Fluoxetine (Prozac) in Chemical Neuroscience: A Classic Examination

Dr M KUMARA SWAMY[1], SHAIK SHAIKRA BEGUM[2], K SWATHI[3]

1,2 & 3, Associate Professor, CHEMISTRY department, Brilliant Institute of Engineering & Technology, Hyderabad, TS.

ABSTRACT:

The introduction of fluoxetine (Prozac) marked a pivotal milestone in the treatment of depression, being the first major advancement since monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs) were developed approximately thirty years earlier. Fluoxetine is recognized for its reduced side effects in comparison to TCAs and MAOIs, making it the first selective serotonin reuptake inhibitor (SSRI) approved by the US Food and Drug Administration. While the exact mechanisms behind fluoxetine's effectiveness in treating depression remain a topic of ongoing research, its importance, along with that of similar SSRIs, is widely acknowledged in the field. The brand name Prozac has gained considerable recognition, playing a role in diminishing the stigma surrounding depression and enhancing public awareness of the disorder. This review will explore fluoxetine's synthesis, pharmacology, drug metabolism, side effects, and historical context, as well as its significant impact on depression treatment and the broader realm of neuroscience.

KEYWORDS: depression, SSRI, serotonin, fluoxetine, Prozac, antidepressant, and

INTRODUCTION

Depression is a widespread and often chronic psychiatric condition characterized by persistent low mood, diminished self-esteem, and a decreased interest or pleasure in most activities. Currently, over 120 million people around the globe are affected, which includes approximately 1 in 10 Americans. The incidence of diagnosed cases is rising at an alarming rate of about 20% annually. The prevalence and severity of depression contribute to significant socioeconomic challenges, with estimated costs exceeding $63 billion per year for U.S. businesses due to low productivity, absenteeism, and treatment expenses.

Depression typically emerges between the ages of 15 and 30, with another incidence peak occurring between 30 and 45 years; however, it can develop at any stage of life. It is vital to recognize that not all instances of depression qualify as psychiatric disorders. Depressive symptoms can arise from specific life events, medical interventions, or as a consequence of non-psychiatric illnesses. Nevertheless, major depressive disorder (MDD) is a debilitating condition that has far-reaching effects on an individual's family life, work performance, sleep and eating behaviors, and overall health. Alarmingly, over 5% of individuals diagnosed with MDD take their own lives, with nearly 60% of suicides in the United States being attributed to patients suffering from depression or related mood disorders.

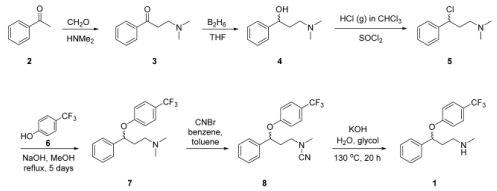
Sadly, many people with depression experience stigma, which often prevents them from seeking help. This reluctance, combined with the absence of validated biological markers for the condition, contributes to the estimate that around 80% of those experiencing clinical depression are currently untreated. The understanding of depression has ancient roots, with its descriptions dating back to the Greek physician Hippocrates, who

referred to it as melancholia, indicative of “black bile,” deriving from the historical concept of the four bodily humors. In his classic text, *Aphorisms*, Hippocrates identified long-lasting "fear and despondencies" as melancholia. This term maintained presence in medical literature until the 17th century, when Delasiauve introduced the term “depression” (from the Latin *deprimere*, meaning "to press down") to describe the reduction of emotional functions.

This terminology gained traction and was officially included in the Diagnostic and Statistical Manual of Mental Disorders (DSM-I) in 1952. The specific designation of “major depressive disorder” was added to the DSM-III in 1980. Diagnostic criteria for depression are outlined in the DSM-5 and the World Health Organization’s (WHO) ICD-10. The latter uses the term “depressive episode” for individual instances and “recurrent depressive disorder” for repeated episodes. According to the ICD-10, three symptoms—depressed mood, anhedonia, and reduced energy—are necessary for diagnosis, with two symptoms required to confirm the condition. Conversely, the DSM-5 stipulates that only two symptoms (depressed mood and anhedonia) need to be present, allowing for diagnosis with just one of these. Major depressive disorder, also known as clinical depression or recurrent depression, is defined by a single or recurrent episode of markedly depressed mood lasting a minimum of two weeks.

Furthermore, the DSM-5 categorizes MDD into five subtypes: melancholic depression, atypical depression, catatonic depression, postpartum depression, and seasonal affective disorder. Treatment approaches for MDD can be classified into three primary categories: psychotherapy (including cognitive behavioral therapy and interpersonal therapy), electroconvulsive therapy, and antidepressant medications. Among the various medications approved for treating MDD, fluoxetine (Prozac) is perhaps the most recognized. This review will delve into the significance of fluoxetine in the management of depression and its application in other central nervous system disorders.

Scheme 1. Original Lilly Synthesis of Racemic Fluoxetine (1) by Molloy and Schmiegel from 1982



CHEMICAL SYNTHESIS

Fluoxetine, formally known as (R,S)-N-methyl-3-phenyl-3-(4-(trifluoromethyl)phenoxy)propan-1-amine (CAS No: [54910-89-3]), is a low molecular weight racemic phenoxyphenylpropylamine with a molecular weight of 309.3 g/mol. It features a single hydrogen bond donor, potentially two hydrogen bond acceptors, and a cLogP of 4.2, thus adhering to Lipinski’s rules and demonstrating favorable drug metabolism and pharmacokinetics (DMPK) as well as good central nervous system (CNS) penetration. The original synthetic pathway for racemic fluoxetine was described by Molloy and Schmiegel in 1982 (filed in 1974) in the patent US 4,314,081 (Scheme 1).

The synthesis commenced with a Mannich reaction using acetophenone (2) to yield β-dimethylaminopropiophenone (3) as an oil. This intermediate was then dissolved in THF and added slowly to a THF solution of 4 equivalents of diborane, allowing it to stir overnight. A further equivalent of

diborane was introduced, promoting additional stirring overnight, leading to the formation of the racemic secondary alcohol (4) following an acidic workup. The alcohol (4) was subsequently dissolved in CHCl3 and saturated with anhydrous HCl gas, with the careful addition of SO2Cl maintaining reflux for approximately 5 hours. After solvent evaporation, the crystalline hydrochloride salt (5) was obtained. This was then reacted with an alkaline solution of (6) and refluxed for five days to produce the phenoxy ether (7). The classical Von Braun degradation of the dimethylamino group was executed through the formation of the N-cyano derivative (8) and subsequent hydrolysis, ultimately yielding racemic fluoxetine (1) as a free base.

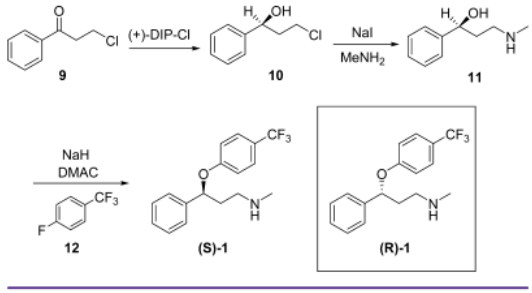
The first salt form of fluoxetine tested for serotonin reuptake in the early 1970s was the oxalate salt; however, the commercially available form is the hydrochloride salt known as fluoxetine hydrochloride (also marketed as Prozac). As a racemate, compound (1) has a reported Ki value of 17 nM for serotonin uptake in rat brain synaptosomes in vitro. Prior studies identified the eudismic ratio (the ratio of affinities or activities of enantiomers) of (1) to be close to unity, with a ratio of (R):(S) being 48:52. This prompted Lilly researchers, led by Robertson, to explore the pharmacological profiles of the individual (R)- and (S)-enantiomers of fluoxetine, specifically (R)-1 and (S)-1.

Leveraging pioneering work in asymmetric reduction chemistry from the Brown lab, Robertson's team facilitated the rapid synthesis of chiral alcohols with high enantiomeric purity. In Scheme 2, the reduction of 3-chloro-1-phenylpropan-1-one (9) with (+)-diisopinocampheylchloroborane ((+)-DIP-Cl) produced the (S)-alcohol (10) in significant enantiomeric excess. Displacement of the chloride with methylamine presented challenges, leading to the application of a Finkelstein reaction to prepare the iodide in situ, followed by successful methylamine displacement to yield (11). The resulting compound was deprotonated with NaH in DMAC, then treated with 1-fluoro-4-(trifluoromethyl)benzene (12) to achieve (S)-fluoxetine ((S)-1) with a substantial 96:4 S:R enantiomeric ratio. Since only (+)-DIP-Cl was available, this asymmetric synthesis enabled access solely to (S)-fluoxetine.

To obtain (R)-fluoxetine (R)-1, Robertson and colleagues utilized classical resolution methods, specifically fractional recrystallization of the D- and L-mandelic acid salts of racemic (1). After conversion to corresponding (R)-1-(1-naphthyl)ethylureas, HPLC and NMR confirmed high enantiomeric purity, achieving (S)-fluoxetine (S)-1 in a >99:1 S:R ratio and (R)-fluoxetine (R)-1 in a 1.5:98.5 ratio. Notably, both enantiomers exhibited comparable potency in vitro, with Ki values of 21 nM for (S)-1 and 33 nM for (R)-1, and were similarly effective across various in vivo preclinical models.

This initial research initiative set in motion numerous asymmetric synthesis strategies for (S)-1 and (R)-1, utilizing more versatile catalysts capable of producing both enantiomers from the prochiral ketone (9). Other asymmetric methodologies have included Sharpless asymmetric epoxidation, hydroxylation, oxidative kinetic resolution, chiral carbonyl-ene reactions, and ruthenium-catalyzed allylic alkylation, among others. Enzymatic methods, such as reduction and lipase-mediated resolutions, have also been employed to establish chirality in

benzyl alcohol. Recently, flow chemistry techniques have been applied in the preparation of fluoxetine (1) as well.



The Finkelstein reaction was utilized to create the corresponding iodide in situ, which was then subjected to displacement with methylamine to form compound (11). This compound underwent deprotonation with sodium hydride (NaH) in DMAC, followed by the addition of 1-fluoro-4-(trifluoromethyl)benzene (12) to yield (S)-fluoxetine ((S)-1) in a significant 96:4 S:R enantiomeric ratio. Given that only (+)-DIP-Cl was available for the synthesis, this method restricted the production to (S)-fluoxetine.

To synthesize (R)-fluoxetine ((R)-1), Robertson and his team employed classical resolution techniques, specifically fractional recrystallization of the D- and L-mandelic acid salts derived from racemic (1). After conversion to the corresponding (R)-1-(1-naphthyl)ethylureas, HPLC and NMR analyses confirmed that (S)-fluoxetine ((S)-1) was achieved in a >99:1 ratio of S:R enantiomers, whereas (R)-fluoxetine ((R)-1) was produced in a 1.5:98.5 ratio of S:R enantiomers. Notably, both enantiomers exhibited nearly equivalent potency in vitro, with Ki values of 21 nM and 33 nM for (S)-1 and (R)-1, respectively, and both were similarly effective across various in vivo preclinical models.

This initial work led to the development of a variety of asymmetric synthesis methods for (S)-1 and (R)-1, utilizing more versatile catalysts capable of generating both enantiomers through the reduction of prochiral ketone (9). Other asymmetric approaches included Sharpless asymmetric epoxidation, Sharpless asymmetric hydroxylation, oxidative kinetic resolution, asymmetric carbonyl-ene reactions, and ruthenium-catalyzed allylic alkylation, among others. Additionally, chirality in the benzyl alcohol was achieved through enzymatic reduction and lipase-mediated enzymatic resolution. Recently, flow chemistry techniques have also been applied to the synthesis of fluoxetine (1).

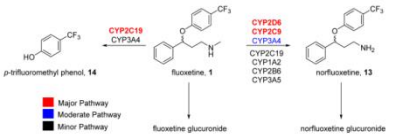


Figure 1.Structures of the oxidative and conjugative metabolites of fluoxetine 1. The major metabolite is N-desmethylfluoxetine 13, equipotent to 1, but with a significantly longer half-life. The phenolic metabolite 14 is inactive. The single enantiomers (S)-1 and (R)-1 showed divergent 2D6 metabolism.

MANUFACTURING INFORMATION

Fluoxetine, the generic name for the drug (1), is produced by Eli Lilly & Co. under the brand name Prozac, though it is also marketed under various other names, including Zactan, Lovan, Fludac, Flutine, Fluoxin, Philozac, Fluxil, Fontex, and several others. Fluoxetine was first synthesized in 1971 and disclosed in 1974 under LY110140. It received approval from the United States Food and Drug Administration (FDA) on December 29, 1987, and was launched commercially as Prozac in January 1988. Eli Lilly offers fluoxetine in 10 mg, 20 mg, and 40 mg tablets, which are yellow and pale green, in addition to a 20 mg/5 mL oral syrup.

Numerous generic manufacturers produce bioequivalent fluoxetine in 10−40 mg doses, including Sandoz (approved in 2001), Dr. Reddy's Lab (approved in 2001), Teva (approved in 2002), Mylan (approved in 2002), Mallinckrodt (approved in 2002), Heritage Pharmaceuticals (approved in 2012), Alembic Pharmaceuticals (approved in 2009), Aurobindo Pharma (approved in 2008), among many others. By 2005, Prozac had been prescribed to over 40 million patients globally, generating sales exceeding $22 billion. Annual sales peaked in 1998 at $2.8 billion; however, following the patent expiration in 2001, Eli Lilly experienced a loss of $35 million in market value in a single day, and approximately 90% of Prozac prescriptions were filled by generic alternatives within the first year of generic competition.

Although obtaining current and precise sales figures for fluoxetine presents challenges, global sales are estimated to surpass $400 million. In 2010, over 24 million generic prescriptions for fluoxetine were filled in the United States, alongside approximately 6 million in the United Kingdom.

DRUG METABOLISM

Fluoxetine (1) exhibits nearly complete absorption following oral administration, with a bioavailability percentage (%F) ranging from 70% to 90%. It demonstrates high central nervous system (CNS) penetration, with a brain-to-plasma ratio in humans of 2.6:1, and possesses the largest volume of distribution (Vd) among selective serotonin reuptake inhibitors (SSRIs), estimated between 14 and 100 L/kg. Fluoxetine also shows low plasma protein binding (Fu = 0.05) and a prolonged half-life, ranging from 1 to 3 days for acute dosing and 4 to 6 days for chronic dosing. Due to this extended half-life, fluoxetine requires between 1 and 22 months to reach a steady state in the body.

Upon administration, fluoxetine is extensively metabolized in the liver by cytochrome P450 enzymes, leading to the formation of several metabolites (Figure 1) and exhibiting nonlinear pharmacokinetics. Approximately 80% of fluoxetine is excreted either as the parent compound (1), N-desmethylfluoxetine (norfluoxetine, 13), or as glucuronides of both (1) and (13). The metabolites have been well-characterized; the phenolic metabolite (14), which is produced through oxidative O-dealkylation primarily by CYP2C19 and CYP3A4, is inactive. In contrast, norfluoxetine (13), mainly produced by CYP2D6 (with contributions from CYP2C9, CYP3A4, and others), exhibits pharmacological activity comparable to fluoxetine but has a notably longer half-life (t1/2 = 4–16 days). Plasma concentrations of norfluoxetine are typically 100% to 130% of those of fluoxetine, and levels of both compounds can remain detectable for over three weeks after treatment discontinuation.

The significant role of CYP2D6 in the metabolism of fluoxetine was highlighted in studies of individuals with poor 2D6 metabolizer status, who presented elevated levels of fluoxetine, while extensive 2D6 metabolizers demonstrated lower levels. This interaction is particularly important since fluoxetine is both a substrate for and an inhibitor of CYP2D6, while norfluoxetine (13) is a substrate for and inhibitor of CYP3A4. Thus, fluoxetine has substantial potential to cause pharmacokinetic drug-drug interactions with a variety of medications, including atypical antipsychotics (such as clozapine, olanzapine, and risperidone), opiates, other antidepressants (including tricyclic

antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and additional SSRIs), as well as benzodiazepines.

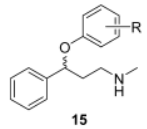
Overall, N-dealkylation is the primary clearance mechanism for fluoxetine. As a racemic mixture, investigations into the metabolism of the individual enantiomers, (S)-fluoxetine (S-1) and (R)-fluoxetine (R-1), revealed that while both enantiomers are nearly equipotent in blocking serotonin reuptake, (S)-1 shows only 1.5 times the potency of (R)-1; however, the norfluoxetine metabolite (S)-13 is 5- to 20-times more potent than (R)-13. The metabolism of (S)-1 and (R)-1, as well as (S)-13, is highly dependent on CYP2D6, whereas (R)-13 metabolism is less variable and not significantly influenced by CYP2D6.

Research assessing the contributions of various CYP enzymes to the metabolism of fluoxetine and its enantiomers revealed significant differences in their metabolic processes. This information prompted discovery programs between Eli Lilly and Sepracor for the individual enantiomers. However, while exploring (R)-1 as a potential antidepressant, concerns about its cardiotoxicity arose, leading to the cessation of these efforts. As of 2002, initiatives to investigate (S)-1 for migraine treatment also appeared to have been discontinued.

MEDICINAL CHEMISTRY, SAR, AND PHARMACOLOGY

After the antidepressant activities of TCAs were discovered, the pharmacological basis of this action was determined to be potent inhibition of monoamine uptake.49−54 Carlsson and coworkers noted that subtle structural modification among TCAs resulted in dramatic differences in serotonin (5-HT) and norepinephrine (NE) uptake in brain slices.53,54 Against this backdrop, Lilly scientists Molloy, Fuller, Rathburn, and Wong initiated a campaign to identify novel antidepressant agents lacking the side-effect profile of TCAs.17,18,21 To access novel chemical space, Molloy then employed a phenoxyphenylpropyl amine (PPA) core from which to develop analogues; moreover, Wong postulated, based on the observations of Carlsson with TCAs, that subtle structural changes within the PPA series might engender selective 5-HT uptake. Molloy then synthesized approximately 60 PPA analogues, which were found by Wong to inhibit 5-HT and/ or NE uptake in synaptosomal preparations, an activity that was confirmed in vivo by Fuller and co-workers.21,22,24 As theorized, subtle structural changes did engender dramatic variations in monoamine uptake selectivity, and Table 1 highlights key

Table 1. Structures and SAR of PPA Analogues 15[a]



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		inhibition of uptake (K_i, nM)	
compd	R	5-HT	NE
15a	H	102	200
15b	2-OCH_3	1371	2.4
15c	2-CH_3	390	3.4
15d	2-F	898	5.3
15e	2-CF_3	1498	4467
15f	3-CF_3	166	1328
15g (1)	4-CF_3	17	2703
15h	4-CH_3	95	570
15i	4-OCH_3	71	1207
15j	4-Cl	142	568
15k	4-F	638	1276

[a]Uptake inhibition data as reported by Wong et al.[19]

structure−activity relationships (SARs). The parent PPA 15a (LY86032) was a potent 5-HT/NE uptake inhibitor (NE IC50 = 200 nM, 5-HT IC50 = 102 nM), while the addition of a 2- OMe moiety afforded 15b (LY94939, nisoxetine), a highly selective NE uptake inhibitor (NE IC50 = 2.4 nM, 5-HT IC50 =1.37 μM). Of the analogues screened, 1 (LY82816, fluoxetine oxalate) was the most potent

and selective 5-HT uptake inhibitor (NE IC50 = 2.7 μM, 5-HT IC50 = 17 nM, >150-fold selective). In this same assay, the N-desmethyl metabolite norfluoxetine 13 displayed equivalent potency and selectivity to 1 (NE IC50 = 2.2 μM, 5-HT IC50 = 17 nM, >125-fold selective). From this point on, studies with 1 were performed on the HCl salt form (LY110140), and 1 was further advanced as a putative candidate.20 Despite screening only a small library of compounds by today's standards, these efforts yielded a candidate that eventually entered the therapeutic marketplace as fluoxetine (Prozac).18 The single enantiomers of fluoxetine, (S)-1 and (R)-1 also displayed comparable potencies in this assay (5-HT IC50's of 16 and 21 nM, respectively); however, the single enantiomers of norfluoxetine, (S)-13 and (R)-13, showed differential activity, with (S)-13 having a 14-fold higher potency than (R)-13 (5-HT IC50's of 20 and 268 nM, respectively). Whereas the earlier TCAs possessed significant activity at adrenergic, muscarinic, opiate, dopamine, GABA, and histamine receptors, leading to adverse events, 1 was generally clean versus these key antitargets: α1-adrenergic (21 μM), α2- adrenergic (22 μM), β-adrenergic (>10 μM), H1 (1.9 μM), M3 (6.6 μM), opiate (>10 μM), GABA (>10 μM), and D2 (2.1 μM).17−23 However, both 1 and 13 do exhibit relatively strong affinities for the 5-HT2A and 5-HT2C receptors. Over the years, numerous reports on the full pharmacology of 1 and 13 have been disclosed; however, in order to allow direct comparisons under standard assay conditions and uniform cell lines, we present recent data from the NIMH Psychoactive Drug Screening Program (Table 2).55

Table 2. Pharmacological Profile of Fluoxetine (1) and *N*-Desmethylfluoxetine (13)[a]

	K_d (nM)	
protein target	fluoxetine (1)	norfluoxetine (13)
SERT	2	38
DAT	6670	4102
NET	1560	6838
5-HT_{2A}	246	295
5-HT_{2B}	>10 000	5063
5-HT_{2C}	398	91
α_1	2262	3900
α_2	3090	>10 000
M_1	702	1200
M_2	2700	4600
M_3	1000	760
M_4	2900	2600
M_5	2700	2200
H_1	1240	>10 000
H_3	7300	>10 000

[a] K_d values as determined by the NIMH Psychoactive Drug Screening Program, http://pdsp.med.unc.edu/pdsp.php (accessed June 22, 2013).

Neurochemical studies demonstrated that, after administration of 1, extracellular 5-HT concentrations were increased 1.5- to 4-fold across multiple brain regions.56,57 In addition to heightened 5-HT concentrations, a concomitant decrease in the synthesis and release of 5-HT, as well as 5-HIAA, was observed.24 Therefore, administration of 1 appears to result in a feedback mechanism to reduce 5-HT turnover.56,57 Many reviews have focused on the preclinical behavioral pharmacology of 1; therefore, we will only list key findings here. Administration of 1 has been shown to suppress feeding, attenuate aggression, reduce amphetamine self-administration, diminish compulsive behaviors, and induce an analgesic response. Fluoxetine has shown efficacy in multiple rodent models of depression, including learned helplessness and social isolation models, as well as in the forced swim and tail suspension tests.18,19 Interestingly, the inhibition of 5-HT uptake by 1 occurs immediately upon accessing SERT, but full antidepressant efficacy is not acquired for 3−6 weeks.58 Thus, the mechanism of action of 1 cannot be attributed exclusively to the acute elevation of 5-HT concentrations; in addition, more than 50% of preclinical studies fail to demonstrate elevated 5-HT

levels after chronic administration with 1 or other SSRIs, suggesting other adaptive mechanisms.59,60 This temporal discrepancy has led to many hypotheses to account for the antidepressant activity of 1 and other SSRIs. For example, down-regulation of other 5-HT receptor subtypes, including as 5-HT1A and 5-HT2C, downstream neural adaptations, such as changes in the brain-derived neurotrophic factor (BDNF)-TrkBsignaling pathway, decreases in plasma glutamate concentrations with concomitant up-regulation of forebrain glutamate receptor subunits, and increases in neurosteroid concentrations, such as 3-α-hydroxy-5-α-pregnane-2-one (3α5α-ALLO), have all been postulated to account for the efficacy of SSRIs.9,61−63 Despite years of investigation and multiple lines of thought, the exact mechanism by which fluoxetine relieves major depression symptoms is still not definitively known. Moreover, while SSRIs such as fluoxetine revolutionized the treatment of depression, they remain only partially effective, failing to relieve symptoms in >50% of depressed patients after multiple treatment regimens.

APPROVED INDICATIONS

Fluoxetine is approved for the treatment of major depressive disorder (adult and pediatric), obsessive-compulsive disorder (adult and pediatric), acute depressive episodes in Bipolar I disorder, panic disorder, bulimia nervosa, and premenstrual dysphoric disorder.41,64

ADVERSE EFFECTS AND DOSAGE

A number of adverse effects have been noted in patients taking fluoxetine. A major issue with 1 and other SSRIs concerns sexual dysfunction: erectile dysfunction, anorgasmia (inability to achieve orgasm), and diminished libido have all been well documented.41,64 However, noting the effects on anorgasmia, 1 has been used to prevent premature ejaculation.41,64 SSRIs, including 1, can elicit discontinuation syndrome, and all SSRIs carry a black box warning for increased risk of suicide (especially for patients under 25). Some studies have found that 1 and other SSRIs can lead patients to commit violent acts and display aggressive behaviors. A host of other mild side effects have been reported and include headache, nausea, drowsiness, diarrhea, tremors, photosensitivity, and weight loss.41,64 However, compared to the early TCAs and MAOIs, the side effect profile is greatly improved, especially in cases of overdose. The FDA has also approved 1 for use during pregnancy, but only recommended when the benefits outweigh the risks and is not recommended for breast-feeding mothers.1,4,13,41

HISTORY AND IMPORTANCE IN NEUROSCIENCE The earliest classes of antidepressant medications, which dominated the clinical landscape from the 1950s through the 1970s, were discovered serendipitously.65 Tricyclic antidepressants (TCAs) were developed in the 1950s in the wake of the discovery that chlorpromazine 16, derived from early synthetic antihistamines, acted as an antipsychotic agent (Figure 2).6,11,65−68 This breakthrough led to the synthesis and pharmacological evaluation of other analogues of 16, such as imipramine 17, the first TCA to be developed.69−74 Numerous efforts followed the development of 17, including the introduction of amitriptyline 18 by Merck in 1961.72−76 For many years, TCAs were the standard of care for depression.72,73 It was later discovered that TCAs exert their antidepressant affects by blocking both the serotonin transporter (SERT) and the norepinephrine transporter

(NET), increasing extracellular concentrations of serotonin 19 and norepinephrine 20, with little effect on dopamine (DA) 21. 49−53,77−81 However, TCAs have promiscuous pharmacology, with agonist or antagonist activity at multiple muscarinic, adrenergic, histamine, serotonin, and NMDA receptor subtypes, which engender significant adverse effects (e.g., agitation, dry mouth, and seizure).72,73,79 Moreover, TCAs are potent inhibitors of L-type calcium and sodium channels, leading to potentially lethal hypertension and arrhythmias.82 Thus, TCA overdose is often fatal, which limits the use of these compounds in a patient population which is at risk for suicidal behavior.

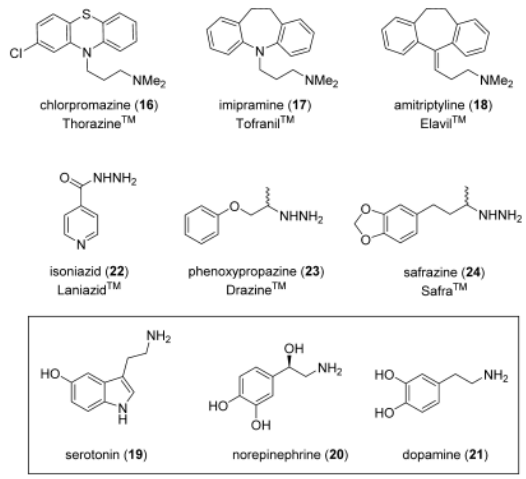


Figure 2.Structures of tricyclic antidepressants 17 and 18, and first generation nonselective, irreversible monoamine oxidase inhibitors 22−24, the first clinically available antidepressants. Also shown are the key neurotransmitters 19−21, via which these early TCAs and MAOIs elicited their antidepressant effects.

The other major class of early antidepressants, monoamine oxidase inhibitors (MAOIs), was developed out of an effort to optimize the antituberculosis drug isoniazid 22, and these compounds were only later found to possess antidepressant activity.83 This discovery led to the development of additional MAOIs, such as 23 and 24. MAOIs act to treat depression via inhibition of monoamine oxidase, which prevents the catabolism of neurotransmitters such as 19−21 (Figure 2).83−86 However, MAOIs also inhibit the breakdown of dietary amines. This can lead to hypertension if large amounts of foods containing tyramine are ingested, and can result in hyperserotonemia if large quantities of foods containing tryptophan are ingested.87−89 Moreover, these first generation MAOIs can engender serious pharmacodynamic drug−drug interactions with a wide variety of prescription and over-thecounter medications, which leads to difficulty in designing effective treatment regimens. Despite the shortcomings of TCAs and MAOIs, their apparent efficacy in the treatment of patients suffering from depression led to the development of the “monoamine hypothesis of depression”. This hypothesis posits that depression results from low brain concentrations of monoamines, such as 5-HT, and catecholamines, such as NE and DA. Overall, most TCAs and MAOIs had a more robust effect on the regulation of NE than on 5-HT or DA; however, 17 and 18 were found to have a more dramatic effect on levels of 5-HT than of NE or DA.6−9,53,54 By combining this observation with clinical data, Carlsson and colleagues proposed that inhibition of 5-HT uptake may be responsible for the mood elevating profile of 17 and 18. 54 Specifically, it had been previously noted in post-mortem studies that concentrations of 5-HT, and its major metabolite 5-hydroxyindole acetic acid (5-HIAA), were found to be lower in depressed patients that committed suicide than in those patients that died from other causes.90,91 Furthermore, MAOIs were found to be

more efficacious if given in combination with precursors to 5-HT synthesis.92,93

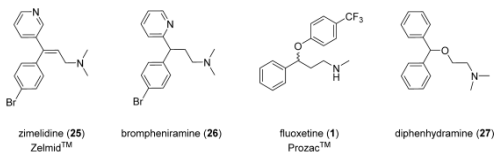


Figure 3. Structures of the first SSRIs 25 and 1, and the antihistamines 26 and 27 from which they were developed.

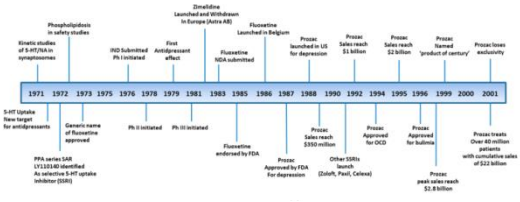


Figure 4.Timeline of the key milestones that led to the development of Prozac (1) and other SSRIs, highlighting key sales figures. Figure adapted from Wong et al.18

Finally, research indicated that depressed patients had lower concentrations of 5-HIAA in their bodily fluids compared to healthy controls. This evidence prompted researchers in the early 1970s to explore serotonin (5-HT) reuptake inhibition as a novel therapeutic strategy for treating depression. Consequently, Carlsson and Astra AB developed zimelidine (25) during the 1970s, launching it in Europe in 1982 as the first selective serotonin reuptake inhibitor (SSRI) approved for the treatment of depression. Like the earlier tricyclic antidepressants (TCAs), zimelidine was derived from the antihistamine brompheniramine (26). However, its market presence was short-lived due to serious adverse events, including the induction of Guillain-Barré syndrome, a potentially fatal neuropathy. Following this setback, Astra AB ceased all SSRI development efforts.

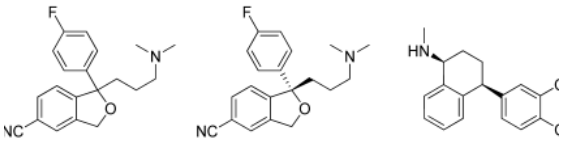
Fortunately, Eli Lilly & Co. was concurrently pursuing its own SSRI discovery program, also inspired by the antihistamine diphenhydramine (27), which ultimately led to the development of fluoxetine (1), known commercially as Prozac. As detailed by Wong et al., the journey to Investigational New Drug (IND) application for fluoxetine was fraught with challenges and hurdles both from within Lilly and external advisors. Shortly after a development team formed in 1973 to oversee product advancement, concerns regarding phospholipidosis—an accumulation of phospholipids in the lungs—almost derailed the project. During this period, Lilly researchers first presented fluoxetine as an SSRI at the 1974 annual meeting of the Federation of American Societies for Experimental Biology and the American Society of Pharmacology and Experimental Therapeutics. In the same year, they published a significant paper detailing the structure-activity relationship (SAR) and pharmacology of the compound.

After a nine-month hiatus, safety studies resumed following consultation with the Neuropharmacology division of the FDA. By 1976, six years after fluoxetine's initial synthesis, all IND-enabling animal studies were completed, and an IND application was filed with the FDA. Later that year, Lemberger, a Lilly clinician, administered fluoxetine to humans for the first time, finding it well tolerated at doses of up to 90 mg. However, during the initial phase II trial with depressed patients, fluoxetine did not show statistically significant improvement over placebo, almost curtailing the clinical development program. The research team was subsequently informed that the lack of efficacy might result from enrolling patients who had previously not responded to other antidepressants.

In light of this, Lilly decided to repeat the trial with non-treatment refractory patients

but faced a two-year challenge in finding an appropriate clinician to lead the study. Eventually, Slater and Stark were recruited to conduct the phase II and III trials, leading to successful outcomes that demonstrated fluoxetine's efficacy in treating major depression without the undesirable side effects associated with TCAs, such as blurred vision, dry mouth, and sedation, and without cardiovascular complications. The results were submitted to the FDA in 1983, seven years after the first human dosage of fluoxetine.

During this time, Astra AB had launched zimelidine in Europe, leading to disappointment in the Lilly team for not being the first to market. However, the eventual withdrawal of zimelidine allowed fluoxetine to become the first SSRI approved in the United States and arguably the most successful SSRI on the market. Nevertheless, FDA approval was not fast-tracked; it took Lilly over two years to receive approval for fluoxetine after submission, with final approval granted on December 29, 1987. This journey from laboratory research to clinical use spanned over 16 years, culminating in the launch of fluoxetine as Prozac in January 1988.



citalopram (**27**) Celexa™ escaitalopram (**28**) Lexapro™ sertaline (**29**) Zoloft™

Fluoxetine (1) was the first selective serotonin reuptake inhibitor (SSRI) to be marketed in the United States, fundamentally transforming both the treatment landscape for depression and the public's perception of the condition. Prozac became recognized as a safe and effective once-daily medication for depression, leading to widespread adoption among healthcare professionals. Although Eli Lilly initially estimated the depression treatment market at around $200 million, fluoxetine quickly exceeded these projections. Within a year of its launch, Prozac generated annual sales of approximately $350 million. By 1992, annual sales exceeded $1 billion, surpassing $2 billion by 1995, and peaking at $2.8 billion in 1998. By the time fluoxetine lost patent protection in 2001, over 40 million individuals had used the medication, totaling an estimated $22 billion in worldwide sales.

From its launch in 1988 to 2001, the approved indications for fluoxetine expanded to include obsessive-compulsive disorder, panic disorder, anorexia nervosa, and bulimia nervosa. Beyond its commercial success, fluoxetine has played a crucial role in enhancing our understanding of serotonin (5-HT) function in the central nervous system (CNS). A search of databases such as PubMed and Web of Science reveals over 10,000 published papers discussing various aspects of fluoxetine, underlining its significance in neuroscience research.

The success of fluoxetine inspired other pharmaceutical companies to develop their own SSRIs, leading to a market that quickly surpassed $10 billion, with several SSRIs achieving blockbuster status (over $1 billion in sales) and millions of prescriptions being written. Depression emerged as a mainstream disorder with a definitive treatment approach. Notable SSRIs that followed in Prozac's footsteps included Lundbeck’s citalopram (27), later marketed as the single (S)-enantiomer escitalopram (28), Pfizer’s sertraline (29), and GSK’s paroxetine (30), all launched in the U.S. prior to 1992.

With the influx of generic versions of fluoxetine in 2001, Eli Lilly rebranded Prozac as Sarafem to target premenstrual

dysphoric disorder (PMDD), a severe form of premenstrual syndrome, in an effort to sustain sales. Prozac also achieved a level of cultural prominence unmatched by any previous medication. It was featured on the cover of Newsweek in 1990, which described it as “a breakthrough drug for depression,” and in 1999, Fortune labeled it one of the “Pharmaceutical Products of the Century.” Additionally, Prozac appeared in book titles, song lyrics, and even band names, gaining further recognition in mainstream media. A notable example is Elizabeth Wurtzel's memoir, "Prozac Nation," published in 1994, which later inspired a feature film. Even Webster’s dictionary includes a definition of Prozac, illustrating its integration into common language.

This extensive exposure has contributed to raising awareness about depression and reducing the stigma surrounding it, encouraging more individuals experiencing depression to seek treatment. Although recent studies, such as the STAR*D study, have indicated that only one-third of depressed patients achieve remission with traditional SSRIs and that the onset of their action can take 3 to 6 weeks, the influence of fluoxetine is unmistakable. It has been a pivotal force in the field of serotonin research, reshaping the therapeutic landscape for major depression and altering societal attitudes toward the condition. For these reasons, fluoxetine (1 or Prozac) stands out as a classic in the realm of chemical neuroscience and serves as a noteworthy example for the Classics in Chemical Neuroscience series.

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