shown in Figure 2. On cochromatography with authentic samples, the radioactive metabolites were characterized as 2,4-diamino-10-deazapteroyl glutamate (peak 1), (2,4-diamino-10-deazapteroyl)glutamyl-γ-glutamate (peak 2), (2,4-diamino-10-deazapteroyl)glutamyl-γ-glutamyl-γ-glutamate (peak 3), and (2,4-diamino-10-deazapteroyl)-γ-glutamyl-γ-glutamyl-γ-glutamyl-γ-glutamate (peak 4). This experiment was repeated with radiolabeled 10-EDAAM under identical conditions. The results are shown in Figure 3.

Hydrolysis of Polyglutamyl Metabolites with Human Plasma Conjugase. The plasma used in this experiment was obtained from one of the investigators (N.T.N.). The second part of the homogenate containing the radioactive metabolites of either 10-DAAM or 10-EDAAM was diluted 10 times (120 mL) with distilled water and applied on a DEAE column. The column was washed with water (200 mL). The water washings were devoid of radioactivity. The column was eluted with 50 mL of 15% NH_4OH. More than 90% of the radioactivity that was present in the original sample was eluted in this fraction. The NH_4OH eluent was evaporated to dryness in vacuum, and the residue was dissolved in 1 mL of distilled water. To this sample was added 0.05 mL of 0.1 M sodium acetate buffer (pH 4.5), and the mixture was kept at 37 °C in a water bath. After 20 min, 0.05 mL of a preparation of plasma FPGH was added, and the mixture was diluted with 0.05 mL of distilled H_2O. The mixture was incubated for 24 h at 37 °C, and 0.05 mL of 10% TCA was added to precipitate the proteins. The mixture was then diluted with 10 mL of distilled water and centrifuged for 90 min. The supernatant was decanted and diluted with 50 mL of H_2O, the pH was adjusted to 7.3 with 0.1 N NaOH, and the resultant mixture was chromatographed on a DEAE-cellulose column. More than 95% of the radioactivity was found in a single fraction, which was identified to be either [^{14}C]-10-DAAM or [^{14}C]-10-EDAAM by comparison with authentic samples.

Molecular Structure of Fluoxetine Hydrochloride, a Highly Selective Serotonin-Uptake Inhibitor

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Fluoxetine, a selective inhibitor of serotonin uptake, is clinically useful in treating depression and may be useful for management of a variety of other psychiatric and metabolic derangements. Using X-ray crystallography, we have determined the three-dimensional structure of fluoxetine hydrochloride. A total of 2394 unique reflections were measured, and full-matrix least-squares refinement of all non-hydrogen coordinates and thermal parameters gave a final discrepancy index of 0.074 for 1759 observed reflections. In the solid state, the planes defined by the two aromatic rings are skewed, precluding the possibility of intramolecular ring–ring interactions. The methylene units of the methylpropanamine moiety adopt the anticipated conformational relationships to minimize torsional strain. An exact antiperiplanar relationship exists between N11 and C3; the N11–C1–C2–C3 dihedral angle is −180°. The C1–C2–C3–O4 dihedral angle is 60.6°, indicating that the propanamine side-chain folds toward the phenoxy moiety rather than adopting a fully extended conformation. This folded three-dimensional relationship may be necessary for high-affinity interaction with the serotonin-uptake carrier and confers considerable structural homology between this portion of fluoxetine and the phenylcyclohexylamine substructure of sertraline and EXP-561. However, the nature of substituents on the phenoxy portion of fluoxetine is also critical in determining potency and selectivity in this series of compounds.

Drugs that enhance serotonergic neurotransmission are useful or potentially useful in treating a variety of major psychiatric and metabolic derangements, including depression, eating disorders, alcoholism, pain, anxiety, and obsessive-compulsive behavior.[1] Serotonin released at synapses is actively removed from the synaptic cleft via a presynaptic serotonin transport carrier in an energy-dependent process, and this uptake is a rapid, efficient mechanism for physiological modulation of serotonin-mediated neurotransmission. Inhibitors of presynaptic reuptake augment physiological signals mediated by serotonin by increasing its availability in the synaptic cleft, thereby increasing postsynaptic receptor activation. The availability of a variety of compounds that selectively inhibit neuronal uptake of serotonin, without an effect on uptake of the catecholamines norepinephrine or dopamine, has been invaluable in elucidating the central role of serotonin in several physiological systems and pathophysiological states.[2]

One of the earliest selective inhibitors of serotonin uptake was fluoxetine (N-methyl-γ-[4-(trifluoromethyl)-

Table I. Crystal Data and Experimental Details for Analysis of Fluoxetine Hydrochloride

formula	$C_{17}H_{18}F_3NO \cdot HCl$
formula wt	345.8
space group	*Pcab*
a, Å	10.457 (2)
b, Å	10.387 (2)
c, Å	32.345 (6)
V, Å^3	3513.1 (1.4)
Z	8
d_{calcd}, g cm^{-3}	1.307
reflections measured	2394
observed reflections	1759
final *R*	0.074

phenoxy]benzenepropanamine; Chart I).[3,4] Fluoxetine is a selective and competitive inhibitor of serotonin uptake both in vitro and ex vivo; the selectivity for the serotonin-uptake carrier vs other monoamine-uptake carriers appear to be greater than 50-fold.[3,4] Fluoxetine antagonizes the neurotoxic effects of p-chloroamphetamine, a com-
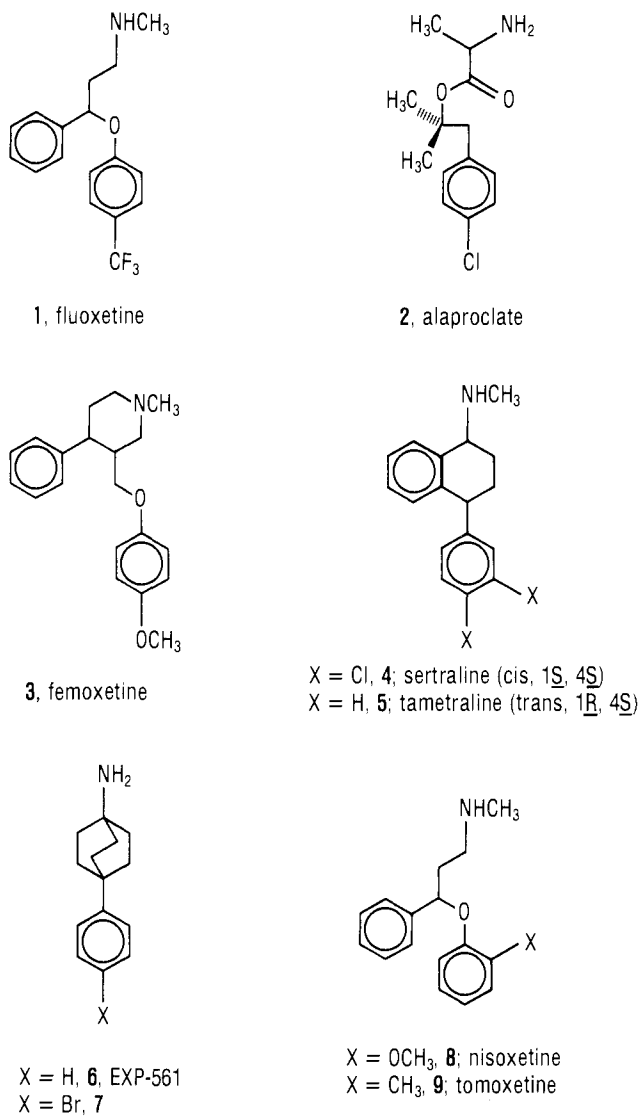
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Chart I

1, fluoxetine

2, alaproclate

3, femoxetine

X = Cl, 4; sertraline (cis, 1S, 4S)
X = H, 5; tametraline (trans, 1R, 4S)

X = H, 6, EXP-561
X = Br, 7

X = OCH₃, 8; nisoxetine
X = CH₃, 9; tomoxetine

Figure 1. Unit cell contents and molecular packing of fluoxetine hydrochloride.

Figure 2. Computer-generated ORTEP drawing of fluoxetine hydrochloride with crystallographic numbering system.

pound that depletes serotonin via a carrier-dependent mechanism.[4-6] Moreover, the pharmacologic actions of fluoxetine are consistent with a drug-induced enhancement of serotonin neurotransmission. For example, in humans fluoxetine is effective in the treatment of depression[7] and may be effective in the management of obesity;[8] in animal studies fluoxetine suppresses alcohol intake[9] and potentiates opiate-induced analgesia.[10,11]

To assist our further monoamine uptake inhibitor drug-design studies, we have mapped the three-dimensional structure of fluoxetine using X-ray crystallography. In this paper we detail these data and, in conjunction with classical structure-activity relationship (SAR) data, discuss some of the structural requirements for high-affinity, selective interaction with the serotonin-uptake carrier.

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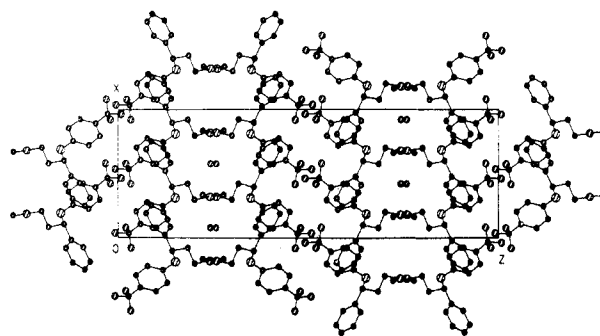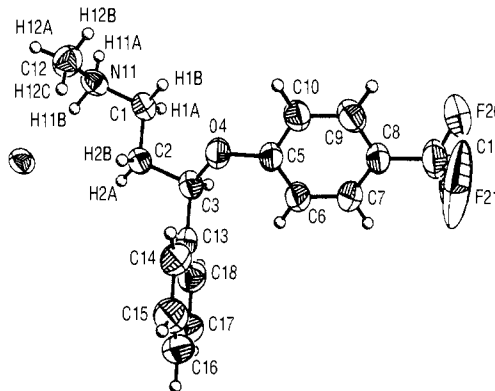(11) Hynes, M. D.; Lochner, M. A.; Bemis, K. G.; Hymson, D. L. *Life Sci.* 1985, *36*, 2317.

Results

Fluoxetine hydrochloride crystallized from water as colorless needles in the orthorhombic space group *Pcab*, and the unit cell constants are summarized in Table I. A total of 2394 unique reflections were measured, and the structure was solved by direct methods. Anisotropic temperature factors were assigned to all non-hydrogen atoms, while the hydrogen atoms were kept isotropic. Full-matrix least-squares refinement of all non-hydrogen coordinates and thermal parameters gave a final discrepancy index of 0.074 for 1759 observed reflections. Atomic coordinates, bond lengths, bond angles, anisotropic temperature factors, and hydrogen atom coordinates for fluoxetine hydrochloride are compiled in Tables III-VII, respectively (supplementary material). No unexpected bond lengths or angles were noted when the standard deviations were considered.

Figure 1 depicts the unit cell contents and molecular packing of fluoxetine hydrochloride. Each unit cell contains eight molecules, and the molecules are arranged in bilayers with the hydrophobic (trifluoromethyl)phenoxy and hydrophilic amine hydrochloride moieties juxtaposed to the corresponding regions of a second fluoxetine molecule. Figure 2 shows a computer-generated ORTEP drawing of fluoxetine hydrochloride as well as the crystallographic numbering system. Although these studies were performed on the racemate, for convenience only one enantiomer is depicted. In the solid state, the planes defined by the two aromatic rings are skewed, precluding the possibility of intramolecular ring-ring interactions. The H3-C3-C13-C18 dihedral angle is -19.9°, indicating the monosubstituted phenyl ring deviates only slightly from the plane defined by C13, C3, and H3. The relationship of this phenyl ring to the trifluoromethyl-substituted phenoxy ring is defined by the C13-C3-O4-C5 and C3-O4-C5-C6 dihedral angles, which are 75.8 and -14.5°, respectively. This conformation minimizes steric inter-
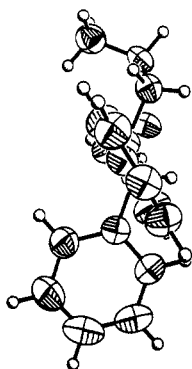
Figure 3. Computer-generated ORTEP drawing of fluoxetine hydrochloride. The view is essentially down the C8–C5–O4 axis, and the trifluoromethyl group has been deleted to permit observation of the skewed phenyl-ring planes. Note also the folding of the propanamine side chain toward the phenoxy moiety.

actions among C18, H3, H18, and H6. The distances from H6 to H3, H18, and C18 are 2.280, 2.811, and 2.795 Å, respectively. The relative orientation of the two phenyl rings is more easily observed from the computer-generated ORTEP drawing of fluoxetine when viewed down the C8–C5–O4 axis (Figure 3). In Figure 3, the trifluoromethyl group has been deleted to permit observation of the skewed phenyl-ring planes. There is no evidence for intramolecular stacking of the aromatic moieties as might be anticipated on the basis of bibenzyl studies,[12] and the distance from center to center of the aromatic rings is 5.079 Å.

Typical orientations of the nonaromatic portions of fluoxetine were observed. As is evident from Figure 2, the trifluoromethyl group appears to be disordered, and positions of the three fluorine atoms should be taken as only approximate. The methylene units of the methylpropanamine moiety adopt the anticipated staggered conformational relationships to minimize torsional strain. For example, H2A–C2–C3–H3, H1A–C1–C2–H2A, H1A–C1–N11–H11B, and H11A–N11–C12–H12A dihedral angles are 62.7, −62.1, 77.8, and 59.2°, respectively. An exact antiperiplanar relationship exists between N11 and C3; the N11–C1–C2–C3 dihedral angle is −180°. The C1–C2–C3–O4 dihedral angle is 60.6°, indicating that the propanamine side chain folds toward the phenoxy moiety rather than adopting a fully extended conformation. The distances from the centers of the phenoxy and phenyl rings to the amine are 6.697 and 6.195 Å, respectively.

Discussion

This X-ray structural determination adds to the group of selective serotonin-uptake inhibitors for which solid-state conformations are known. Previous reports have depicted the X-ray structures of zimelidine,[13] (R)-ala-proclate,[14] and femoxetine[15] (Chart I). Moreover, an X-ray crystallographic study of sertraline has been performed, although the data have not been published.[16]

A variety of techniques have been used to examine which structural features of uptake inhibitors are necessary for high-affinity, selective interaction with uptake carriers of serotonin and the catecholamines dopamine and nor-

Table II. In Vitro Inhibition of Serotonin (5-HT) and Norepinephrine (NE) Uptake in Crude Rat Brain Synaptosomes by Monoamine-Uptake Inhibitors

no.	in vitro IC_{50}, nM		selectivity: NE/5-HT	ref
	5-HT	NE		
1	70	>10000 (10)[a]	>143	4
2[b]	700	>34000	>49	18
3	8.3	410	49	37
4	60	1200	20	16
5	840	18	0.02	16
6	95	80	0.84	30
7	680	54000	79	30
8	2000	10	0.005	32
9	1500	4	0.003	32
10	3000	>10000 (26)	>3	32

[a] Values in parentheses represent percent inhibition of uptake at the maximum concentration tested. [b] Data were obtained from brain slices rather than synaptosomes.

epinephrine.[17–21] One of the pioneering, and perhaps most convincing, studies of this type was that by Sarges and co-workers, in which the structural features of tametraline ((+)-trans-(1R,4S)-N-methyl-4-phenyl-1-aminotetralin; compound 5, Chart I), a sertraline progenitor, were compared to those of the tricyclic antidepressants through the use of classical SAR and molecular modeling techniques.[22] From the structural resemblance of tametraline with desipramine, these workers hypothesized that the active conformation of the tricyclics was that in which the aminoalkyl side chain was folded toward one of the aromatic rings. This hypothesis was supported by the potent uptake inhibition observed with the nontricyclic compound 1-amino-4-phenylbicyclo[2.2.2]octane (compound 6, Chart I; EXP-561), suggesting that a key feature for uptake inhibition involved a phenylbutylamine, with the proper spatial orientation between the amine and the aromatic ring. This work was subsequently extended by Koe to include possible topological overlap of a variety of antidepressants with one another.[23]

From the X-ray data described herein for fluoxetine, it is apparent that the (trifluoromethyl)phenoxypropanamine moiety adopts a folded orientation; a synclinal conformation exists about the C2–C3 bond. This folded three-dimensional relationship between the alkylamine moiety and the trifluoromethyl-substituted phenoxy portion of fluoxetine may be necessary for high-affinity, selective interaction with the serotonin-uptake carrier and confers considerable structural homology between this portion of fluoxetine and the phenylcyclohexylamine substructure of the phenylaminotetralins (e.g., 5) and compound 6. Since there is a close three-dimensional structural relationship among 6, tametraline, and fluoxetine, why is the last compound selective for inhibition of serotonin uptake whereas the former compounds have considerable affinity for the norepinephrine-uptake carrier (Table II, note the NE/5-HT selectivities)? Some of the answer may be subtle differences in conformations among these compounds, particularly in solution, but the most cogent ex-

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planation is the effects of substituents on the phenoxy ring. From classical SAR studies it was noted that para substituents, particularly the strongly electronegative and hydrophobic trifluoromethyl substituent (σ_p = 0.54; π = 0.88), is primarily responsible for the selectivity of fluoxetine for the serotonin-uptake carrier.[4] In fact, by removal of this substituent and addition of either a methoxy or a methyl substituent in the ortho position [nisoxetine (8) and tomoxetine (9), respectively; Chart I], the selectivity of the compound is changed such that inhibition of norepinephrine uptake becomes the predominant pharmacological action.[24-27] It should be noted that, in addition to the electronic effects of these ortho substituents, they may alter the spatial relationship of the two aromatic rings,[28] and we are currently investigating the nature and magnitude of conformational differences between the solid-state conformations of tomoxetine and fluoxetine.

Conferring high degrees of potency and selectivity for the serotonin-uptake site by lipophilic, electronegative substituents has been noted in several other series of compounds. For example, compound 6 inhibited serotonin and norepinephrine uptake in rat brain synaptosomes with IC_{50} values of 9.5 × 10^{-8} and 8 × 10^{-8} M, respectively. Compound 7, the *p*-bromo analogue of 6, inhibited serotonin and norepinephrine uptake with IC_{50} values of 6.8 × 10^{-7} and 5.4 × 10^{-5} M. Thus, the *p*-bromo substituent increased serotonin selectivity approximately 95-fold since the affinity of 7 for the norepinephrine-uptake carrier was attenuated to a much greater degree than affinity for the serotonin-uptake carrier (Table II).[29,30] IC_{50} values for the in vitro inhibition of serotonin accumulation by the *p*-chloro compound alaproclate (2) and its deschloro congener were reported to be 0.7 and 2.7 μM, respectively, indicating this para substituent increased potency 4-fold. Moreover, the substituent increased selectivity (serotonin vs norepinephrine uptake inhibition) 6-fold.[18] Finally, in the 1-amino-4-phenyltetralin series, Welch and co-workers reported that 4-chloro substitution, and especially 3,4-dichloro substitution, enhanced potency for the serotonin-uptake carrier dramatically.[16] Most remarkable was the fact that 3,4-dichloro substitution was able to transform the relatively inactive *cis*-1-amino-4-phenyltetralin to the highly potent serotonin-uptake inhibitor sertraline [4, Chart I; (+)-*cis*-1S,4S isomer]; IC_{50} values for inhibition of serotonin uptake for these compounds were 3.5 and 0.06 μM, respectively, a 58-fold substituent-induced increase in potency.[16] Moreover, this substitution pattern increased the norepinephrine/serotonin selectivity 39-fold.[16]

In addition to these substituent effects, the spatial relationship of the amine group to the plane defined by the trifluoromethyl-substituted phenoxy ring may enhance the selectivity of fluoxetine for the serotonin-uptake site. In 6, the phenyl ring and the amine are constrained to be coplanar by the rigid bicyclo[2.2.2]octane system, and the compound is a rather balanced inhibitor of both serotonin

and norepinephrine uptake (selectivity for serotonin uptake is 0.84, Table II). The amine of fluoxetine lies out of the trifluoromethylphenyl plane, and this is easily observed from the orientation depicted in Figure 3; this nonplanar relationship may maintain or accentuate fluoxetine's affinity for the serotonin-uptake carrier while attenuating its ability to inhibit the norepinephrine carrier. As judged from molecular models and the calculated conformation of sertraline,[31] the amine of this inhibitor is maintained in a pseudoaxial orientation and lies even further out of the nonfused phenyl-ring plane than is the case with fluoxetine. When examined by the same laboratory in cortical synaptosomes, the NE/5HT selectivity ratios of 6, fluoxetine, and sertraline were 0.84, 35, and 50, respectively.[32] These data also lend credence to the observations that the norepinephrine-uptake site has rigid requirements for the proper spatial orientation between the aromatic nucleus and the amine moiety of inhibitors, whereas the serotonin-uptake site accepts a wider range of spatial orientations.[16,19] For example, in the 4-phenyl-1-aminotetralins, appropriately substituted cis and trans isomers both inhibit the serotonin-uptake carrier, whereas the norepinephrine-uptake site is most profoundly affected by the trans series.[16]

Although the folded [(trifluoromethyl)phenoxy]-propylamine moiety of fluoxetine appears to play a crucial role in the expression of its pharmacology, the 3-phenyl group is also critical. In vitro studies employing rat brain synaptosomal preparations indicated that serotonin uptake inhibition IC_{50} values for fluoxetine and the analogous desphenyl compound, *N*-methyl-3-[4-(trifluoromethyl)-phenoxy]propanamine (10) were 70 and 3000 nM, respectively, a 62-fold phenyl-induced increase in potency (Table II). One logical explanation for the phenyl-ring enhancement of potency would be its interaction with an hydrophobic pocket on the serotonin-uptake carrier. It should also be noted, however, that *both* enantiomers of fluoxetine are potent and selective serotonin-uptake inhibitors; in vitro studies indicated that the dextrorotatory isomer is only about 50% more potent than the levorotatory isomer (K_i values = 21 and 33 nM, respectively).[33] Moreover, in vivo experiments in rats indicated that the dextrorotatory antipode of fluoxetine was only slightly more potent in antagonizing the depletion of brain serotonin concentrations by *p*-chloroamphetamine.[34] If the aforementioned hypothesis is correct, and a phenyl-uptake carrier hydrophobic interaction is important, then it is enigmatic that the magnitude of this interaction could be similar for both enantiomers.

An alternative and perhaps more plausible explanation of the phenyl-induced enhancement of potency of 10 is an alteration in the three-dimensional orientation between the propanamine and (trifluoromethyl)phenoxy moieties. The steric effects of the phenyl group may be responsible for adoption of the folded orientation between the two aforementioned moieties, rather than a more fully extended orientation. This conformational effect would not be stereodependent, and thus would explain the similar bioactivities of the two fluoxetine enantiomers. Moreover, it would explain the high potency of both fluoxetine and 6 as serotonin-uptake inhibitors (IC_{50} values = 70 and 95

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nM, respectively, Table II) even though 6 possesses no analogous phenyl ring; this conformational anchor is not necessary in 6 since the rigid bicyclo[2.2.2]octane enforces the proper spatial relationship between the aromatic ring and the alkylamine. We are currently conducting substituent studies on this phenyl ring of fluoxetine, as well as replacing it with other sterically demanding groups, to determine the importance of steric bulk, hydrophobicity, and electron density in this region of fluoxetine.

Grunewald and Creese recently described their calculations on the theoretically preferred conformation of fluoxetine.[28] They concluded that fluoxetine has a sharply defined global minimum, and approximately 8 kcal/mol are required to access other local minima. Their calculations are interesting, particularly with respect to the side-chain conformation of fluoxetine. The calculations predicted an approximate C1–C2–C3–O4 dihedral angle of 60°, and this angle in the solid state was found to be 60.6°. However, whereas we found an antiperiplanar relationship between N11 and C3, the CAMSEQ calculations predicted a synclinal orientation; the N11–C1–C2–C3 dihedral angles were −180 and 300°, respectively. The synclinal orientation predicted by Grunewald appears to permit a more favorable overlap between the phenoxy, dichlorophenyl, and amine moieties of fluoxetine and sertraline, but extensive molecular modeling studies are required to determine the extent of the overlap and the energetic differences between the antiperiplanar and synclinal conformations about the C1–C2 bond of fluoxetine.

In this discussion we have hypothesized that the [(trifluoromethyl)phenoxy]propanamine portion of fluoxetine and the synclinal orientation about the C2–C3 bond are key factors in the potency and selectivity of this agent as a serotonin-uptake inhibitor. However, it should be noted that the conformation of the phenylpropylamine moiety of fluoxetine bears a modest resemblance to the side-chain conformation of serotonin, as determined by X-ray crystallography and molecular orbital calculations.[35,36] In these studies the side chain of serotonin was perpendicular to the plane of the indole ring and adopted either an antiperiplanar conformation or a synclinal orientation such that the aliphatic nitrogen projected toward C-2 of the indole ring. The C1–C2–C3–C13 and H3–C3–C13–C18 dihedral angles in fluoxetine are −175.7 and −19.9°, respectively, indicating the propanamine side chain does project almost directly away from the plane of the phenyl ring, and it is possible to achieve reasonable overlap between the phenyl rings and aliphatic amines of serotonin and fluoxetine. Thus, regions of fluoxetine *may* resemble serotonin, and other workers, using a variety of compu-

tational techniques, have stressed the possible structural overlaps of natural uptake carrier substrates (e.g., serotonin, norepinephrine, and dopamine) with those of uptake inhibitors.[18,19] It should be noted that although fluoxetine is a competitive inhibitor of serotonin uptake and interacts with the same portion of the carrier responsible for the transport of serotonin, the structural features responsible for substrate–carrier recognition may be different from those responsible for inhibitor–carrier recognition. Additional studies will be required to determine whether the structural overlap between fluoxetine and serotonin is biochemically and pharmacologically meaningful.

In conclusion, the key conformational findings from this X-ray analysis of fluoxetine are (1) the planes defined by the two aromatic rings are skewed, precluding the possibility of intramolecular ring–ring interactions, (2) an exact antiperiplanar relationship exists between N11 and C3, and (3) a synclinal orientation exists about the C2–C3 bond such that the propanamine side chain folds toward the phenoxy moiety. Further studies are needed to define the extent and pharmacological relevance of the overlap among this three-dimensional conformation of fluoxetine, the conformations of other serotonin-uptake inhibitors, and the various conformations of serotonin.

Experimental Section

X-ray Crystallography. Fluoxetine hydrochloride crystallized from water as colorless needles in the orthorhombic space group *Pcab*, with eight molecules in a unit cell having the dimensions $a = 10.457$ (2) Å, $b = 10.387$ (2) Å, $c = 32.345$ (6) Å; calculated density was 1.307 g cm^{-3}.

Intensities of 2394 unique reflections with 2θ less than 116.0° were measured in the automated θ–2θ scan mode on a Nicolet P3F four-angle diffractometer using monochromatic copper radiation. Positions of the atoms were obtained by interpretation of an E map phased by the direct methods routine SOLV of the SHELXTL program.[38] The structure was refined by the least-squares method with anisotropic temperature factors for all atoms except hydrogens, which were included at calculated positions with isotropic temperature factors. The final R factor was 0.0738 for 1759 observed reflections. Tables III–VII (supplementary material) contain atomic coordinates, bond lengths, bond angles, anisotropic temperature factors, and hydrogen atom coordinates, respectively.

Supplementary Material Available: Atomic coordinates, bond lengths, bond angles, anisotropic temperature factors, and hydrogen atom coordinates for fluoxetine hydrochloride (Tables III–VII, respectively) (5 pages). Ordering information is given on any current masthead page.

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