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Central & Peripheral Nervous Systems

Triple uptake inhibitors: therapeutic potential in depression and beyond

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Drugs that interfere with the uptake and/or metabolism of biogenic amines have been used to treat depression for > 4 decades. Early medications such as tricyclic antidepressants and monoamine oxidase inhibitors are effective but possess many side effects that limit their usefulness. Selective serotonin reuptake inhibitors (SSRIs) or selective noradrenaline reuptake inhibitors (SNRIs) are the results of rational design to find drugs that are as effective as the tricyclic antidepressants, but with more selectivity towards a single monoamine transporter. The SSRI class of drugs, which includes fluoxetine, paroxetine and sertraline, were previously viewed as the agents of choice for treating major depression. Recently, inhibitors of both serotonin and noradrenaline uptake ('dual uptake inhibitors'; SSRI/SNRI such as venlafaxine, duloxetine and milnacipran) have gained acceptance in the market. However, neither the SSRIs nor the SSRI/SNRI are fully satisfactory due to a delayed onset of action, low rate of response and side effect that can affect compliance. An important recent development has been the emergence of the triple uptake inhibitors (SSRI/SNRI/selective dopamine reuptake inhibitor), which inhibit the uptake of all three neurotransmitters that are most closely linked to depression: serotonin, noradrenaline and dopamine. Preclinical studies and clinical trials indicate that a drug inhibiting the uptake of all three of these neurotransmitters could produce a more rapid onset of action and possess greater efficacy than traditional antidepressants. This review discusses the evolution of biogenic amine-based therapies, the emerging strategies involved in the design and synthesis of novel triple uptake inhibitors as antidepressants and the therapeutic potential of triple uptake inhibitors.

Keywords: antidepressant, depression, drug design, dual uptake inhibitors, monoamine uptake inhibitor, SDRAI, SNRI, SSRI/SNRI, SSRI, structure–activity relationship, synthesis, tricyclic antidepressant, triple uptake inhibitors

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1. Introduction

Depression is a common mental disorder that is characterized by sadness, loss of interest in activities and decreased energy. Feelings of depression, unhappiness or disappointment are common in the general population, affecting up to one-third of all people at some time in their lives. Major depressive disorder (MDD; also called unipolar major depression) is the most extreme and common form of depression. Other common types of depression include dysthymic disorder, double depression (dysthymia with a superimposed major depressive episode), adjustment disorder, seasonal affective disorder and minor depression. MDD is ranked by the World Health Organization as among the most burdensome diseases to society, with lifetime prevalence rates in the US as high as 17% and 12-month prevalence rates estimated

Table 1. Classification of monoamine-based antidepressants.

MAOIs	TCAs	SSRIs	SNRIs	SSRI/SNRIs	SDARI/SNRI
Isocarboxazid (Marplan®) Phenelzine (Nardil®) Tranylcypromine (Parnate®) Moclobemide (Aurorix®; Roche)	Imipramine Desipramine Clomipramine Trimipramine Amitriptyline Nortriptyline Doxepin Dothiepin Amoxapine Protriptyline Tetracyclic: Maprotiline Mirtazapine	Fluoxetine (Prozac®; Eli Lilly) Citalopram (Celexa; Forest Laboratories) Escitalopram (Lexapro™; Forest Laboratories) Fluvoxamine (Luvox™) Paroxetine (Paxil®; Novo Nordisk) Sertraline (Zoloft®; Pfizer) Zimelidine§	Desipramine Reboxetine Maprotiline Viloxazine Atomoxetine*	Duloxetine (Cymbalta®; Eli Lilly) Venlafaxine (Effexor®; Solvay Pharmaceuticals and Wyeth) Milnacipran (Dalcipran®; Pierre Fabre; Ixel®; sanofi-aventis) Sibutramine‡ (Meridia®; Abbott GmbH) Nefazodone§	Bupropion (Wellbutrin®; GlaxoSmithKline and Biovail)

Brand names are in brackets.

*Attention-deficit/hyperactivity disorder.

‡Antiobesity.

§Discontinued.

MAOI: Monoamine oxidase inhibitor; SDARI: Selective dopamine reuptake inhibitor; SNRI: Selective noradrenaline reuptake inhibitor; SSRI: Selective serotonin reuptake inhibitor; TCA: Tricyclic antidepressant.

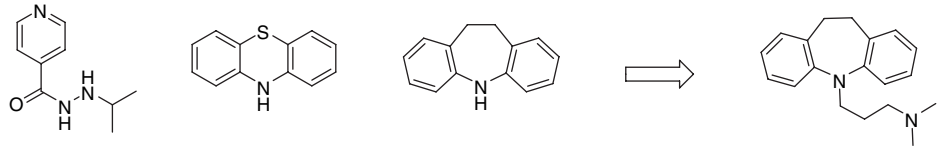

Figure 1. Design of the first marketed antidepressant.

at 1.7 – 8.6% [1,2]. Nearly 30 million of the US adult population may be affected by MDD, with approximately one-third being classified as severely depressed [2,3].

At present, there are six major classes of monoamine based drugs that are used in the treatment of depression (**Table 1**) [3,4]. Monoamine oxidase (MAO) inhibitors slow the breakdown of dopamine (DA), noradrenaline (NA) and serotonin (5-HT) in the brain [5], allowing them to continue sending messages for a longer time. Many tricyclic antidepressants (TCAs) alter the balance of neurotransmitters (NA and 5-HT) in the brain by blocking the uptake of NA and 5-HT. Selective serotonin uptake inhibitors (SSRIs) enhance the activity of 5-HT, but do not affect other chemical messengers and are generally associated with fewer and less severe side effects than TCAs or MAO inhibitors. Recently, inhibitors of both 5-HT and NA uptake ('dual uptake inhibitors': SSRI/SNRI (selective noradrenaline reuptake inhibitor) such as venlafaxine, duloxetine and milnacipran) have also gained acceptance in the market place. Nefazodone has been discontinued as a brand name product, but it is still available as a generic in some countries.

An important recent development in the therapy of depression has been the emergence of the triple uptake inhibitors (SSRI/SNRI/SDARI [selective dopamine re-uptake inhibitor]), which inhibit the uptake of the three neurotransmitters that are most closely linked to depression: 5-HT, NA and DA. Preclinical studies and clinical trials using combinations of existing uptake inhibitors or receptor agonists suggest that a drug inhibiting the uptake of all three of these neurotransmitters could produce a more rapid onset of action and greater efficacy than traditional antidepressants. In this review, the structure–activity relationship of monoamine-based antidepressants, the evolution of biogenic amine-based therapies, emerging strategies involved in the design and synthesis of novel triple uptake inhibitors as antidepressants and the therapeutic potential of triple uptake inhibitors are discussed.

2. Evolution of the biogenic amine-based therapies

The first two classes of drugs used to treat major depression – the monoamine oxidase inhibitors (MAOIs) and the TCAs – were discovered by serendipity. Iproniazid (**Figure 1**), the first modern antidepressant, was originally developed as an antitubercular drug (an analog of isoniazid) in the early 1950s by Roche. In addition to its ability to treat tuberculosis, iproniazid elevates mood and stimulates activity in many patients [6]. These effects led researchers to investigate the ability of iproniazid to treat the symptoms of depression.

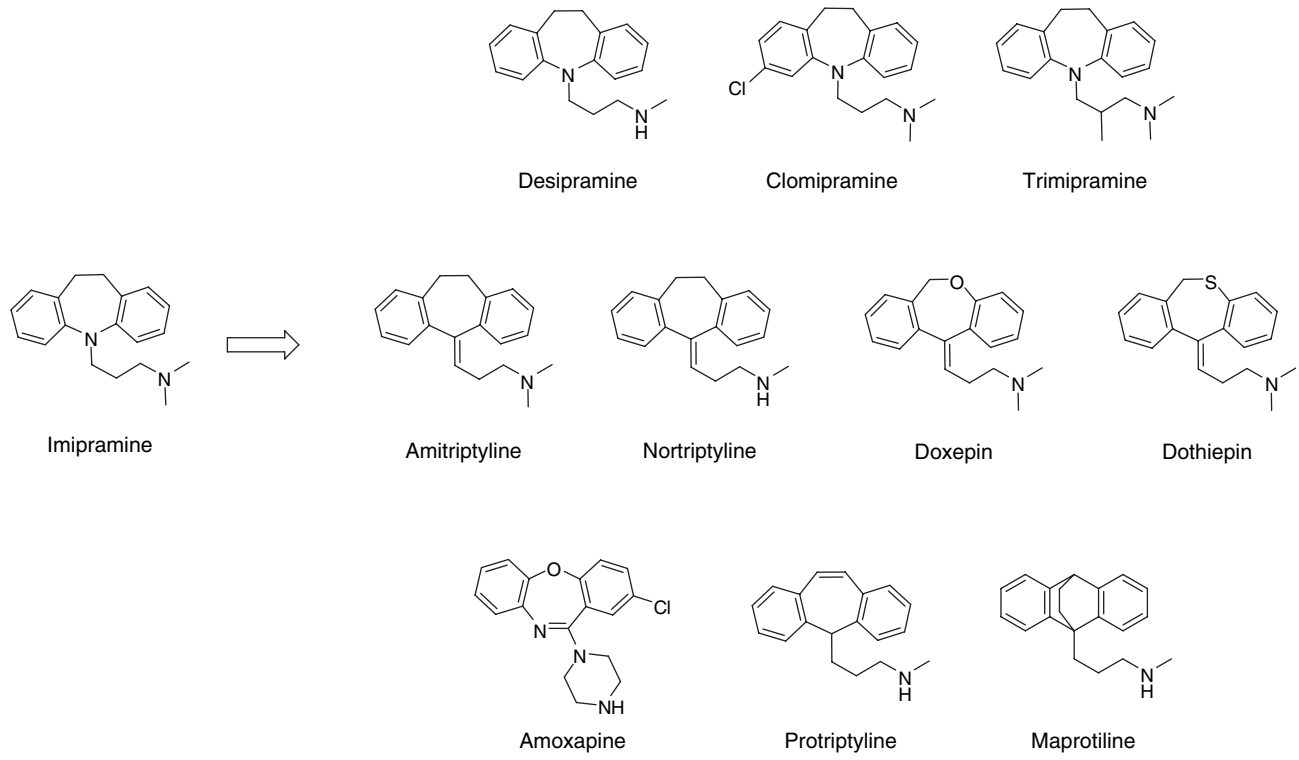

Figure 2. Design of tricyclic antidepressants.

The psychiatrist N Kline [6] proposed that there were drugs, iproniazid among them, that functioned as 'psychic energizers'. These drugs would increase the 'id energy' available to the ego, resulting in a sense of joy and optimism. After promising preliminary findings were reported in 1957, iproniazid was prescribed widely to patients with major depression. Within the first year it was available as an antidepressant and 400,000 depressed people were treated with iproniazid. Subsequent studies demonstrated the ability of this drug to block the activity of monoamine oxidase, the enzyme that metabolizes the monoamine neurotransmitters (NA, 5-HT and DA). Although iproniazid is no longer used as an antidepressant because of its toxicity, the efficacy of this drug led to further interest in the idea that depression may be alleviated by appropriate drugs.

During the 1950s, the chief of pharmacology at Geigy, R Domenjoz encouraged the company to explore compounds with structures similar to phenothiazines (**Figure 1**). Geigy had a number of compounds developed over the years and those with phenothiazine-like structures were re-examined. One such compound, iminodibenzyl (**Figure 1**), was synthesized in 1898 but no use was found for it at that time. It was this compound that was to become the prototypical TCA. Geigy chemists synthesized a series of 42 other compounds based on iminodibenzyl. R Kuhn of Muensterlingen Psychiatric Clinic tested a compound labeled G-22335 [7-9]. The results were dramatic and Kuhn proposed that the compound was an effective treatment for depression. The compound was named imipramine and was the first marketed antidepressant (**Figure 1**). As seen in the case of imipramine, analog research plays an important role in medicinal chemistry. One of the most frequently used approaches is to further optimize and improve an existing drug. The discovery of a series of TCAs is an excellent example of analog research in drug discovery. This analog research around the structure of imipramine resulted in the class of antidepressants that is termed as TCAs (**Figure 2**).

In 1972, a research team at Eli Lilly (B Molloy, DT Wong and R Fuller) synthesized and characterized an agent labeled LY-82816 (fluoxetine oxalate) that seemed to have the desired effects of TCAs without many of the side effects that are common to TCAs. The compound was developed into fluoxetine hydrochloride (LY-110140; **Figure 3**) [10,11]. After its initial release in South Africa, fluoxetine was released in the US in 1988. Although two serotonergic agents (trazodone and zilmelidine) had been developed in the early 1980s, they did not have the efficacy or receive the marketing that fluoxetine did. Several other SSRIs were later launched and have become the treatments of choice since the late 1990s (**Figures 3** and **4**). A new generation of antidepressants, SSRI/SNRIs such as venlafaxine, milnacipran and duloxetine (**Figures 3** and **4**), were launched in 1993, 1998 and 2004, respectively [12-16]. It is interesting to note that SSRIs, SNRIs (desipramine) and dual uptake inhibitors (SSRI/SNRI and

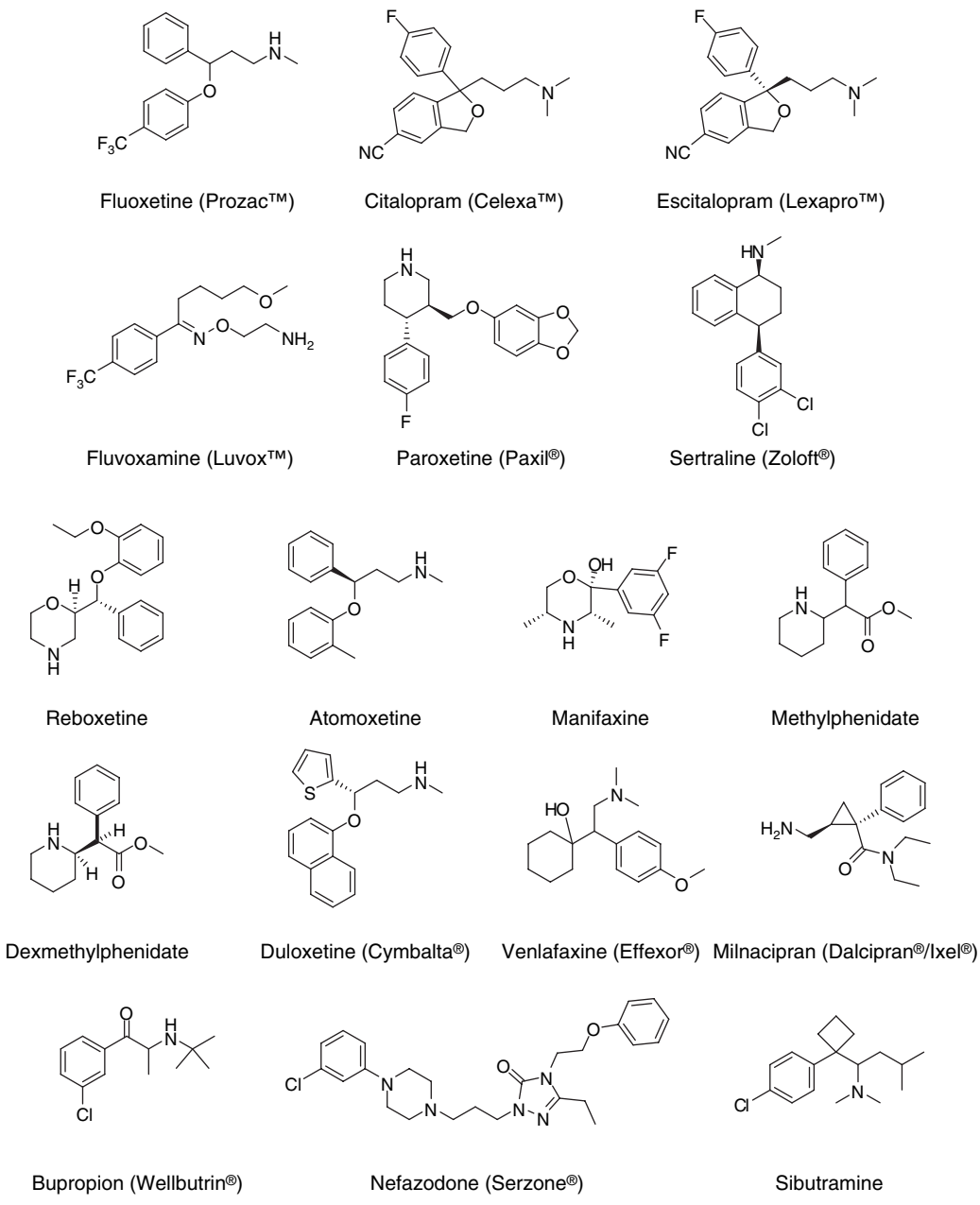

Figure 3. Selective uptake inhibitors (SSRIs, SNRIs, DARIs) and dual uptake inhibitors.

Brand names are listed in parentheses.

DARI: Selective dopamine reuptake inhibitor; SNRI: Selective noradrenaline reuptake inhibitor; SSRI: Selective serotonin reuptake inhibitor.

SDARI/SNRI) have all been approved as antidepressants (**Figure 3**). SNRI/SDARIs (methylphenidate and dexmethylphenidate) and SNRI (atomoxetine) have been approved for use in treating attention-deficit/hyperactivity disorder (ADHD) [17]. It is also interesting to note that sibutramine has been marketed as an antiobesity agent for some time, although it is a dual SSRI/SNRI. The therapeutic uses of dual 5-HT and DA uptake inhibitors and triple uptake inhibitors are still under investigation.

3. Preclinical and clinical status of novel triple uptake inhibitors

The evolution of antidepressants over the past 40 years has involved the replacement of drugs with a multiplicity of effects (e.g., TCAs) by those with selective actions (i.e., SSRIs) [18-20]. This strategy was employed to reduce the adverse effects of TCAs and MAOIs, largely by eliminating interactions with certain neurotransmitters or receptors. Although these more

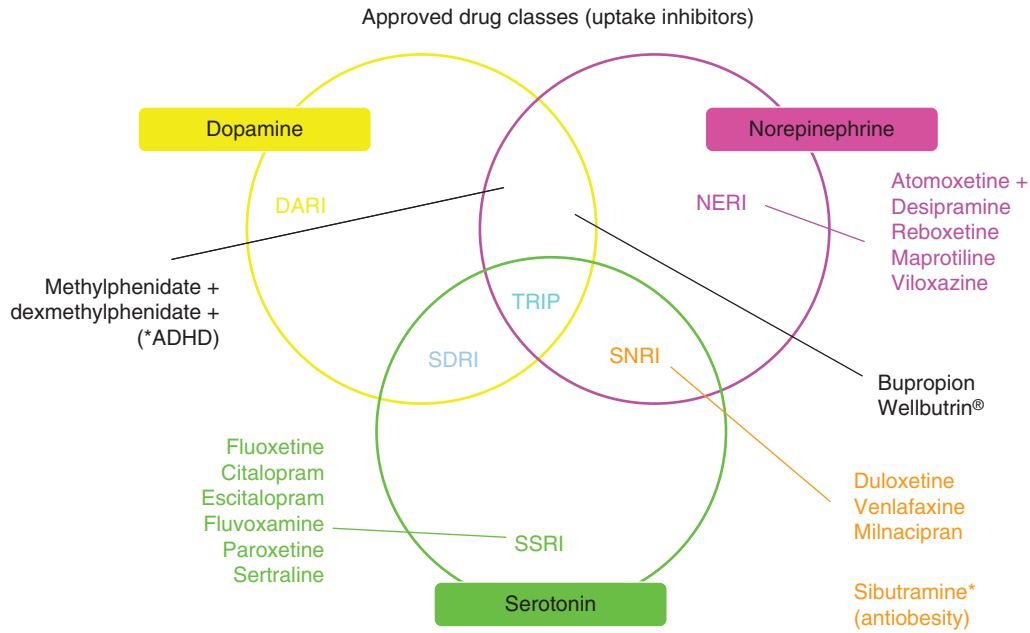

Figure 4. Approved drug classes.

*and + are notes to indicate different uses for some compounds.

ADHD: Attention-deficit/hyperactivity disorder; DARI: Dopamine re-uptake inhibitor; NERI: Norepinephrine re-uptake inhibitor; SDRI: Serotonin and dopamine re-uptake inhibitor; SNRI: Serotonin and norepinephrine re-uptake inhibitor; SSRI: Selective serotonin re-uptake inhibitor; TRIP: Triple reuptake inhibitor.

selective compounds may be better tolerated by patients, selective drugs – specifically SSRIs – are not superior to older drugs in treating depressed patients as measured by response and remission rates. In fact, it may be an advantage to increase synaptic levels of both serotonin and noradrenaline, as, for example, with venlafaxine (at high doses). Using single and dual uptake inhibitors, most placebo-controlled, double-blind trials require 3 or more weeks of therapy to produce a clinically meaningful improvement in depressive symptomatology [21]. Moreover, it is estimated that only ~ 60 – 70% of patients respond to approved antidepressants and only 30 – 50% of those responding achieve a full remission [22-24]. This suggests that ~ 60% of patients are not satisfactorily treated by present therapies. Another, perhaps more insidious, drawback of present therapies is the frequency of relapse in a significant proportion (estimated at 15 – 40% in long-term clinical trials) of patients, despite drug maintenance [25]. An improved side effect profile over the available antidepressants, such as eliminating the sexual dysfunction associated with SSRI administration, would be likely to improve patient compliance. A drug superior to the present therapies in one or more of these dimensions would significantly affect the treatment of MDD [26,27].

A triple uptake inhibitor adds the element of dopamine transporter (DAT) blockade to a dual (i.e., serotonin and noradrenaline; SSRI/SNRI) uptake inhibitor. It has been hypothesized that such a molecule will be superior to present therapies in one or more dimensions, including speed of onset, efficacy (defined here as an increase in the proportion of patients exhibiting a meaningful clinical response, including remission) and side-effect profile. The rationale behind a 'broad-spectrum' antidepressant that is capable of inhibiting amine uptake of the 5-HT, NA and DA transporters comes from both preclinical and clinical studies [28,29]. A 2006 paper has a comprehensive review of pharmacotherapies for depression with pro-dopaminergic activity [29]. For example, co-adminstering dopamine receptor agonists such as bromocriptine, pergolide or pramipexole with traditional antidepressants has shown to improve clinical syptoms in depressed patients [30]. Moreover, a recent randomized controlled clinical trial has demonstrated that augmentation of citalopram (a SSRI) with sustained-release bupropion (a SDARI/SNERI [norepinephrine re-uptake inhibitor]) has certain advantages in patients who did not respond adequately to a sustained regimen of citalopram, including a greater reduction in the number and severity of symptoms and fewer side effects and adverse events [31]. However, treatment with multiple drugs may introduce pharmacokinetic confounds. The ideal drug would be a single molecule that inhibits the uptake of serotonin, noradrenaline and dopamine.

Triple uptake inhibitors are expected to be the next generation of drugs for the treatment of major depression after SSRI/SNRIs. They are believed to offer clinically significant advantages in efficacy and/or in tolerability. As the authors describe below, a number of compounds with the ability to bind to all three monoamine transporters and block the reuptake have been identified during the research for selective

Table 2. Triple uptake inhibitors in preclinical and/or clinical development.

Company	Compounds	Clinical trial status
DOV Pharmaceutical, Inc.	DOV 216, 303	Phase II completed
DOV Pharmaceutical, Inc.	DOV 21,947	Phase II planned in 2007
DOV Pharmaceutical, Inc.	DOV 102,677	Phase I
GlaxoSmithKline/NeuroSearch	NS-2359 (GSK-372475)	Phase II
Sepracor	SEP-225289	Phase I
Bristol-Myers Squibb/Albany Molecular Research, Inc.	Albany Molecular Research, Inc. CNS-1 and -2	Preclinical
Eli Lilly & Co.	Multiple series	Preclinical
Acenta Discovery, Inc.	One series	Preclinical
Mayo Foundation	PRC-025 and PRC-050	Preclinical

or mixed inhibitors. Although the clinical efficacy of such a broad-spectrum antidepressant has not yet been fully demonstrated, several compounds have entered clinical trials for depression and/or ADHD, including DOV 216,303 and DOV 21,947 (DOV), NS-2359 (Neurosearch/GlaxoSmithKline) and SEP-225289 (Sepracor) (**Table 2**).

3.1 DOV 216,303, DOV 21,947 and DOV 102,677

DOV Pharmaceutical, Inc. is developing a series of 3-azabicyclo[3.1.0]hexanes as novel antidepressants. DOV 216,303 ([+/-]-1-[3,4-dichlorophenyl]-3-azabicyclo[3.1.0]hexane hydrochloride; **Figure 5**) is the prototype of a class of compounds referred to as 'triple' uptake inhibitors. Such compounds inhibit the uptake of 5-HT, NA and DA: the three neurotransmitters that are most closely linked to MDD. DOV 216,303 inhibits [^{3}H]5-HT, [^{3}H]NA and [^{3}H]DA uptake to the corresponding human recombinant transporters (expressed in human embryonic kidney [HEK] 293 cells), with IC_{50} values of ~ 14, 20 and 78 nM, respectively (**Table 3**) [26-28,32,33]. DOV 216,303 is active in tests predictive of antidepressant activity including the mouse forced-swim test and reversal of tetrabenazine-induced ptosis and locomotor depression. DOV 21,947, the (+)-enantiomer of DOV 216,303, inhibits the uptake of [^{3}H]5-HT, [^{3}H]NA and [^{3}H]DA in HEK 293 cells expressing the corresponding human recombinant transporters with IC_{50} values of 12, 23 and 96 nM, respectively. DOV 21,947 also inhibits [^{125}I]RTI-55 (3β-[4-iodophenyl]tropane-2β-carboxylic acid methyl ester) binding to the corresponding transporter proteins in membranes prepared from these cells (dissociation constant for an inhibitor [K_i] values of 100, 260 and 210 nM, respectively). DOV 21,947 reduces the duration of immobility in the forced-swim test in rats with an oral minimum effective dose of 5 mg/kg. This antidepressant-like effect manifests in the absence of significant increases in motor activity at doses of up to 20 mg/kg. DOV 21,947 also produces a dose-dependent reduction in immobility in the tail-suspension test, with a minimum effective oral dose of 5 mg/kg. The ability of DOV 21,947 to inhibit the uptake of three biogenic amines that are closely linked to the etiology of depression may result in a therapeutic profile different from antidepressants that inhibit the uptake of 5-HT and/or NA [33].

DOV 102,677 is also a triple uptake inhibitor that blocks [^{3}H]5-HT, [^{3}H]NA and [^{3}H]DA uptake in recombinant human transporters with IC_{50} values of 130, 100 and 130 nM, respectively. Radioligand binding to the 5-HT, NA and DA transporters is inhibited with K_i values of 740, 1000 and 220 nM, respectively [34]. DOV 102,677 (20 mg/kg i.p.) increased extracellular levels of DA and 5-HT in the prefrontal cortex to 320 and 280% above baseline 100 min after administration. DA levels were increased in a stable fashion for the duration (240 min) of the study, but 5-HT levels declined to baseline by 200 min after administration. NA levels increased linearly to a maximum of 348% at 240-min post-dosing. DOV 102,677 dose-dependently reduced the amount of time spent immobile by rats in the forced-swim test, with a minimum effective dose of 20 mg/kg and a maximal efficacy comparable to imipramine. This decrease in immobility time did not seem to result from increased motor activity. Furthermore, DOV 102,677 was as effective as methylphenidate in reducing the amplitude of the startle response in juvenile mice, without notably altering motor activity. DOV 102,677 also potently blocked volitional consumption of alcohol and reduced operant responding for alcohol.

3.1.1 *Clinical data*

In a dose-escalating, placebo-controlled, double-blind, Phase Ia trial, DOV 216,303 (5 – 150 mg) was rapidly absorbed following oral administration, with blood levels proportional to the administered dose. No adverse effects were observed after doses several times higher than the projected therapeutic doses [35]. In a Phase Ib, clinical trial, 10 subjects were given either placebo (n = 3) or drug (n = 7) at 3 doses (25 mg b.i.d., 25 mg t.i.d. and 50 mg b.i.d.) for 10 days. No severe side effects were noted, although diarrhea, vomiting and nausea were observed. The drug was well tolerated and no accumulation was observed after the treatment period. DOV 216,303 was rapidly absorbed with a plasma T_{max} of 0.7 – 1.2 h and $t_{1/2}$ of 3.3 – 4.4 h. In addition, no remarkable differences

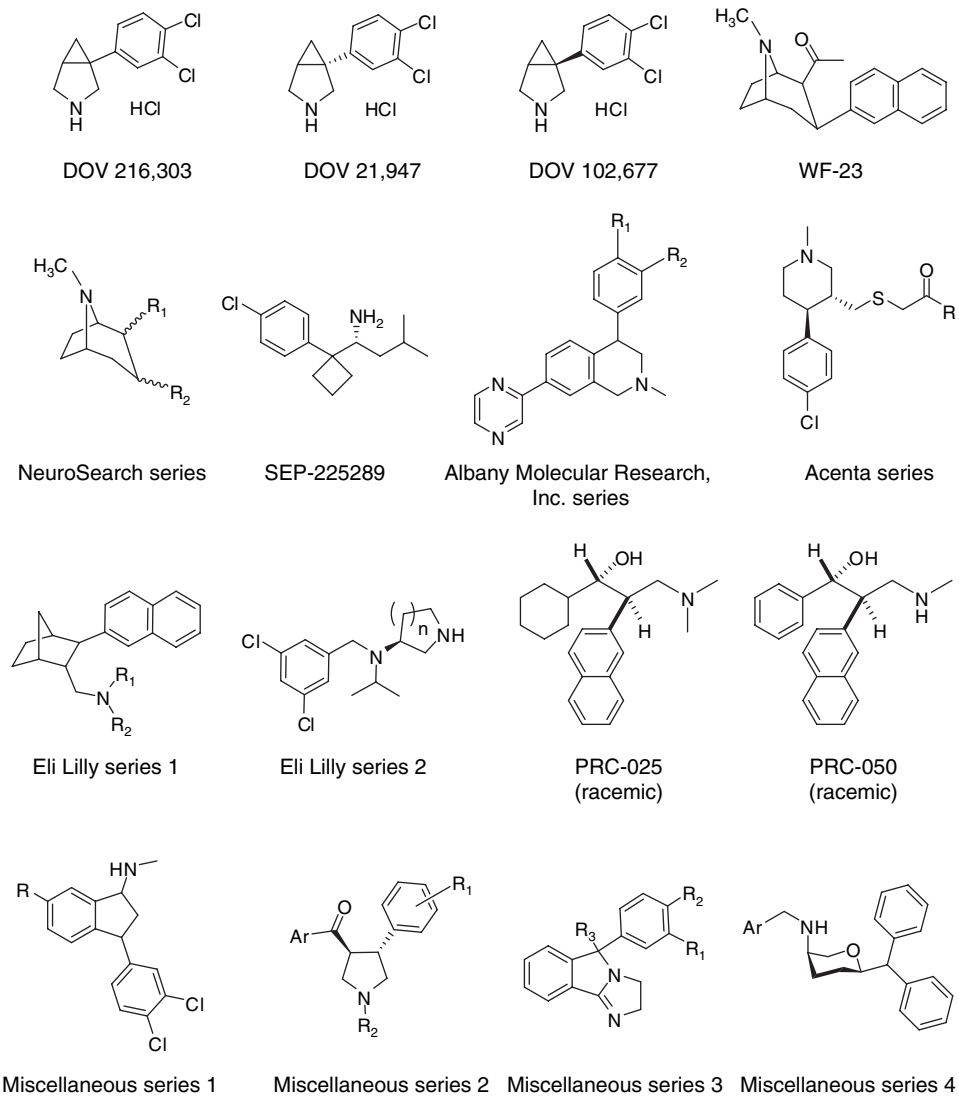

Figure 5. Structures of triple uptake inhibitors in preclinical studies and/or clinical development.

Table 3. *In vitro* binding and uptake inhibition of DOV compounds.

Compound	Inhibition of neurotransmitter uptake (IC_{50} [nM])			Inhibition of radioligand binding (K_i [nM])		
	[^{3}H]5-HT	[^{3}H]NA	[^{3}H]DA	SERT	NET	DAT
DOV 216303	14 ± 1.5	20 ± 6.1	78 ± 15	190 ± 28	380 ± 43	190 ± 40
DOV 21947	12 ± 2.8	23 ± 3.3	96 ± 20	100 ± 16	260 ± 41	210 ± 56
DOV 102677	130 ± 26	100 ± 27	130 ± 15	740 ± 140	1000 ± 76	220 ± 43

5-HT: Serotonin; DA: Dopamine; DAT: Dopamine transporter; K_i: Dissociation constant for an inhibitor; NA: Noradrenaline; NET: Noradrenaline transporter; SERT: Serotonin transporter.

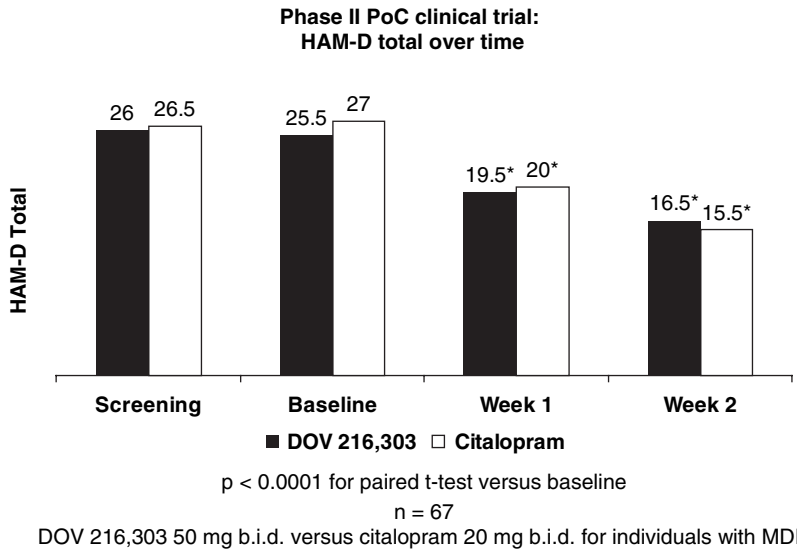

Figure 6. Phase II clinical trial results for DOV 216,303. DOV 216,303 and citalopram produce time-dependent reduction in HAM-D values. Patients received either DOV 216,303 (50 mg/kg b.i.d.) or citalopram (20 mg b.i.d.); administered medications were identical in appearance.

*Statistically significant.

HAM-D: Hamilton depression scores; MDD: Major depressive disorder; PoC: Proof-of-concept.

were apparent in either the C_{max} or AUC of DOV 216,303 following 1 and 10 days of dosing. The present data indicate that DOV 216,303 is safe and well tolerated both at single doses of up to 100 mg and multiple doses of up to 100 mg/day for 10 days. Plasma concentrations of DOV 216,303 after doses > 10 mg exceed its reported IC_{50} values for the inhibition of biogenic amine uptake [35].

A Phase II trial of DOV 216,303 examined the safety, tolerability and efficacy of DOV 216,303 in severely depressed patients using the SSRI with citalopram as an active control. The trial was of 2 weeks duration and the Hamilton Depression Rating Scale (HAMD) was the primary outcome measure; the Beck Depression Rating Scale and the Zung Self-Rating Depression Scale were the secondary measures. Results from this study indicated that DOV 216,303 is as effective as citalopram. Patients treated with either citalopram or DOV 216,303 showed > 40% reductions from baseline HAMD scores (**Figure 6**).

3.2 NS-2359 (GSK-372475)

NS-2359 (GSK-372475) is another triple uptake inhibitor that has entered clinical development. This compound was discovered at NeuroSearch and subsequently out-licensed to GlaxoSmithKline as part of a 5-year R&D alliance in CNS diseases. Although the structure of NS-2359 is not published yet, it is believed that NS-2359 is a tropane analog.

Phase I trial results of NS-2359 are reported in a press release by NeuroSearch. In an imaging study in 6 healthy volunteers receiving daily doses of NS-2359 (0.25 – 1.0 mg), single photon emission computed tomography (SPECT) showed very clear and specific binding in relevant areas of the brain and clear dose dependency (NeuroSearch press release, 3 July 2003). In a single-dose Phase I trial in the UK, this compound is well tolerated (Company Web Page, NeuroSearch, 25 Oct 1999; Ann. Rep., NeuroSearch, 1999). In a trial in 54 volunteers, NS-2359 increased attention and improved the ability to recall verbal information. Following these results, NeuroSearch had proposed to focus on the development of NS-2359 for treating ADHD (Ann. Rep., NeuroSearch, 2002).

In December 2006, NeuroSearch announced that GlaxoSmithKline initiated a Phase II clinical development of its drug candidate NS-2359 in patients diagnosed with MDD. The studies will be conducted in multiple centers worldwide involving several hundred patients.

3.3 SEP-225289

SEP-225289 is another triple uptake inhibitor that is under development by Sepracor for treatment of refractory depression and for generalized anxiety disorder (JP Morgan, 22nd Annual Healthcare Conference, San Francisco, USA, 2004; 3rd Quarter Release, Sepracor, 2004). In preclinical studies, SEP-225289 was believed to be a potent and balanced SSRI/SNRI/SDARI. It is in a randomized, single-blind, placebo-controlled Phase I safety, tolerability and pharmacokinetic trial for the treatment of depression (Sepracor press releases, 25 Apr & 19 Oct 2005). The structure is believed to be as shown in **Figure 5**.

3.4 AMRI CNS-1 and CNS-2

Albany Molecular Research Institute Inc. (AMRI) is developing biogenic amine transporter inhibitors for the treatment of CNS disorders. Compounds with various combinations of amine transporter inhibition profiles acting selectively or in

Table 4. *In vitro* binding and uptake inhibition of AMRI compounds.

Compound	Uptake inhibition (K_i [nM]) Rat synaptosomes			Binding inhibition (K_i [nM]) Rat transporters		
	[^{3}H]5-HT	[^{3}H]NA	[^{3}H]DA	SERT	NET	DAT
AMRI CNS-1	14	12.7	22.4	1.3	8.2	21.6
AMRI CNS-2	24	12.6	24.6	3.5	10.4	31.7
Duloxetine	2.8	3.21	202	0.6	3.9	888

AMRI: Albany Molecular Research, Inc.; DA: Dopamine; DAT: Dopamine transporter; K_i: Dissociation constant for an inhibitor; NA: Noradrenaline; NET: Noradrenaline transporter; SERT: Serotonin transporter.

Table 5. *In vitro* binding and uptake inhibition of Mayo Foundation compounds.

Compound	Inhibition of neurotransmitter uptake (K_i [nM])			K_d values for binding to human SERT, NET and DAT (nM)		
	[^{3}H]5-HT	[^{3}H]NA	[^{3}H]DA	SERT	NET	DAT
PRC-025	6.0 ± 0.8	10 ± 0.5	53 ± 1	6.0 ± 0.8	19 ± 2	100 ± 10
PRC-050	12 ± 2	1.2 ± 0.1	43 ± 7	6.0 ± 0.3	0.40 ± 0.05	120 ± 10
Venlafaxine	39 ± 3	210 ± 20	5300 ± 600	9.0 ± 0.3	1060 ± 40	9300 ± 50

5-HT: Serotonin; DA: Dopamine; DAT: Dopamine transporter; K_d: Dissociation constant; K_i: Disassociation constant for an inhibitor; NA: Noradrenaline; NET: Noradrenaline transporter; SERT: Serotonin transporter.

combination to increase brain levels of 5-HT, NA or DA are under investigation. Bristol-Myers Squibb has an exclusive worldwide license to develop and commercialize the compounds [36]. Preclinical data for compounds AMRI CNS-1 and -2 was disclosed in a recent presentation [37]. Both AMRI CNS-1 and -2 (**Table 4**) are believed to be novel 4-phenyl tetrahydroisoquinolines with nomifensine as its prototype compound (**Figure 5**). In June 2007, AMRI announced that it has received the first milestone payment, of US$1.5 million, from Bristol-Myers Squibb for milestones met in preclinical development according to their agreement [38].

3.5 Acenta series

Acenta Discovery, Inc. has synthesized a library of piperidine-based nocaine/modafinil hybrids and some ligands were found to display an improved potency at all three monoamine transporters [39,40]. Some compounds were reported to be more active in tail-suspension tests than desipramine (**Figure 5**). It was reported at a recent American Chemical Society meeting that more systematic *in vivo* assessments of the selected compounds from this library were ongoing in order to identify potential drug candidates for advancement to pre-development characterization in preparation for a potential investigational new drug submission.

3.6 PRC-025 and PRC-050

The Mayo Foundation and the Virginia Polytechnic Institute and State University are investigating analogs of venlafaxine as potential antidepressants. The two lead compounds, PRC-025 ([2*RS*,3*RS*]-*N*,*N*-dimethyl-3-cyclohexyl-3-hydroxy-2-[2′-naphthyl]propylamine) and PRC-050 ([2*RS*,3*RS*]-*N*-methyl-3-cyclohexyl-3-hydroxy-2-[2′-naphthyl]-3-phenylpropylamine), are racemic compounds (**Figure 5**) [41,42]. Preclinical data on PRC-025 and PRC-050 were reported recently [42]. The K_i values for inhibition of [^{3}H]5-HT, [^{3}H]NA and [^{3}H]DA uptake were 6, 10 and 53 nM for PRC-025 and 12, 1.2 and 43 nM for PRC-050, respectively (**Table 5**). These compounds were tested in animal models and their potentials as antidepressants were evaluated. In the forced-swim test with male Sprague-Dawley rats, both PRC-025 and PRC-050 (5 and 10 mg/kg i.p.) reduced the time spent immobile and increased the time spent swimming, comparable to the effects seen with imipramine (15 mg/kg). In addition, both PRC-025 and PRC-050 were effective in reducing the time spent immobile in the tail-suspension test (intraperitoneally), again with effects comparable to imipramine. Studies using the enantiomers of these compounds are reported to be underway.

3.7 Triple uptake inhibitors from Eli Lilly

Starting from a known SSRI/SDARI, WF-23 (Wake Forest University; **Figure 5**), the Lilly group developed a pharmacophoric model for each of the three transporters, which led to the design of several series of bicyclic analogs such as dimethyl-(3-naphth-2-yl-bicyclo[2.2.1]hept-2-yl methyl)-amine (**Figure 5**; Lilly series 1) [43,44]. The (2*S*,3*S*) diastereomer showed

Table 6. Top 10 antidepressants in 2005 with their patent and formulary status.

Drug name	Patent expiration date	Formulary or non-formulary	Generic or brand
Fluoxetine	Expired	Formulary	Generic
Paroxetine	Expired	Formulary	Generic
Bupropion	Expired	Formulary	Generic
Citalopram	Expired	Formulary	Generic
Sertraline	Expired	Formulary	Generic
Paxil CR® (paroxetine; Novo Nordisk)	2009	Formulary	Brand (generic)
Wellbutrin XL® (bupropion; GlaxoSmithKline/Bioavail)	2018	Formulary	Brand (generic)
Cymbalta® (duloxetine; Eli Lilly)	2008	Formulary	Brand (generic)
Lexapro™ (escitalopram; Forest Laboratories)	2009	Non-formulary	Brand (generic)
Effexor XR® (venlafaxine; Solvay Pharmaceuticals and Wyeth)	2008	Non-formulary	Brand (generic)

the best balance of the desired activities with IC_{50} values of 9, 86 and 28 nM as an inhibitor of 5-HT, DA and NA, respectively, in rat synaptosomes. It also exhibited oral activity in rat models of depression at oral doses of 20 – 25 mg/kg; the 1-naphthyl analog was much less active, but the 2-naphthyl derivative had only 2 – 3% oral bioavailability due to ring hydroxylation. Exploration of the activity of other bicyclic aromatics led to 5- or 6-substituted benzothiophenes, which provided enhanced potency and improved bioavailability, especially by the use of a *N*-methylamino substituent. The dimethyl amino derivative of the benzothiophen-5-yl analog was progressed into further development, displaying oral activity of 2.5, 5 and 10 mg/kg in models of 5-HT, DA and NA activity, respectively. However, this compound encountered unspecified problems later in development and was discontinued.

Lilly has also reported the synthesis and discovery of 3-amino piperidine and pyrolidine-based inhibitors of neurotransmitter re-uptake transporters in a 2005 American Chemical Society meeting (**Figure 5**; Lilly series 2) [45]. No further preclinical data has been published.

3.8 Miscellaneous series

Miscellaneous mixed biogenic amine transporter inhibitors were reported from a number of academic groups. For example, Rice and colleagues reported the synthesis of a series of 3-(3,4-dichlorophenyl)-1-indanamine derivatives (**Figure 5**; miscellaneous series 1) [46]. Wang *et al.* have reported the synthesis of 3,4-disubstituted pyrrolidines (**Figure 5**; miscellaneous series 2) [47,48]. Houlihan *et al.* have reported a series of nonselective mazindol and homomazindol analogues in their effort to develop a selective inhibitor of cocaine binding (**Figure 5**; miscellaneous series 3) [49,50]. Dutta *et al.* have reported a series of series of asymmetric pyran derivatives (**Figure 5**; miscellaneous series 4) [51,52]. No preclinical development activities have been reported from these academic groups.

4. Expert opinion and conclusion

4.1 Depression market and patent status of top 10 antidepressant drugs

At present, the depression market is the largest segment of the CNS market, with global sales of US$15.9 billion in 2004, which is a growth of 1.2% in comparison to the 2003 figures. In 2005, total world revenues for the top 10 antidepressant drugs (fluoxetine, paroxetine, bupropion, citalopram, sertraline, Paxil CR® [paroxetine; Novo Nordisk, licensed to GlaxoSmithKline], Wellbutrin XL (bupropion; GlaxoSmithKline), Cymbalta® [duloxetine; Eli Lilly], Lexapro™ (escitalopram; Forest Laboratories), Effexor XR® (venlafaxine; Solvay Pharmaceuticals and Wyeth) were > US$13 billion. However, because 9 out of these 10 drugs will have generic versions before 2010 (**Table 6**), there are reasons to believe that the world market for antidepressants will shrink to US$7 billion in 2010 due to these patent expirations. This is a loss of over 50% of the total revenues of 2004. The present pipeline seems lackluster with no drugs likely to be launched that will halt this massive revenue loss. Even the recent launch of Cymbalta can not overcome the revenue loss to generics. One development candidate that is closest to reaching pharmacy shelves for the treatment of MDD is the new SSRI/SNRI by Wyeth (desvenlafaxine). In January 2007, Wyeth announced that the Company has received an approvable letter from the FDA for desvenlafaxine succinate. Desvenlafaxine is the active metabolite of venlafaxine and is developed as a replacement for the older drug, which will lose patent protection in 2008. The triple uptake inhibitors are believed to have the potential to be the next blockbusters for treating major depression.

4.2 'Rule of 7' for designing new antidepressants

During the authors' endeavors to design novel triple uptake inhibitors, a detailed analysis of all 25 marketed transporter based antidepressant drugs was performed (**Figures 2 – 4**) and the following 7 knowledge-based rules for the design of new transporter-based antidepressants have been summarized [53]:

- Rule 1. It has to be a basic compound.
  All 25 marketed transporter-based drugs have a basic amino group in the molecule, 2 of them have a primary amino group, 14 of them have a secondary amino group (methylamino group: 8; piperidine ring: 3; piperazine: 1; morpholine: 1; t-butylamine: 1) and 9 of them have a tertiary amino group (all dimethylamino group).
- Rule 2. The presence of one or two phenyl groups.
  A total of 18 of the 25 have 2 phenyl groups; 6 of them have one phenyl group with a hydrophobic ring or chain; 1 has 2 aryl groups (duloxetine).
- Rule 3. A low molecular weight.
  The molecular weight of all 25 marketed transporter-based drugs (antidepressants) ranges from 233 to 329 with an average of 285 and a median of 280.
- Rule 4. A narrow C log P window.
  C logP ranges from 2.12 to 4.76 with an average of 3.52 and a median of 3.53.
- Rule 5. It can be flexible.
  The number of rotatable bonds ranges from 1 to 9 with an average of 4.4 and a median of 4.0.
- Rule 6. A low number of hydrogen bond acceptors.
  The number of hydrogen bond acceptors range from 1 to 4 with an average of 2.3 and a median of 2.0.
- Rule 7. A low number of hydrogen bond donors.
  The number of hydrogen bond donors range from 0 to 2 with an average of 0.8 and a median of 1.0.

Of all these rules, the most striking is the presence of a basic amino group such as dimethyl amino group or methyl amino group. It is also interesting to note that all novel triple uptake inhibitors discussed in this review contain one basic amino group as well. One direct application of these knowledge-based rules in the design and synthesis of future antidepressants is that these rules will help us to narrow down our searching scope to basic small molecules with certain physical characteristics. The findability of a new lead candidate for this drug discovery program will be much higher than regular combinatorial synthesis and high-throughput screening.

4.3 Therapeutic versatility of triple uptake inhibitors

The key approved indications of blockbuster antidepressants often include generalized anxiety disorder, MDD, obsessive–compulsive disorder, panic disorder and social anxiety disorder. In addition, a majority of primary care physicians and psychiatrists also prescribe antidepressants off-label for treatment across a broad range of clinical conditions outside the five key approved indications. It was believed that pain disorders are among one of the most often off-label antidepressant use. Emerging antidepressants in the pipeline at present will eventually find success in the antidepressant market and other related markets as well. For example, duloxetine, a SSRI/SNRI, was approved by the FDA in August 2004 for the treatment of MDD and in September 2004 for the treatment of diabetic peripheral neuropathic pain. In February 2007, duloxetine was also approved for generalized anxiety disorder. Other antidepressants approved for generalized anxiety disorder include escitalopram, venlafaxine and paroxetine.

Preclinical and clinical studies of triple uptake inhibitors suggest that these compounds will have use as treatment for MDD, diabetic peripheral neuropathic pain and generalized anxiety disorder. Other uses, such as treatment of ADHD, smoking, alcohol abuse and obesity, are also under active investigation by a variety of pharmaceutical companies.

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