCH_2O), 5.7 (d, 1, CH=CHN), 2.8 (s, 6, CH_3). Anal. ($C_{11}H_{12}N_2O_4$) C, H, N.

4,5-(Methylenedioxy)indole (20a). To a solution of 3.5 g (0.015 mol) of **19a** in 200 mL of benzene in a Parr hydrogenation bottle was added 0.35 g of 10% Pd/C, and the mixture was shaken under 3 atm of H_2 for 7 h. The mixture was filtered from the catalyst, and the filtrate was rapidly washed with cold 2 N H_2SO_4, aqueous $NaHCO_3$, and H_2O, dried, and filtered. The benzene was evaporated to yield 1.2 g (50%) of **20a**: mp 111 °C after recrystallization from benzene–petroleum ether; NMR δ 10.1 (br s, 1, NH), 7.2 (dd, 1, C-2 H), 6.8 (dd, 2, C-6 H, C-7 H), 6.3 (t, 1, C-3 H), 5.6 (s, 2, OCH_2O). Anal. ($C_9H_7NO_2$) C, H, N.

(*E*)-6-(Methylthio)-2-nitro-β-pyrrolidinostyrene (19b). 2-Nitro-6-(methylthio)toluene (**18b**;[25] 21.5 g, 0.118 mol) was dissolved in 150 mL of freshly distilled DMF, and 36 mL (0.25 mol) of dimethylformamide dimethyl acetal and 19.2 mL (0.24 mol) of pyrrolidine were added. The mixture was refluxed under N_2 for 4 h. After the mixture was cooled, the DMF was removed at 0.5 mm in order to maintain the temperature below 40 °C. Trituration of the oily red residue with ice-cold MeOH induced crystallization to give crude **19b**, mp 85–90 °C. Recrystallization from EtOH gave 24 g (77%) of red needles, mp 91–92 °C. NMR indicated 11% of the *N*,*N*-dimethylaminostyrene, an impurity which was removed from the analytical sample by further recrystallization from EtOH, mp 91–92 °C, but was not removed from the material reduced in the next step: NMR δ 7.0–7.4 (m, 3 H), 6.8 (d, 1 H), 5.2 (d, 1 H), 3.3 (t, 4 H), 2.8 (s, ~0.5 H, from the NCH_3), 2.5 (s, 3 H), 2.0 (t, 4 H). Anal. ($C_{13}H_{16}N_2O_2S$) N, S.

4-(Methylthio)indole (20b). Pyrrolidinostyrene **19b** (18.1 g, 0.068 mol) was stirred under N_2 with 57.2 g (1.02 mol) of iron powder in 208 mL of 50% EtOH–AcOH.[28] The reaction mixture was warmed to 85 °C and maintained at this temperature for 10 min. During this time, a vigorous reaction occurred, acompanied by evolution of gas and the formation of a white precipitate; the red suspension changed to a gray paste. After an additional 10 min, the paste was poured into a solution of 291 g of $Na_2S_2O_5$ in 1750 mL of H_2O, and the organic material was extracted with Et_2O. The extract was washed with aqueous $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated to give a green oil, which was applied to a column of alumina (100 g of neutral Al_2O_3, 60 mesh) and eluted with 300 mL of 1:1 benzene–petroleum ether, followed by 300 mL of benzene. Removal of the solvent afforded 7.0 g (63%) of **20b** as a nearly colorless solid: mp 36–38 °C. The analytical sample (from EtOH) melted at 37–38 °C (lit.[26] mp 44–47 °C): NMR δ 8.1 (br s, 1, NH), 6.9–7.2 (m, 4, C-5 H, C-6 H, C-7 H, C-2 H), 6.7 (t, 1, C-3 H), 2.5 (s, 3, SCH_3). Anal. (C_9H_9NS) C, H, N.

***N*,*N*-Dimethyl-4,5-(methylenedioxy)indole-3-glyoxalamide (22a).** To a stirred solution of 4.8 g (0.03 mol) of **20a** in 60 mL of anhydrous Et_2O cooled in an ice bath was added a solution of 5 mL (0.038 mol) of oxalyl chloride so that the temperature did not exceed 10 °C. The red solid acid chloride was collected by filtration, washed with Et_2O, returned to the original flask, and suspended in 60 mL of fresh anhydrous Et_2O. Dimethylamine (7 mL, 0.1 mol) was added at 10 °C, and the mixture was stirred for 30 min. The crude solid product was collected and slurried in 50 mL of H_2O to dissolve dimethylamine hydrochloride. Crude

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22a was filtered off and dried in a vacuum oven to yield 5.7 g (77%) of a colorless solid, mp 240–243 °C, which was reduced without further purification.

4,5-(Methylenedioxy)-*N*,*N*-dimethyltryptamine (1). To a stirred and cooled (ice bath) mixture of 3.8 g (0.1 mol) of $LiAlH_4$ in 100 mL of anhydrous THF was added a solution of 3.7 g (0.015 mol) of **22a** in 500 mL of anhydrous THF during 1 h. The mixture was then stirred and refluxed for 1 h, cooled in an ice bath, and hydrolyzed by the cautious sequential addition of 3.8 mL of H_2O, 3.8 mL of 5 N NaOH, and 10.4 mL of H_2O and allowed to stir for 30 min. The inorganic salts were removed by filtration, the filtrate was dried ($MgSO_4$) and filtered, and the solvent was evaporated to leave a residual yellow oil. Kugelrohr distillation (100 °C, 0.5 mm) of this oil gave, after recrystallization from benzene–petroleum ether, 250 mg (8%) of a colorless solid: mp 93–95 °C; NMR [$(CD_3)_2C{=}O$] δ 9.9 (br s, 1, NH), 7.0 (br s, 1, C-2 H), 6.7 (q, 2, C-6 H and C-7 H), 5.9 (s, 2, OCH_2O), 2.9 (br t, 2, CH_2N), 2.6 (br t, 2, CH_2CH_2), 2.2 (s, 6, NCH_3). Anal. ($C_{12}H_{16}N_2O_2$) C, H, N.

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Potential Affinity Labels for the Opiate Receptor Based on Fentanyl and Related Compounds

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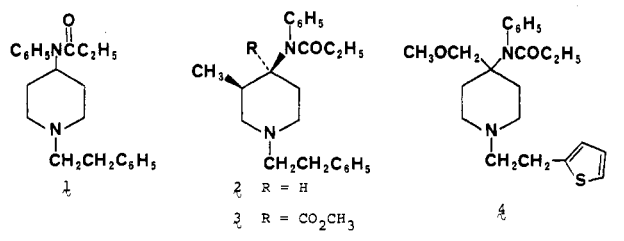
Derivatives of fentanyl, 3-methylfentanyl, sufentanil, and lofentanil, possessing chemo- or photoaffinity functionalities, were synthesized as potential affinity reagents for the opiate receptor. Opiate receptor binding constants (IC_{50}) were determined in competition experiments with [^{3}H]naloxone and [^{3}H]naltrexone. Affinity-labeling experiments were generally unsuccessful, although some irreversible attachment was achieved with α-diazoamide **17** and aryl azide **23**.

Opiate receptors, stereospecific binding sites for narcotic analgesic drugs in the central nervous system of vertebrates, have been extensively studied[1] since their initial identification[2] in 1973. Much research[1] has focused on the determination of membrane components responsible for the high-affinity binding, especially following the discovery[3] of endogenous opioid ligands, the enkephalins and endorphins. The isolation and characterization of opiate receptors would aid in understanding their function on a molecular level and their role in neuromodulation.

Attempts to isolate and purify membrane-bound opiate receptors in an active state have been plagued with problems.[1,4] Simon et al. found that preformed ligand–receptor complexes allowed receptor solubilization, but the dissociated receptor isolate was unable to bind opiate ligands.[4a] A receptor–enkephalin complex solubilized by Zukin and Kream, using the same procedure, was also inactive.[4b] More recently, active solubilized receptors have been reported by three research groups.[5] In 1977 we became interested in applying affinity-labeling methods, which had proved to be useful in the isolation of various biological macromolecules,[6] to the opiate receptor. Although several unsuccessful approaches to affinity labels for the opiate receptor had already been explored at that time,[7] we sought to synthesize a new series of compounds based on fentanyl (**1**) and its congeners [(+)-*cis*-3-methylfentanyl (**2**, R-26,800),[8] lofentanil (**3**, R-34,995),[9] and sufentanil (**4**, R-30,730)[9]], possessing either chemo- or



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