Triple reuptake inhibitors as potential next-generation antidepressants: a new hope?

The current therapy for depression is less than ideal with remission rates of only 25–35% and a slow onset of action with other associated side effects. The persistence of anhedonia originating from depressed dopaminergic activity is one of the most treatment-resistant symptoms of depression. Therefore, it has been hypothesized that triple reuptake inhibitors (TRIs) with potency to block dopamine reuptake in addition to serotonin and norepinephrine transporters should produce higher efficacy. The current review comprehensively describes the development of TRIs and discusses the importance of evaluation of *in vivo* transporter occupancy of TRIs, which should correlate with efficacy in humans.

Background

Depression is a debilitating illness characterized by symptoms like **anhedonia**, depressed mood leading to suicidal thoughts, impaired cognitive functions, slowing of speech and other actions. Other symptoms include insomnia, loss of appetite and psychomotor agitation. Major depressive disorder (MDD) is a severe form of depression characterized by multiple episodes of depressed mood that must persist for at least 2 weeks accompanied by at least four other symptoms [1,2]. MDD can be differentiated as either melancholic depression or atypical depression based on the classification system set by the diagnostic and statistical manual of mental disorders IV (DSM-IV) [2,3]. While patients with melancholic depression manifest above symptoms, atypical depression patients have contrasting characteristics like increase in appetite, weight gain, hypersomnia and improved mood in response to positive events. Other depression types include dysthymic disorder, adjustment order, double depression, seasonal affective disorder and minor depression. MDD is however the most prevalent form of depression affecting 15–20% of population in the USA [4]. It is estimated that approximately 20% of all individuals suffer from a major mood disorder at least once in their lifetime. According to the

WHO by 2020, MDD would be the second-most leading cause of disability worldwide, affecting 121 million people, thus making it a global health problem [5]. Currently, there are six major classes of antidepressant drugs, targeting mainly monoamine serotonin and norepinephrine transporters (NET), available. Although a plethora of antidepressants are in market, it is believed that approximately 30–40% of patients do not respond to the therapy, thus reflecting an unmet need to develop novel therapeutics to combat MDD.

In this review, we provide a functional and structural overview of the monoamine transporters (MAT) and summarize major classes of antidepressant drugs with an emphasis on triple reuptake inhibitors (TRIs) as the next-generation antidepressants. In the first part, we have detailed the design and development of novel TRIs undergoing preclinical development in our laboratory. Next, we have detailed literature drug candidates that have transcended to preclinical and clinical evaluation.

The monoamine deficiency hypothesis

The modulation of serotonergic and noradrenergic systems laid the foundation of the ‘monoamine deficiency’ hypothesis of depres-

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Key terms

Anhedonia: A core symptom of major depressive disorder and other neuropsychiatric illness. It is characterized by an inability to feel pleasure from normally enjoyable acts.

Attention-deficit/hyperactivity disorder (ADHD): A common neurobehavioral disorder found mostly in children. It is characterized by difficulty in paying attention, hyperactivity and impulsive behavior.

sion that presumes a deficit of monoamine neurotransmitters in the synaptic cleft. The hypothesis has been extended by studies that found reduced levels of secondary messenger system, cAMP and phosphatidylinositol, for serotonergic and noradrenergic neurotransmission in depressed patients [1,6–8]. Thus, impaired monoaminergic transmission resulting from either a deficit of monoamine neurotransmitters in the synaptic cleft and/or disturbed receptor signaling are some of the key contributors that lead to depression [1,9,10]. While alterations in serotonin (5-HT) transmission correlated with aggressive, impulsive and anxious behavior; abnormalities in norepinephrine (NE) are related to motor activity, attention, memory and arousal [11–14]. The role of dopamine was not clearly understood and initially gained less interest in the pathophysiology of depression. Major drug developmental efforts have thus been directed toward inhibiting serotonin transporter (SERT) and NET proteins to increase the 5-HT and NE levels in the brain synapses. Studies have shown that other neurotransmitters like glutamate and gamma-aminobutyric acid (GABA) might also be involved in the neurobiology of mood disorder [15,16]. Other theories include abnormal circadian rhythms [17], lower brain cholesterol levels [18], impaired cholinergic-muscarinic functions [19], dysregulated endogenous opioid functions [20] and low cerebrospinal transthyretin levels [21]. However, the ‘monoamine deficiency’ is by far the most accepted and validated hypothesis and considered the gold standard in the treatment of depression. Although, in some recent findings, ketamine which is an antagonist at *N*-methyl-D-aspartate (NMDA) receptor, produced promising antidepressant effects in clinical trials in treatment-resistant depressed population [22,23]. More studies are underway to understand the effect of this drug in greater detail.

Structural & functional understanding of the MAT

MAT are integral membrane proteins which perform the transport of the monoamines: serotonin, NE and dopamine. The MAT – SERT, NET and dopamine transporter (DAT) – are important pharmacological targets of many psychostimulant and antidepressant drugs. MAT are members of Na-dependent neuro-

transmitter symporters (NSS) that use cotransport of Na^+ as a driving force for transport. The NSS family, referred to as the Na- and Cl-dependent solute carrier 6 (SLC6) families in the human genome organization, also include transporters for the amino acid neurotransmitters GABA and glycine [24]. The SLC6 family is the largest members of the SLC superfamily with 20 genes encoding a group of structurally similar transporter proteins with 20–30% sequence similarity between bacterial and their mammalian counterparts. First structural information on the SLC6 transporters became available in early 1990s with the cloning of the gene encoding the rat GABA transporter type 1 (GAT1) followed by cloning of the other family members [25–28]. It revealed 40% sequence identity between their primary structures suggesting that these transporters share a similar general structure. The amino acids range from 599 for GAT1 to nearly 700 for the two glycine transporters and are arranged as 12 transmembranes (TMs) helices. Significant efforts were made in the following studies to unearth the functional roles of these transporters in ion transport and elucidate residues implicated in substrate specificity and inhibitor binding. Heterologous expression system and mutagenesis experiments mainly the substituted cysteine assessment method (SCAM) were performed to identify the structural and functional role of individual residues of the transporters. It was shown that tyrosine in the TM3 of GAT1 was implicated in GABA binding and transport. Moreover, a tyrosine residue was found to be highly conserved across the SLC6 family implying its common role in the recognition of all substrates of the family [29]. Furthermore, an aspartate in TM1 (Asp79 in human DAT) is conserved among the monoamine proteins indicating its common role in monoamine substrate recognition [30]. This aspartate is substituted with glycine in other transporter proteins for amino acid transport. In another study, SCAM analysis on SERT revealed that Ile172 is involved in substrate recognition [31]. Although these studies provided a wealth of information, absence of a tertiary structure has presented challenge in structure-based drug design and further our understanding of the SLC6 transporter proteins at a molecular level.

In a significant breakthrough in 2005, the first x-ray crystal structure of the membrane protein, the leucine transporter (LeuT), from the bacterium *Aquifex aeolicus* in complex with Leu was determined to a 1.65-Å resolution [32]. Later, structures of LeuT in complex with different substrates and inhibitors were also published [33–36]. Crystal structure of LeuT mutant in the outward- and inward-open states in the absence of substrate has also been reported [37]. LeuT shared 20, 21 and 24% sequence identity with DAT, SERT and

NET, respectively, with several structurally conserved domains including TMs 1, 3, 6 and 8 accounting high sequence similarity (55–67%) near the substrate-binding site [38]. Furthermore, functional similarities to the SLC6 transporter proteins suggest a common mechanism for transport making LeuT a surrogate model for studying the functional properties and pharmacology of human membrane proteins.

Traditional antidepressants

The history of antidepressants dates back to nearly seven decades when the earliest antidepressant agents were discovered serendipitously in 1950s [39]. Iproniazid, a monoamine oxidase inhibitor (MAOI), originally discovered for tuberculosis in 1951 was observed to produce euphoria like effect. Its antidepressant property was later presented at a regional meeting of the American Psychiatric Association in 1957. In the same year, the therapeutic effect of imipramine, the first tricyclic antidepressants (TCA), which was discovered based on the antipsychotic agent chlorpromazine, was published in the Swiss Medical Journal [39]. MAOIs like phenylzine, isocarboxazid and tranylcypromine were among the earliest class of antidepressants used [40]. They block the degradation of monoamines by nonselectively and irreversibly inhibiting enzymes MAO-A and MAO-B. Although MAOIs are effective in treating major depression disorder, they also inhibit the catabolism of dietary amines and can precipitate hypertensive crisis the ‘cheese effect’ when taken with foods rich in tyramine [41]. Moreover, their liability to produce severe drug–drug interactions further limits their clinical use as the last resort. Moclobemide and transdermal selegiline were later developed as reversible inhibitors of MAO-A and MAO-B, respectively, to address some limitations associated with the use of MAOIs as antidepressant agents. Another structurally different class, belonging to TCAs, increased synaptic monoamine levels by preventing the reuptake of 5-HT and NE into the nerve terminals. Although for several years TCAs stood as a classical therapeutic class for treating depression; they were hampered by their non-specific interactions in the CNS including antagonism of adrenergic, histamine and cholinergic receptors [42]. TCAs can also cause fatal side effects including hypertension and arrhythmias by blocking L-type calcium and sodium channels. Despite these limitations, the first-generation antidepressants laid the genesis of the ‘monoamine deficiency’ hypothesis, which has been the mainstay in the treatment of depression.

Second-generation antidepressants

Over the past few decades, selective serotonin reuptake inhibitors (SSRIs) and dual serotonin/norepinephrine

reuptake inhibitors (SNRIs) were developed (Figure 1) and hailed as second-generation antidepressants [43–47]. Prozac (fluoxetine) developed by the Eli Lilly company was the first SSRI approved in 1988, which grossed US\$22 billion worldwide before its patent expired in 2001 [48]. Following fluoxetine, several SSRIs including citalopram, escitalopram, sertraline and paroxetine also went on to attain the status of blockbuster drugs. Concurrently, research by the Danish University Anti-depressant Group (DUAG) showed that clomipramine, a TCA that inhibited both serotonin and NE was much more effective than citalopram and paroxetine and fueled the concept, and controversy, of dual reuptake inhibitors for the management of MDD [49]. Later, in support of dual inhibition strategy, it was shown that venlafaxine, an SNRI, exhibited greater response and remission rates than SSRIs [50,51]. Inhibition of SERT is a common feature that TCAs, SSRIs and SNRIs possess to elicit an antidepressant response; however, selective NE reuptake inhibitors (NRIs): reboxetine, atomoxetine and viloxazine have also been developed for depression and are prescribed for **attention-deficit/hyperactivity disorder (ADHD)** [52–54]. SSRIs and SNRIs by virtue of their selective interactions at the SERT and NET exhibited an improved adverse effect profile; however, they were not without shortcomings [55,56]. These agents have failed to improve the efficacy and exhibit delayed onset, typically 4–6 weeks, of antidepressant response [57]. Moreover, because of relapse and unwanted sexual dysfunction side effects associated with SSRIs, it is imperative to discover newer targets for the treatment of depression [58,59]. Table 1 enlists monoamine inhibition data of approved MAT inhibitors including vortioxetine and livomilnacipran that have recently been approved for the treatment of depression.

Triple reuptake inhibition: a panacea for depression?

To address the failure in full symptom remission following an antidepressant therapy, various augmentation approaches to SERT inhibition have been pursued. Triple reuptake inhibitors (TRIs) have emerged as a promising class that target SERT, NET and DAT at the same time. The concept of triple uptake inhibition has gained credence based on the evidence from the preclinical and clinical studies that dopamine plays an important role in the pathophysiology and treatment of depression. Dopaminergic neurons innervate brain in cortex, limbic and the pituitary gland the regions that are linked with cognition, motivation and reward [65–68]. Role of dopamine is suggested by the fact that depression amounts to 50% in patients with Parkinson’s disease (PD), a disease characterized

Figure 1. Representative structures of inhibitors from different classes of antidepressants.

DRI: Dopamine reuptake inhibitor; NRI: Norepinephrine reuptake inhibitor; SMS: Serotonin modulator and stimulator; SNRI: Serotonin/norepinephrine reuptake inhibitor; SSRI: Selective serotonin reuptake inhibitor; TCA: Tricyclic antidepressant.

by loss of dopaminergic neurons and reduction in DA transmission [69]. Biochemical evidences result from observations that concentration of homovanillic acid, a major metabolite of dopamine, is diminished in the cerebrospinal fluid of depressed patients [70]. It is reasoned that mesolimbic dopamine is associated with motivation and reward-related behavior and, there-

fore, including the dopamine activity should improve anhedonia that is the central component in depression [71,72]. Furthermore, it has been demonstrated that antidepressants could sensitize mesolimbic postsynaptic dopamine receptors, thereby implicating dopaminergic component in the therapy. This is validated by the findings that adjunct administration of a DAT

Inhibitor class	Inhibitor	Year approved	SERT (K _i , nM) [†]	NET (K _i , nM) [†]	DAT (K _i , nM) [†]
TCAs	Imipramine	1951	1	37	8500
	Trimipramine	1966	149	2450	1690
	Amitriptyline	1961	4	35	3780
	Opipramol	1961	N/A [‡]	N/A	N/A
	Desipramine	1964	18	1	3190
	Nortriptyline	1963	18	4	1140
	Protriptyline	1966	20	1	2100
	Dosulepin	1969	9	46	5310
	Butriptyline	1974	1360	5100	3940
	Clomipramine	1970	0.3	38	2190
	Doxepin	1969	68	30	12,100
	Maprotiline	1974	5800	11	1000
	Amoxapine	1980	58	16	4310
	Lofepramine	1980	70	5	18,000
	Amineptine	1983	N/A	N/A	N/A
SSRI	Zimelidine	1982	152	9400	11,700
	Fluoxetine	1987	1	240	3600
	Paroxetine	1992	0.1	40	490
	Fluvoxamine	1994	2	1300	9200
	Citalopram	1989	1	4070	28,100
	Sertraline	1991	0.3	420	25
	Escitalopram	2001	1	>10000	>10,000
SNRI	Sibutramine	1997	N/A	N/A	N/A
	Venlafaxine	1993	9	1060	9300
	Milnacipran	2009	420	77	6100
	Duloxetine	2004	1	8	240
	Desvenlafaxine	2008	40	558	>100,000
	Levomilnacipran [§]	2013	11	92	>10,000
NRI	Viloxazine	1977	17,300	155	>100,000
	Mirtazapine	1994	>100,000	4600	>100,000
	Atomoxetine	2002	152	5	658
	Reboxetine	1999	242	3	>100,000
DRI	Bupropion	1985	9100	52,000	520
NRI/DRI	Nomifensine	1977	1010	16	562

[†]Activity values taken from [60] and [61] unless otherwise specified.
[‡]N/A, no data available for human-cloned receptors.
[§]Activity values taken from [62].
^{*}Serotonin modulator and stimulator.
[#]Activity values taken from [63].
^{††}Activity values taken from [64].
 DAT: Dopamine transporter; DRI: Dopamine reuptake inhibitor; NET: Norepinephrine transporter; NRI: Norepinephrine reuptake inhibitor; SERT: Serotonin transporter; SNRI: Serotonin/norepinephrine reuptake inhibitor; SMS: Serotonin modulator and stimulator; SSRI: Selective serotonin reuptake inhibitor; TCA: Tricyclic antidepressant.

Table 1. List of monoamine transporter inhibitors as antidepressant agents (cont.).

Inhibitor class	Inhibitor	Year approved	SERT (K _i , nM) [†]	NET (K _i , nM) [†]	DAT (K _i , nM) [†]
SMS [¶]	Trazodone	1981	160	8500	7400
	Nefazodone	1994	200	360	360
	Vilazodone [#]	2011	0.1	56	37
	Vortioxetine ^{††}	2013	1.6	113	>1000

[†]Activity values taken from [60] and [61] unless otherwise specified.
[†]N/A, no data available for human-cloned receptors.
[§]Activity values taken from [62].
[¶]Serotonin modulator and stimulator.
[#]Activity values taken from [63].
^{††}Activity values taken from [64].
 DAT: Dopamine transporter; DRI: Dopamine reuptake inhibitor; NET: Norepinephrine transporter; NRI: Norepinephrine reuptake inhibitor; SERT: Serotonin transporter; SNRI: Serotonin/norepinephrine reuptake inhibitor; SMS: Serotonin modulator and stimulator; SSRI: Selective serotonin reuptake inhibitor; TCA: Tricyclic antidepressant.

blocker bupropion potentiates the action of both SSRI and SNRI in rat **forced-swim tests (FST)** and, furthermore, produced greater symptomatic improvement than when either drug is administered alone [73–76]. Moreover, piribedil a D2/D3 agonist and Pramipexole, a D3-preferring agonist, was found to be effective in both unipolar and bipolar depression [77,78]. Nomi-fensine, a DNRI, possesses significant dopaminergic activity and was formally approved as antidepressant [79]. Thus, an attractive strategy entails inhibiting the reuptake of all three monoamines: serotonin, noradrenaline and dopamine and developing TRIs for the treatment of depression [80,81]. Furthermore, it has been shown that treatment with bupropion improves SSRI-induced sexual dysfunction side effects [82,83]. A new era in the antidepressant therapy was ushered on the premise that a broader spectrum inhibition of the three major MAT would lead to enhanced efficacy and faster antidepressant response. Moreover, TRIs should be able to address broad range of depressive symptoms including anhedonia and also exhibit reduced side effects, which are associated with currently available antidepressants [84].

Our progress to the development of novel tris as potential antidepressant agents revisited

The research on TRIs in our laboratory stemmed from our interest in developing a pharmacotherapy for cocaine addiction. Initial attempts were directed in exploring the structural determinants in GBR 12909,

Key term

Forced-swim test (FST): A widely used behavioral animal model for evaluation of efficacy of antidepressant drugs. The test involves forcing a rodent to swim in a tank filled with water and their mobility behavior for escape is then scored. The reduction of immobility is a measure of antidepressant activity of a drug given that it does not increase the locomotor activity.

a known DAT inhibitor, which conferred high and selective affinity for the DAT. The foremost study demonstrated that piperazine ring of GBR 12909 could be replaced by a piperidine ring with retention of activity [85]. Our next effort led to the identification of highly selective and more potent DAT inhibitors than the parent molecule GBR12909 with a promise as cocaine antagonists [86–88]. In subsequent efforts, with an aim to elucidate the bioactive conformation of our lead compounds, medicinal chemistry approach came to forefront in the design of conformationally rigid piperidine analogs (**Figure 2**), which emerged as more potent and selective at DAT than GBR12909 [89–91]. Further efforts led to the bioisosteric substitution of the piperidine ring with a pyran moiety resulting in the discovery of the *cis*-3,6-disubstituted pyran template, which became an established pharmacophore moiety of our lead TRIs, representing a unique structural class not known to any existing MAT [92]. It was observed that the *cis*-pyran analogs, like their piperidine counterparts, were more active than the *trans*-isomer. Although the pyran-based compounds did not significantly interfere with activity at the DAT in some cases, they were in general more active at the NET and SERT compared with the piperidine counterpart [92].

In a related field, neurotransmitter blockers like SNRI and SSRI were widely used for the treatment of depression; albeit, applications of a broad spectrum approach to an effective antidepressant therapy were still nascent. In 2003, pioneering work from DOV Pharmaceuticals showed a TRI exhibiting an antidepressant effect [93,94]. Concurrently, research on neurotransmitter inhibitors in our laboratory have started to shed more light on molecular determinants important for developing a TRI [95]. In 2005, we reported the asymmetric synthesis and characterization of novel tri-substituted pyran derivatives (2*S*,4*R*,5*R*) as potent TRIs [96]. The synthesized compounds were evaluated

Figure 2. Structural transformation in the design of pyran-based triple reuptake inhibitors.

for their affinities for the DAT, SERT and NET by measuring their potencies in inhibiting the uptake of [^{3} H]DA, [^{3} H]5-HT and [^{3} H]-NET. Compounds were also tested for their binding affinity at the DAT by their inhibition of [^{3} H]-WIN 35,428. In general, tri-substituted pyran derivatives exhibited high affinity for SERT. Moreover, as expected, both regiospecificity and stereo selectivity played important roles as only derivatives with (-) 2*S*,4*R*,5*R* absolute configuration demonstrated appreciable activities particularly for SERT and NET. Furthermore, a *cis*-relationship between the benzhydryl moiety and the amino group was maintained for the SERT and NET interactions. Thus, starting from DAT selective inhibitors our endeavors have resulted in the discovery of several derivatives, including **D142**, which exhibited triple uptake inhibition profile with (K_i) values of 29.3, 14.7 and 59.3 nM (Table 2) for NET, SERT and DAT, respectively. Affinity of **D142** for SERT was comparable to fluoxetine, a well-known SSRI. Furthermore, SAR studies led to the development of several tri-substituted (2*S*,4*R*,5*R*) pyran derivatives including **D161** and **D185** that exhibited potent triple uptake inhibitory activities [97]. **D-161**, a TRI, was most potent for NET followed by its affinity for SERT and DAT (Table 2). In the monoamine uptake assays, the potency of **D-161** for NET and SERT was comparable to desipramine and fluoxetine or imipramine, respectively, indicating potent transporter blockade.

Empowered by molecules (Figure 3) with three different biological profiles belonging to SNRI, NRI and TRI classes of drugs, the TRIs were then evaluated for their potential antidepressant effect in standard *in vivo* animal models. **D-161** demonstrated potent *in vivo* activity in reducing immobility in preclinical antidepressant rat FST, and mouse tail suspension test [101]. In the FST, compared with vehicle control, **D-161** at 10 and 20 mg/kg doses caused significant reduction of immobility. The result from the tail suspension test

(TST) indicates that **D-161** could reduce immobility dose-dependently with the highest dose (20 mg/kg) producing maximum reduction of immobility. At a dose of 15 mg/kg, the effect of **D-161** in reduction of immobility was comparable to reference imipramine. Moreover, the results suggest that reduction of immobility did not result from activation of motor system as **D-161** failed to increase motor activity when tested in the same dose range under the same experimental conditions. **D-142** has emerged as another lead compound from our laboratory exhibiting potent antidepressant activity. In the rat FST, the dose range tested (2.5, 5 and 10 mg/kg), **D-142** was much more efficacious than the reference imipramine. In the mouse TST, **D-142** reduced immobility in a dose (2.5, 5 and 10 mg/kg)-dependent manner, suggesting potent antidepressant effect. In locomotor activity tests, compound **D-142** did not exhibit any stimulation in the same dose ranges. Our continued efforts [98,99] have resulted in the discovery of several TRIs, which are listed in Table 2. In a recent report, we have described the development of an orally active TRI, **D-473**, which demonstrated *in vivo* efficacy in FST and showed elevated levels of all three monoamines in microdialysis study [100]. We have also developed a pharmacophore model for TRIs and have also embarked on a novel tetrahydrofuran template which demonstrated balanced, albeit moderate, activities at all three neurotransmitters [102].

TRIs in preclinical & investigational stage

The structural differences between the MAT represent an enormous challenge to synthesize small molecules which can inhibit all three transporters appreciably while having improved bioavailability as well as broad safety profile. Moreover, until recently, no clinical data are available to pinpoint the optimal selectivity ratio of TRIs for all three transporters. However, it has been hypothesized that varying potency ratios can render different clinical outcomes which may be helpful for

Table 2. Binding affinity at dopamine transporter, serotonin transporter and norepinephrine transporter in rat brain.

Compound	[³ H]DA [†] uptake	[³ H]5-HT [†] uptake K _i (nM)	[³ H]DA [†] uptake
D-185 [‡]	62.4 ± 5.6	16.1 ± 1.6	12.6 ± 3.7
D-411 [§]	15.9 ± 1.7	12.9 ± 1.3	29.3 ± 4.8
D-161 [‡]	42.0 ± 3.3	29.1 ± 3.5	30.5 ± 7.8
D-142 [§]	37.4 ± 3.9	14.7 ± 2.1	29.3 ± 7.9
D-199 [¶]	34.6 ± 6.5	19.9 ± 1.5	54.3 ± 9.8
D-391 [§]	31.3 ± 10.6	40.1 ± 4.9	38.5 ± 6.0
D-471 [‡]	38.4 ± 2.6	58.4 ± 4.0	4.20 ± 2.3
D-478 [‡]	11.2 ± 2.8	3.0 ± 0.3	6.1 ± 2.4
D-451 [‡]	32.5 ± 4.3	69.1 ± 19.8	8.4 ± 1.7
D-168 [‡]	85.2 ± 8.2	25.0 ± 8.4	25.5 ± 9.6
D-473 [#]	70.4 ± 16.7	9.18 ± 1.69	39.7 ± 7.6
Fluoxetine	1092 ± 98	12.2 ± 2.4	158 ± 58
Reboxetine	>10,000	503 ± 61	0.69 ± 0.21

[†]For uptake by DAT, SERT and NET, [³H]DA, [³H]5-HT and [³H]NE accumulation were measured. Results are average ± SEM of three to eight independent experiments assayed in triplicate.

[‡]See [98].

[§]See [99].

[¶]See [96].

[#]Uptake into cells expressing human DAT, SERT and NET [100].

specific symptom-related treatments. For example, a specific potency ratio could be useful to treat pain, whereas another specific potency ratio may be useful to treat attention-deficit hypertension disorder. Here, we summarize TRIs that have been pursued to pre-clinical and investigational stage (Figure 4 & Table 3). **DOV 220,075** or 1-(4-methylphenyl)-3-azabicyclo [3.1.0] hexane (Bicifadine) was developed as analgesic and for the treatment of pain by the DOV Pharmaceuticals [103]. It has stronger affinity toward SERT and NET compared with DAT (Table 3). Phase III clinical trial for this compound for pain indication has been completed. The half-life and $T_{\max}$ of Bicifadine were found to be 1.6 and 1.06 h, respectively [104].

Ro 8-4650 or diclofensine is a tetrahydroisoquinoline-based TRI that showed somewhat stronger potency for DA and NA compared with 5-HT (Table 3). In clinical trials, the compound showed efficacy to be an antidepressant with relatively less side effects [131]; however, due to its potential drug abuse issue, the compound was withdrawn from further clinical trials.

NS-2214 or **BMS-204756** (brasofensine) is a phenyltropane-based TRI [107] and developed by the Bristol–Meyers Squibb company and later codeveloped by the NeuroSearch company. Due to its strong potency toward DAT over SERT and NET, it was a potential candidate to treat L-DOPA-induced dyskinesia in PD [132]. It was able to increase locomotor

activity in MPTP-treated marmosets dose dependently. The compound is found to be metabolically more stable in human than rat [133]. Although it had shown good tolerability in Phase II clinical trial, further development was halted due to *cis-trans* isomerization of the aldoxime. Maintaining the same pharmacophore, GSK developed **NS-2359** which is an analog of **NS-2214**. Primarily, it was being developed for ADHD [134]; however, further development was halted due to disappointing results from Phase II study [135]. **NS-2330**, the ethoxy analog of **NS-2214**, was initially developed for AD and PD [136]. Corresponding *N*-desmethyl compound (**NS-2360**) is the only metabolite and has slightly longer half-life (374 h) compared with the parent compound. Due to its comparatively low potency for DAT and weight loss side effect, later it was dropped from further development for PD.

Another azabicyclohexanes-based TRI, **DOV 216,303** was also developed by the DOV Pharmaceuticals. It was able to inhibit the transport of all three monoamines in recombinant human MAT *in vitro* with desired potency [109]. In *in vivo* study, **DOV 216,303** was able to reduce observed immobility both in the FST as well as in the tail suspension test. In clinical trial, **DOV 216,303** was able to improve the **Hamilton Depression Rating Scale (HAM-D)** scores significantly when citalopram was used as a control [109]. The concern related to addiction of

Figure 3. Structures of lead pyran-based triple reuptake inhibitors.

TRIs was alleviated in a recent study indicating that **DOV 216,313** has much less addictive-like properties compared with cocaine [137].

DOV 102,677 is the (-)-isomer of **DOV 216,303** and according to prediction, it showed nearly five- to eight-fold less potency at all three transporters; however, it has a 'balanced' potency, in other words, almost equal potency at all three transporters over the racemate and the (+)-isomer (Table 3) [110].

EB-1020 is being developed by the Euthymics company. It is a norepinephrine and dopamine-preferring TRI which modulates reuptake of NE, DA and 5-HT in a 1:6:14 ratio. It is being developed as a drug to treat attention-deficit hypertension disorder [111]. It has been hypothesized that it may exhibit potential less risk of drug abuse liability. In TST, a lowest dose of 20 mg/kg was able to produce significant inhibition of immobility. In microdialysis experiment, **EB-1020** was able to increase the extracellular concentration of NE significantly and DA to a lesser extent. In locomotor activity, only at a higher dose of 30 mg/kg it produced slight activity. **EB-1020** also has strong ability to cross the blood–brain barrier (BBB), which is evident from a preferential blood to plasma ratio of 8 [111].

Lu AA24530 or tedatoxetine, a serotonin and NE-preferring TRI which is being developed by the Lundbeck and Takeda company [112]. According to the Montgomery–Asberg depression rating scale (MADRS) for primary efficacy end point study, the compound showed significant improvement compared with placebo. It was able to increase acetylcholine (ACh), NE, 5-HT and DA in brain. In addition, **Lu**

AA24530 showed affinity to 5HT_{2C}, 5HT_{2A} and alpha 1A receptors [138]. Corresponding piperazine analog **Lu AA21004** or Vortioxetine has been recently clinically approved for the treatment of MDD. It is a serotonin and NE-preferring TRI [113,139]. Typical side effects are dizziness, vomiting, headache, nausea, diarrhea and lower sexual side effects [140].

Racemic analogs of venlafaxine, **PRC-025** and **PRC-050**, are serotonin- and NE-preferring TRIs developed by the Mayo Foundation [141]. Both compounds were able to reduce total immobility time in the TST and the FST when they were administered via intraperitoneally (ip.) at 5 and 10 mg/kg doses. The results were comparable to imipramine although at a higher dose (15 mg/kg). Both compounds did not stimulate locomotor activity at 5 mg/kg dose. However, **PRC-050** produced significant locomotor activity at 10 mg/kg dose in rat. On the other hand, **PRC-025** produced a significant increase in locomotor activity in mice at 10 mg/kg dose [114]. **PRC200-SS**, an optically pure enantiomer of **PRC-050**, inhibited uptake of serotonin, NE and dopamine with *K_i* values of 2.1, 1.5 and 61 nM, respectively. Similar to imipramine, **PRC200-SS** also reduced immobility in both FST in rats and tail suspension test in mice in a dose-depen-

Key term

Hamilton Rating Scale for Depression (HAM-D or HDRS): HAM-D is a widely used scale for measuring severity of depression in patients. It involves answering 17 or 21 questions related to mood disorder. The higher the HAM-D score the more severe is the depression.

Figure 4. Example of triple reuptake inhibitors in preclinical and investigational stage (cont. from facing page).

dent manner. Furthermore, peripheral administration of **PRC200-SS** (5 and 10 mg/kg ip.) significantly enhanced extracellular concentrations of NE and serotonin in the medial prefrontal cortex and dopamine and serotonin levels in the core of nucleus accumbens [115]. Its clinical development was terminated because of dose-proportional kidney toxicity observed in preclinical studies in cynomolgus monkeys [142]. Tropane scaffold-based compound **WF-23** is an analog of cocaine and exhibited nearly 1000-fold potency for all three transporters [116]. It has very high bioavailability and exhibited slow metabolism [143]. With a dose of 1 mg/kg, it was able to produce significant increase in locomotor activity [144].

The Sunovion Pharmaceuticals has a series of TRIs based on novel cyclohexane alkyl amine scaffold. The *N*-monomethyl compound **1** is a dopamine and NE-preferring TRI which has a half-life of 0.53 h and inhibited hERG at 23 μ M (IC_{50}) [117]. Corresponding *N*-dimethyl compound **2** is a NE and serotonin-preferring TRI which has a half-life of 0.32 h and inhibited hERG at 40 μ M (IC_{50}). Compound **3** in which the 3,4-dichlorophenyl group was replaced by a 2-naphthyl group, is a serotonin and NE-preferring TRI which has half-life of 0.28 h and inhibited hERG at 4.3 μ M (IC_{50}). The three compounds (**1**, **2** and **3**) were tested at 5, 10 and 30 mg/kg in animal study, respectively. They have good BBB crossing capability and have shown good concentration in brain levels. All three compounds were able to reduce total immobility time by 30–33% in the TST at 30 mg/kg and also reduced locomotor activity to a great extent [117].

SKF83959, (3-methyl-6-chloro-7,8-hydroxy-1-(3-methylphenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine), has been discovered as a competitive inhibitor of SERT but a noncompetitive inhibitor of DAT and NET [118]. **SKF83959** was also active in animal models as it reduced immobility in the FST and the TST [118].

Han *et al.* have developed azetidine-based TRIs. 3-(Naphthalen-2-yl(propoxy)methyl)azetidine (**4**) and 3-(naphthalen-2-yl(phenoxy)methyl)azetidine (**5**) are serotonin- and norepinephrine-preferring TRIs.

Both compounds have good half-life (4.8 and 3.3 h, respectively, p.o.) and the BBB crossing ability. In FST, compound **5** was able to reduce total immobility in a dose-dependent manner [119]. However, due to the presence of naphthyl groups, both compounds have inhibited hERG at low concentrations which may lead to serious side effects.

The Sunovion Pharmaceutical has been developing octahydro-1-H-isoindole-based TRIs which are structurally similar to DOV analogs. Compound **6** exhibited ‘balanced’ potency at all three transporters and was able to reduce total immobility time dose-dependently in the TST at 10 and 30 mg/kg doses. It did not increase locomotor activity compared with the control groups. The compound has good human microsomal stability (262 h) and inhibited hERG at 5.28 μ M (IC_{50}). In addition, the compound exhibited good BBB crossing ability and brain to plasma level (25:1) [120].

5-APB or 5-(2-aminopropyl)benzofuran was originally synthesized at the Eli Lilly. Most recently, it was reported by Iversen *et al.* that **5-APB** has a ‘balanced’ TRI activity [121]. However, the compound may lead to severe cardiotoxicity because of its potent 5-HT_{2B} agonist property. Due to its structural similarity with MDA, the compound is sold as a controlled substance in the market [145].

The Accenta Pharmaceuticals has utilized a hybrid approach to combine key pharmacophoric moieties of Nocaine (DAT/NET selective) and Modafinil (DAT preferred) to develop the TRI **JZ-IV-10**, which exhibited improved potency for all three transporters. The compound has ‘balanced’ affinity for all three transporters [122].

Lu 19-005 or Indatraline was developed by the Lundbeck company [146]. It is a serotonin and NE-preferring TRI [147]. It has a similar mode of action like cocaine, but slower onset of action and potentially be useful to treat various drug addiction issues [148].

JNJ-7925476 is an isoquinoline scaffold-based TRI that is being developed by the Johnson and Johnson company [124]. This racemic compound is a serotonin

Table 3. Physicochemical properties, binding affinities and functional potencies of investigational triple reuptake inhibitors.

Compound	Mol wt.	CLogP	LE	Potency [3H]DA:[3H]NA:[3H]5-HT IC ₅₀ (nM)	Affinity DAT:NET:SERT Ki (nM/I)	Ref.
DOV-220,075	209.72	2.2	27.4	910:55:117	5.2:5.0:2.4 (μM)	[103]
RO-84650	322.23	5.0	15.3	16.8:15.7:51	10.9:8.8:10.3	[105,106]
NS-2214	327.25	4	54	03:13:13	0.79:3.13:18.0 (EC ₅₀)	[107,108]
NS-2330	314.25	3.6	10.9	01:07:7	N/A	[80]
DOV-216,303	228.12	3	22.5	78:21:14	210:260:100	[109]
DOV-102,677	228.12	3	22.5	129:103:133	220:1000:740	[110]
EB-1020	209.29	2.9	30.5	0.2773495	N/A	[111]
Lu-AA24530	283.43	4.5	22.6	X:23:8	DA<NE<SE	[112]
Lu-AA21004	298.45	4.9	27.3	890:140:5.4	N/A:113:1.6	[113]
PRC-025	311.46	4.9	14.3	53:10:06	53:10:06	[114]
PRC-050	291.3	4.2	13.6	53:10:06	53:10:06	[114]
PRC200-SS	291.3	4.2	13.6	18:0.6:2 (Kd)	61:1.5:2.1	[115]
WF-23	307.43	4.2	17.9	0.65:0.32:0.47	0.12:0.39:2.9	[116]
1	286.24	5.3	13.9	30:57:81	N/A	[117]
2	300.27	5.8	14.8	36:10:12	N/A	[117]
3	281.44	5.7	21.6	28:21:<1	N/A	[117]
SKF83959	317.81	4	6.7	1375:381:242	9010:600:1430	[118]
4	255.35	2.9	17.4	12:06.8	N/A	[119]
5	289.37	4.6	21.3	35:8.3:3.1	N/A	[119]
6	270.2	4.7	17.9	140:150:53 (pIC ₅₀)	N/A	[120]
5-APB	175.23	2.3	16.2	6.3:6.3:5.8 (pKi)	265:180:811	[121]
JZ-IV-10	354.94	3.1	13.2		1.0:0.8:1.1	[122]
Lu 19-005	292.2	4.8	21	11.9:8.8:5.1	1.7:5.8:0.42	[105,123]
JNJ-39836966	273.37	4.4	37.1		1.6:4.1:0.3	[124]
7	300.19	2.7		19:08.8	N/A	[125]
8	328.28	5.6	7.3	N/A	8.6:8.2:8.2 (pKi)	[126]
9	279.42	5.5	25.3	86:28:9	41:99:3.6	[127]
10	306.23	4.9	19.7	72:73:107	N/A	[128]
11	300.22	3.6	10.6	8.0:8.9:8.6	7.0:8.4:9.5	[129]
DOV-21,947	228.12	3	22.5	96:23:12	213:262:99	[93]
SEP-225289	251.79	4.9	15.9	8 ± 7:17 ± 3:10 ± 8	49:20:15	[130]

DA:NA:5-HT: Dopamine:norepinephrine:serotonin; IC₅₀: Concentration required for 50% inhibition *in vitro*; Ki: Binding affinity of the inhibitor; LE: Ligand efficiency; N/A: Not available.

and dopamine-preferring TRI and corresponding (+)-diastereomer **JNJ-39836966** is the most potent. **JNJ-7925476** exhibited higher potency toward human transporter than rat. In microdialysis study, it was able to increase the extracellular level of all three neurotransmitters at 1 mg/kg dose; however, for DAT occupancy, a higher dose (10 mg/kg) was required to reach the same level as SERT and NET. The compound has good $T_{\max}$ of 1 h and good BBB penetration ability. It has a half-life

of 1.8 h in plasma and was able to increase extracellular level of all the neurotransmitters considerably [124].

Lee *et al.* have recently reported that tetrazole-containing compound **7** exhibited a 'balanced' TRI profile [125]. The compound has relatively higher half-life in human liver microsome compared with rat. It showed very moderate or minimal activity against other aminogenic receptor and CYP isoforms. It was able to increase 5-HTP-induced head-switches dose dependently. In FST and

TST experiments, it was able to reduce the total immobility time significantly. In addition, the compound has a 5.48 h of elimination half-life and approximately 43% bioavailability when it was administered via PO [125].

Lucas *et al.* at the Roche company have developed pyrrolidine scaffold-based TRI. The 3,3-substituted pyrrolidine (**8**) has ‘balanced’ potency at all three neurotransmitter, however, it exhibited short half-life in human liver microsome [126].

Based on the scaffold of WF-23, the Eli Lilly company has developed TRIs. The 2-naphthyl-based bicyclic analog (**9**) is a serotonin and NE-preferring TRI and was able to exhibit good oral activity [127]. However, it has very low oral bioavailability due to CYP-450-mediated hydroxylation of the naphthyl moiety. Several other analogs of this series were made by replacing the naphthyl moiety with benzothiophene; however, further development was halted due to unspecified reasons.

Researchers at the Sepracor company modified the tetrahydronaphthalene-based structure of *trans*-setraline and developed a positional isomeric compounds, which exhibited TRI-like profile. However, majority of the TRIs in this series have shown strong inhibition of CYP and hERG. Overall, they chose compound **10** for *in vivo* studies based on its potency and liability. In TST, compound **10** was able to reduce overall immobility time in a dose-dependent manner and showed no effect on locomotor activity. The compound also showed good BBB crossing ability and at a dose of 30 mg/kg, a plasma ratio of 40 was observed [128].

Bettati *et al.* have developed oxa-azspiro derivatives as novel TRIs. Among these derivatives, compound **11** has exhibited high affinity for all three transporters. In functional potency assay, **11** showed comparable values similar to indatraline. In CYP₂D₂ bacosome P450 inhibition assay, **11** exhibited an excellent inhibition profile ($IC_{50} > 10 \mu M$). The compound has long *in vivo* half-life (2.2 h) and exhibited a good brain to plasma ratio of 9.6. However, the compound undergoes extensive metabolism and as a result only overall 9% bioavailability was observed [129].

TRIs in clinical trials

Based on the promising results from the animal model of depression, a number of TRI drug candidates have been advanced to clinical development [109,115,149]. These include DOV 216,303, DOV 21,947, BMS 820836, GSK 1360707, GSK 372,475 and SEP 225289 (Figure 5).

DOV 216,313 was safe and well tolerated in both single and multiple dose studies with healthy individuals. Safety and tolerability of DOV 216,303 was explored in a randomized, double-blind Phase II study on 67 subjects with moderate to severe MDD. Subjects received either 100 mg DOV 216,303 (50 mg b.i.d.) or 40 mg citalopram (20 mg, b.i.d.) for 2 weeks. Time-dependent reductions in HAM-D scores were observed in both the DOV 216,303 and citalopram groups compared with baseline scores ($p < 0.0001$). There was no significant difference in the side effect profiles of DOV 216,303 and citalopram. It should be noted that in this study a placebo control was not used [109,150].

DOV 21,947 (amitifadine) is the (+)-isomer of DOV 216,303 and has triple reuptake inhibitory profile with preference for SERT. It was able to reduce immobility both in the FST and in the TST [93]. It did not increase the locomotor activity significantly at doses 10 and 20 mg/kg compared with the positive control MK-801 [93]. In a 6-week randomized, double-blind, placebo-controlled study, amitifadine (EB-1010/DOV 21,947) was given in doses of 50 and 100 mg/day to a group of 63 patients with MDD [151]. The results demonstrated significant antidepressant activity and tolerability relative to placebo. Recently, amitifadine has undergone Phase IIb/IIIa clinical trial in the treatment-resistant 342 MDD patient group. The clinical trial (NCT01318434) involving paroxetine- and placebo-controlled study of 50 and 100 mg/day of amitifadine. The study did not show a statistically significant difference from placebo in the primary end point of a change in MADRS. How-

Figure 5. Triple reuptake inhibitors in clinical trials.

ever, the lack of side effects and signs of efficacy suggest that higher doses may show benefit in the target MDD patient group.

SEP-225289 was developed by the Sunovion Pharmaceuticals Inc. It has a very long half-life of 14 h and showed promising pharmacokinetic properties and tolerability in Phase I study. Although in *in vitro* study the compound exhibited good potency for all three transporters [152]; *in vivo* drug occupancy studies using PET revealed that it has low potency for serotonin and thus may not impart strong antidepressant effect [130]. SEP-225289 in a Phase II clinical trial (NCT00584974) on a double-blind, placebo- and active-controlled study on a 523-patient group with MDD did not result in reduction of symptoms of depression following 8 weeks of treatment. In contrast, positive control venlafaxine did achieve a significant reduction in MDD symptoms as compared with placebo. Furthermore, the serum concentration of SEP-225289 was observed to be lower than that expected at both doses (0.5 and 2 mg) as compared with exposure profiles observed in its Phase I study. Development of this drug has been discontinued.

In two placebo- and active-controlled, double-blind 10-week studies, the safety, efficacy and tolerability of GSK-372,475 were evaluated. Study 1 had patients with MDD while study 2 required patients with decreased pleasure and interest. In both studies, GSK-372,475 did not differ significantly from placebo. Furthermore, GSK-372,475 exhibited side effects like dry mouth, headache, insomnia and was not well tolerated [135].

In a Phase I PET study (NCT01153802) in healthy subjects pharmacokinetics and safety of single dose of GSK-1360707 was evaluated [153]. The study demonstrated that GSK-1360707 was well tolerated up to a dose of 150 mg. However, its further development was halted due to strategic reasons of the company.

BMS-820836 is a triple reuptake inhibitor developed by the Bristol-Myers Squibb. It exhibited high binding affinity for SERT, DAT and NET with IC_{50} values of 1.08, 5.67 and 7.99 nM, respectively, in human embryonic kidney 293 cells. It has a half-life of 34–57 h and T_{max} of 5.0–7.2 h [154]. BMS-820836 had undergone two Phase IIb clinical trials. BMS-820836 indicated high level of safety and tolerability in human [154,155]. Development of BMS-820836 has been terminated as it turned out that the compound acted as a SNRI due to low occupancy of DAT, underscoring importance of *in vivo* receptor occupancy study [154].

It is promising that a number of TRIs drug candidates have entered Phase II clinical trials with a

strong evidence of supporting the hypothesis that a TRI could be efficacious in patients with MDD. However, there have been set backs in Phase IIb clinical trials some of which are attributed to a number of assumptions not tested early on in the clinical studies. The doses selected for the SEP-225289 and DOV 21,947 studies were calculated on allometric scaling between rodents and humans and the target occupancy in humans was not determined. Furthermore, it was assumed that healthy volunteers and patients with treatment-resistant depression would have the similar level of target occupancy [156]. The success of a clinical trial also depends upon patient compliance. The Phase Ib study of DOV-21,947 faced an issue of patient noncompliance which had the effect in assessing the safety and efficacy of drug [157]. It is perceived that better understanding of the optimal inhibition of the three MAT, integration of translational research including a biomarker for functional neuroimaging of reward circuits, well-characterized dosing regimen and target population may provide a breakthrough in getting a TRI in clinic for the effective management of MDD.

Proposed transporter occupancy for a TRI

From various receptor occupancy, PET imaging studies with clinically approved antidepressants in humans, it has been established that an occupancy of 70–80% is required for the SERT and 50–70% is required for the NET for production of an antidepressant effect [153,158–160]. Also it has been established that to produce a dopamine effect without producing any addiction liability, an occupancy of 30–40% is required for the DAT [161]. This occupancy was exhibited by a DAT-blocking antidepressant, bupropion, in a PET imaging study in humans [161]. Therefore, it is hypothesized that an ideal TRI with above *in vivo* transporter occupancy at DAT, SERT and NET should produce optimal efficacy.

Potential of TRIs beyond antidepressant

Although the involvement of dopaminergic component in depression led to the development of TRIs having varied scaffold diversity, several recent clinical and preclinical studies revealed that they may have good potential to treat a broad range of diseases as described below.

- Obesity: during the preclinical and clinical studies of tesofensine, weight loss was an adverse side effect [162]. This side effect had prompted the researchers at NeuroSearch to find out the efficacy of the compound in weight loss by using various doses

and studying its metabolic profile. The compound proved to be efficacious in weight loss. Sibutramine, was approved by the US FDA to treat obesity but was later withdrawn from market owing to its cardiovascular side effects [163]. Antiobesity effect of another TRI, DOV 21,947 has also been demonstrated in both preclinical and clinical studies [164,165].

- Antinociceptive effect: several preclinical studies indicated that the TRIs have strong potentials to act as antinociceptive agents. For example, bicipadine showed promising effect in animal model of pain and inflammation [103].
- Treatment of addiction: DOV 102,677 has a 'balanced' TRI profile and in preclinical study, it was able to reduce alcohol consumption habits in rats without affecting others habits. Since dopamine has been linked to reward-related behaviors, it has been hypothesized that the TRIs can provide improved treatment regimen for addiction due to their dopamine reuptake inhibition property [166].
- Treatment of ADHD: several dopamine and NE-preferring TRIs in preclinical trials for the potential use in ADHD [167].

Conclusion

The previous survey reveals that development of TRIs is an active field of research. Many compounds showed promising results in clinical as well as preclinical studies. Although the optimal potency ratio for all three transporters is yet to be established, it has become apparent that optimal *in vivo* occupancy of transporters has a greater significance to produce efficacy in the clinic. Thus, further systematic future

clinical studies will be able to shed more light into it. In addition, these future clinical studies will also reveal whether the TRIs have any broader advantages over the SSRIs or SNRIs in terms of efficacy, early onset and side effects. We hope that this review will stimulate further interest in the development of TRIs.

Future perspective

Despite having about 30 drugs available for the treatment of MDD, there still exist a significant pressing unmet need for development of novel drugs with improved efficacy. The coming decade will present opportunities for major pharmaceutical players and researches to tap into this unmet need for developing efficacious agents demonstrating rapid antidepressant response with higher efficacy. Coming years may see a personalized treatment approach for patients with depression in order to increase remission rates. Development of suitable TRIs can provide solution to such challenges. Further research will lead to a better understanding of optimal receptor occupancy for the SERT, DAT and NET resulting in clinical success of future TRI as drug candidates. In the next decade or so, it is estimated that number of late-stage products will enter the MDD market including TRIs.

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Executive summary

- Monoamine deficiency has been proposed as one of the validated hypotheses for development of major depressive disorder.
- Structure of leucine transporter, from the bacterium *Aquifex aeolicus* is currently used as a surrogate model for human monoamine transporters.
- Selective serotonin reuptake inhibitors and serotonin/norepinephrine reuptake inhibitors are the current first-line treatment options for major depressive disorder.
- Preclinical and clinical studies have demonstrated that dopamine plays an important role in the pathophysiology and treatment of depression.
- Triple reuptake inhibitors inhibiting uptake of SERT, NET and DAT in a calibrated way are a novel class of drug and has been hypothesized to produce greater efficacy than existing antidepressants.
- D-473 is a novel triple reuptake inhibitor (TRI) with potent *in vivo* efficacy which is currently under development.
- Several inhibitors have been investigated in preclinical stage. Few candidates including DOV 216,303, DOV 21,947, BMS 820836, GSK 1360707, GSK 372,475 and SEP 225289 have gone through clinical trials.
- Proposed occupancy required for a TRI for producing superior antidepressant effect is 70–80% for SERT, 50–70% for NET and 30–40% for DAT.

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