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Design, synthesis, and biological evaluation of new monoamine reuptake inhibitors with potential therapeutic utility in depression and pain

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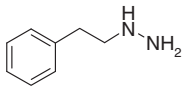
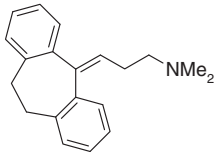
ABSTRACT

Two new series of monoamine triple reuptake inhibitors (TRIs) have been discovered through scaffold homologation of our recently reported series of 3,3-disubstituted pyrrolidine TRIs. The regioisomeric 2- and 3-ketopyrrolidines demonstrated high levels of potency against all three monoamine transporters as well as good human in vitro stability, low drug–drug interaction potential and a decreased propensity for hERG channel binding. Representative compounds from these series displayed good in vivo pharmacokinetics and high monoamine receptor occupancies which are indicators of good brain penetration.

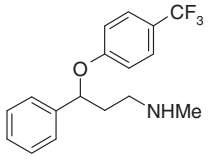
Depression is an often disabling condition that will affect greater than 15% of the general population in their lifetime.[1] The etiology of the disease was widely considered to be due to sub-optimal concentrations of the monoamine neurotransmitters serotonin (5-HT) and norepinephrine (NE) in the central nervous system (CNS). Although it is now apparent that depression is a consequence of dysfunctional endocrine, immune and neurotransmitter systems, the majority of antidepressants have been developed based on the assumption that an imbalance in monoamine concentration is the primary cause.[2] Early therapeutics, which included the monoamine oxidase inhibitors (MAOIs, e.g., Nardil®, **1**) and the tricyclic antidepressants (TCAs, e.g., Elavil®, **2**), treated a broad array of symptoms commonly associated with depression (Fig. 1). This broad activity was largely due to their ability to increase the synaptic concentration of either two (5-HT and NE) or all three (5-HT, NE and dopamine (DA)) neurotransmitters. These therapeutics are far from ideal due to side effects caused by their 'off-target' pharmacology (muscarinic, α-adrenergic, histaminergic) and a potential to cause drug–drug interactions. One breakthrough in modern antidepressant therapy came in the 1980's with the introduction of the serotonin selective reuptake inhibitor (SSRI) class of drugs (e.g., Prozac®, **3**).[3] The serotonin reuptake transporter (SERT) is located on the pre-synaptic nerve terminals and is



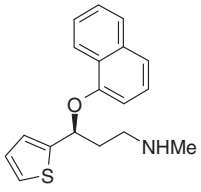
1, Phenelzine (Nardil), a MAOI



2, Amitriptyline (Elavil), a TCA



3, Fluoxetine (Prozac), an SSRI



4, Duloxetine (Cymbalta), an SNRI

Figure 1. Some marketed antidepressants.

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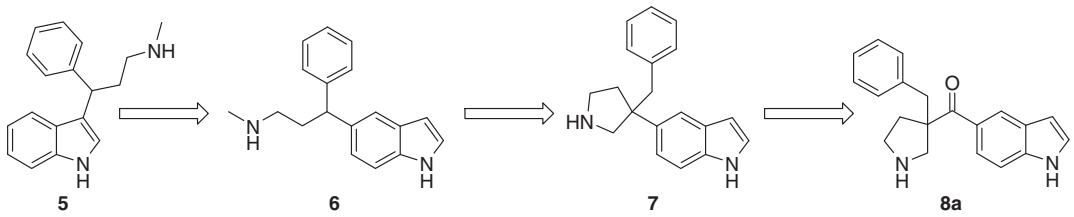


Figure 2. Design of novel triple reuptake inhibitors.

responsible for signal termination and recycling of 5-HT that is released into the synaptic cleft during synapse firing. Inhibition of this transporter results in the increased synaptic concentration of 5-HT and its duration of action, thus alleviating some of the symptoms of major depressive disorder (MDD) associated with low levels of 5-HT (mood dysregulation, anxiety and sleep disorders). Although these therapeutics targeted a smaller range of symptoms, their high level of selectivity led to improved safety and tolerability through a much reduced side effect profile. Unfortunately, full remission is only observed in approximately one third of treated patients. To address the remaining clinical need, serotonin norepinephrine selective reuptake inhibitors (SNRIs, e.g., Cymbalta®, **4**) have emerged that are able to treat a broader array of symptoms compared to the SSRI class. SNRIs augment synaptic NE levels as well as 5-HT while showing limited affinity for the receptors responsible for many of the side effects observed with the tricyclics.[4] This results in a tolerability and safety profile comparable to SSRIs while also addressing symptoms such as lack of energy, concentration, and co-morbid pain.[5] The SNRI class of antidepressants are more likely to result in remission, and some (e.g., Cymbalta®, **4**) are also approved to treat other diseases such as fibromyalgia and neuropathic pain.[6] Evidence also suggests that the SNRIs reduce anxiety as well as depression (through noradrenergic (NA) pathways) and are therefore more likely to benefit a set of patients that commonly suffer from anxiety in addition to depression (approximately 60% of patients).

Elevating DA levels in addition to 5-HT and NE have generated a class of therapeutics referred to as broad spectrum antidepressants or triple reuptake inhibitors (TRIs) which are expected to show superior efficacy to the SSRI and SNRI classes.[7] Increasing brain DA levels through dopamine transporter (DAT) inhibition should address the anhedonia, lack of motivation, and lack of attention components of MDD that the SSRIs and SNRIs do not, and may also result in a faster onset of action.[8] Recent clinical studies have suggested that combination therapy using bupropion, a DAT inhibitor, and an SSRI or SNRI offers improved efficacy in the treatment of depression compared with monotherapy alone.[9] Thus, TRIs may provide the next major advancement in antidepressant and pain therapy.

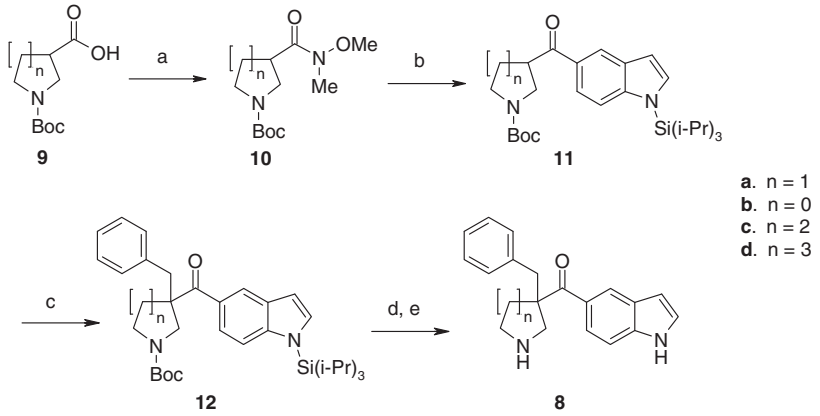
We recently reported on a series of 3,3-disubstituted pyrrolidine monoamine triple reuptake inhibitors (e.g., **7**)[10] that evolved from indolyl phenyl propylamine SNRIs (e.g., **5**)[11] which were developed into analogs with low nanomolar potency at all three transporters. Examples from this series also demonstrated good human in vitro microsomal and hepatocyte stability, high permeability and low drug–drug interaction potential. To further improve upon the pharmacological properties of this scaffold, we sought to reduce the *c*log *P*.[12] To this end, insertion of a carbonyl linker between the indolyl moiety and the pyrrolidine ring to generate analogs such as **8a** was investigated (Fig. 2). In addition to reducing lipophilicity, we hypothesized that the reduction in pK_a of the cyclic amine imparted by the carbonyl moiety might offer the additional benefit of reducing affinity at the hERG channel. Furthermore, the modular chemistry required to access this type of scaffold could facilitate the investigation of an expanded set of aryl or heteroaryl moieties as potential indole replacements.

Early results of this exercise were encouraging; **8a** showed comparable potency to **7** and in vitro screening demonstrated a significant improvement in stability in human liver microsomes

Table 1
Affinity at SERT[a], NET[a], DAT[a], human liver microsome clearance, hERG, and Caco-2 permeability measurements of **7** and **8a**

Compound	SERT	NET	DAT	HLM (μL/min/mg)	hERG IC_{20} (μM)	BA/AB (ER) (cm/s × 10^{-6})
7	8.0	9.0	7.9	100	>1	14/17 (0.82)
8a	8.2	7.7	8.1	36	6.1	19/17 (1.1)

[a] Monoamine reuptake pK_i values are the geometric mean of at least three experiments.



Scheme 1. First generation synthesis of 3-substituted 3-keto cyclic amines. Reagents and conditions: (a) MeONHMe·HCl, EDC, HOBt, DMF; (b) 5-bromo-1-(triisopropylsilyl)indole, *t*-BuLi, THF, −78 °C then **10**; (c) LiHMDS, BnBr, THF, 0 °C; (d) TBAF, THF; (e) HCl, MeOH.

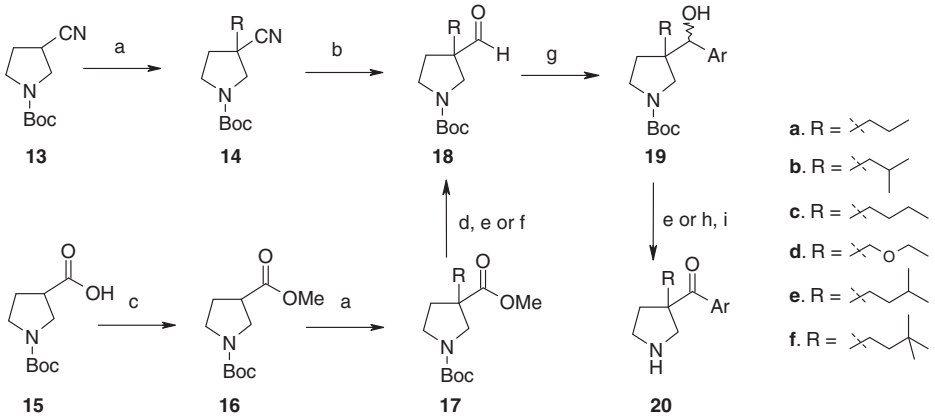
(HLM),[13] reduced hERG channel[14] affinity, and excellent membrane permeability[15] (Table 1). Herein we describe the development of several new scaffolds derived from the homologated ketopyrrolidine **8a**. We also highlight significant SAR and show selected in vivo data.

Our initial synthesis of 3-substituted 3-ketopyrrolidines is described in Scheme 1. It commenced with the commercially available *N*-Boc-β-proline **9a** which was converted to the Weinreb amide **10** and added to the lithiate generated from 5-bromo-1-(triisopropylsilyl)indole via halogen exchange. The resulting ketone **11** was alkylated by treatment with lithium hexamethyldisilazane (LiHMDS) and benzyl bromide to install the quaternary center to provide **12**. Finally, the protecting groups were removed using tetrabutylammonium fluoride (TBAF) followed by hydrogen chloride in methanol to provide **8a**. This methodology was readily extended to synthesize the analogous 3,3-disubstituted azetidines, piperidines, and azepines (**8b–d**) beginning with the appropriate *N*-Boc-amino acids. It should be noted that the 4,4-disubstituted piperidines were also accessible using this synthetic sequence.

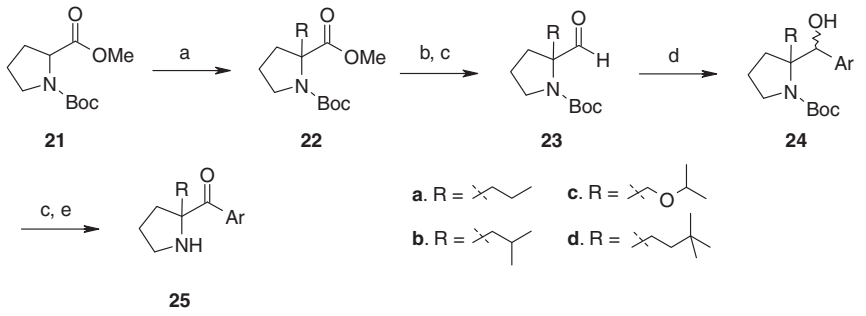
Although the ketone alkylation methodology was useful for the synthesis of preliminary analogs, it became apparent that the alkylation step was limited by the necessity for activated electrophiles such as benzylic or allylic halides in order to achieve reasonable yields of the hindered quaternary ketones. The alkylation became far less efficient or failed entirely when less reactive aliphatic halides were employed. An alternative and more general strategy toward 3-substituted 3-ketopyrrolidines was thus developed and is outlined in Scheme 2. The aliphatic side-chains **a–f** were installed through alkylation of the 3-pyrrolidinyl nitriles **13** or esters **16** with LiHMDS in the presence of the appropriate alkyl halides. With the quaternary center in place, the nitriles were converted to the corresponding aldehydes **18** via direct reduction using diisobutylaluminum hydride (DIBAL). Alternatively, the esters were subjected to a two step protocol involving initial lithium aluminum hydride ($LiAlH_4$) reduction to the alcohol followed by oxidation with either manganese dioxide or Dess–Martin periodinane. Subsequent treatment of **18** at low temperature with an aryl lithiate or Grignard reagent afforded the secondary alcohols **19** as a mixture of diastereomers. Finally, oxidation to the ketone and deprotection of the *tert*-butyl carbamate afforded the desired pyrrolidinyl ketones **20**. Scheme 3 outlines an analogous sequence that was employed to synthesize a variety of aryl and heteroaryl containing 2-pyrrolidinyl ketones (**25**) starting with commercially available *N*-Boc proline methyl ester **21**.

Seebach's Self-Regeneration of Stereochemistry (SRS) methodology was employed to access enantiopure 2-ketopyrrolidines (Scheme 4).[16] (2*R*,5*S*)-2-*tert*-Butyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one **26** (derived from L-proline) was allylated under kinetic conditions with retention of stereochemistry to give **27**. Mild silica gel mediated hydrolysis provided **28** which was elaborated to the desired product **31** through the usual sequence in reasonable overall yield and 90% ee (determined by chiral HPLC analysis of final product, **31**).

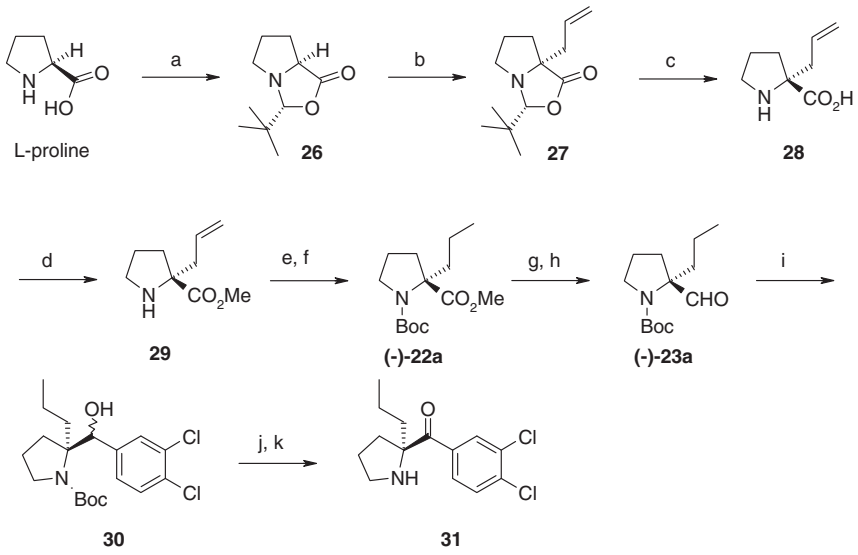
Encouraged by the initial results of 3-ketopyrrolidine **8a**, we proceeded to develop the SAR and identified the 7-fluoroindole **32** which had sufficiently good potency, stability, and permeability for us to investigate its in vivo properties (Table 2). In the mouse tail suspension assay, an assay used to evaluate antidepressant agents,[17] activity was observed at both 10 and 30 mg/kg, ip (data not shown). With this data in hand, our optimization focused on



Scheme 2. General synthesis of 3-substituted 3-ketopyrrolidines. Reagents and conditions: (a) LiHMDS, THF, −78 °C then RI or RBr; (b) $(i\text{-Bu})_2AlH$; CH_2Cl_2, −40 °C; (c) Cs_2CO_3, MeI, DMF; (d) $LiAlH_4$, THF, 0 °C; (e) Dess–Martin periodinane, CH_2Cl_2; (f) oxalyl chloride, DMSO, Et_3N; (g) ArLi or ArMgX, Et_2O, −78 °C; (h) MnO_2, toluene, 100 °C; (i) HCl, MeOH.

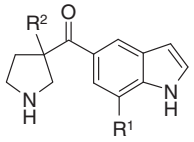


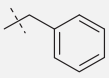
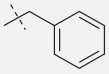
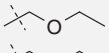
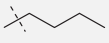
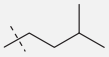
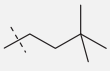
Scheme 3. General synthesis of 2-substituted 2-ketopyrrolidines. Reagents and conditions: (a) LiHMDS, THF, −78 °C, then RX; (b) $LiAlH_4$, THF, 0 °C; (c) Dess–Martin periodinane, CH_2Cl_2; (d) ArLi or ArMgX, Et_2O, −78 °C; (e) HCl, MeOH.



Scheme 4. Asymmetric synthesis of **31**. Reagents and conditions: (a) see Ref.[11]; (b) (i) *n*-BuLi, *i*Pr$_2$NH, THF, −78 °C; (ii) CH$_2$=CHCH$_2$Br, −78 °C to −30 °C, 58%; (c) silica gel, H$_2$O, MeOH, rt, 85%; (d) TMS–CH$_2$N$_2$, MeOH, 27%; (e) Boc$_2$O, DMAP, DCM, 93%; (f) H$_2$, 20% Pd(OH)$_2$/C, MeOH, 81%; (g) LiAlH$_4$, THF, 0 °C, 76%; (h) Dess–Martin periodinane, DCM, 0 °C, 75%; (i) 3,4-dichlorophenylMgBr, THF, 0 °C, 79%; (j) Dess–Martin periodinane, DCM, 0 °C, 78%; (k) HCl, MeOH, 100%.

Table 2
Affinity at SERT[a], NET[a], DAT[a], CYP2D6, and human liver microsome clearance



Compound	R^1	R^2	SERT	NET	DAT	CYP2D6 IC$_{50}$ (μM)	HLM (μL/min/mg)
8a	H		8.2	7.7	8.1	—	36
32	F		8.2	8.0	7.9	0.08	35
(+)-**32**			8.4	8.1	8.1	0.07	25
(−)-**32**			6.7	7.7	6.7	0.34	—
33	F		8.5	8.2	7.8	2.3	6
(+)-**33**			8.8	8.6	7.8	0.8	6
34	F		9.0	8.6	8.1	1.0	3
(+)-**34**			9.4	8.9	8.3	1.3	8
35	F		8.5	7.7	7.1	4.1	10
36	F		8.9	8.6	8.2	0.75	27
(+)-**36**			9.2	9.0	8.4	0.04	12
37	F		9.1	9.1	8.8	0.05	43
(+)-**37**			9.4	9.3	9.0	—	32
38	F		9.1	8.9	9.1	1.8	15
(+)-**38**			9.3	8.9	9.2	0.6	18

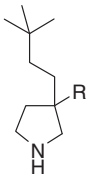
[a] Monoamine reuptake pK_i values are the geometric mean of at least three experiments.

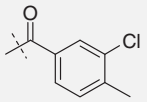
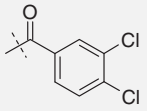
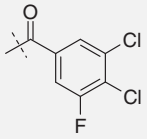
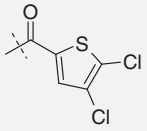
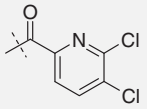
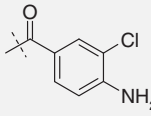
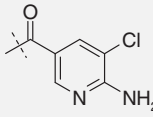
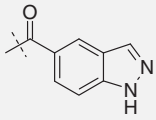
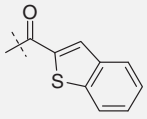
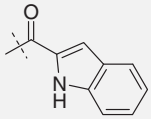
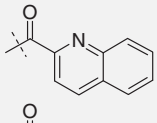
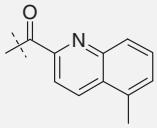
improving the stability in HLM and reducing the potent CYP2D6 inhibition[18] observed for this compound. Separation of the enantiomers by chiral HPLC revealed that one enantiomer (+)-**32** not only retained most of the potency for binding to the monoamine transporters, but also for CYP2D6. Replacing the benzyl side-chain with a propyl group offered a significant advantage with respect to stability in HLM, providing molecules that were equipotent at the transporters and predicted to be of high stability. While there was a 10-fold improvement with respect to the CYP2D6 inhibition, it still remained at an undesirable level (see (+)-**32** and (+)-**33**). Insertion of a polarizing heteroatom (**35**) reduced CYP2D6 affinity slightly, but at the expense of binding. During the course of this side-chain exploration an interesting trend was discovered. We found that by sequentially increasing the steric size of the hydrophobe, we were able to increase binding at all three transporters and in particular at DAT. Disappointingly, this trend was also accompanied by stronger CYP2D6 binding and increased clearance likely due to increased lipophilicity (see **33**, **34**, **36** and **37**). The dimethylbutyl side-chain, however, proved to be an outlier; **38** was notable for its excellent binding properties, moderate clearance, and somewhat weaker CYP2D6 affinity.

While indole analogs have proved to provide potent monoamine reuptake inhibitors,[10,19] there are potential safety issues related to certain indoles which could give rise to reactive metabolites.[20] We hypothesized that the indole itself, as opposed to the side-chain, might be implicated in the persistent CYP2D6 inhibition. Our attempts to substitute either the 2- or 3-position on the indole resulted in loss of potency at the transporters (data not shown). As a consequence we designed, synthesized, and evaluated a series of isosteric indole replacements, many of which possessed significantly reduced CYP2D6 inhibition (Table 3). We were successful in replacing the indole with a variety of simple monocyclic aryl and heteroaryl groups (e.g., **39**–**45**) while maintaining potent triple reuptake inhibitory profiles. Several heterobicyclic analogs with interesting in vitro profiles were also identified (e.g., **46**–**50**); among them was quinoline **49**, which proved particularly potent and balanced in its inhibition at all three transporters but demonstrated high affinity for CYP2D6. Installation of a 5-methyl group to generate **50** provided one of the our most potent compounds and concurrently improved the CYP2D6 and HLM profile relative to **49**.

While the 3-ketopyrrolidine compounds described in Tables 2 and 3 had excellent binding affinities for the monoamine reuptake transporters and acceptable hERG safety windows, the development of an asymmetric synthesis proved challenging. We had been simultaneously investigating modifications to the basic heterocycle and discovered several plausible alternatives that might either provide achiral molecules or, alternatively, offer established enantioselective syntheses. Some of these modifications relative to the 3-ketopyrrolidine **8a** are summarized in Table 4. Ring contraction to the azetidine **8b** met our desire for an achiral scaffold but also caused significant loss of potency. On the other hand, ring expan-

Table 3
Affinity at SERT[a], NET[a], DAT[a], CYP2D6, and human liver microsomal clearance

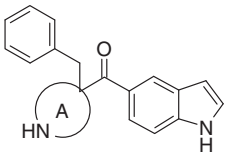


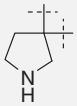
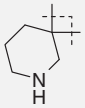
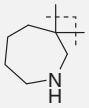
Compound	R	SERT	NET	DAT	CYP2D6 IC_{50} (μM)	HLM (μL/min/mg)
39		9.0	8.4	7.9	13	29
40		8.6	8.2	8.2	7.5	7
(+)-**40**		9.0	8.5	8.4	10	27
41		8.5	8.0	8.0	13	15
42		8.3	8.1	8.7	15	17
43		7.9	8.0	8.0	3	9
44		9.0	8.3	8.5	4	12
45		7.5	7.3	7.2	36	26
46		9.2	8.2	8.1	14	18
47		9.0	8.6	8.9	12	22
48		8.2	8.6	8.6	23	8
49		8.7	8.5	8.4	0.6	35
50		9.7	9.2	9.0	9	25

[a] Monoamine reuptake pK_i values are the geometric mean of at least three experiments

sion to the 3- or 4-ketopiperidines **8c** and **52** was very encouraging. These compounds were selected for additional SAR studies to be reported in due course. Further ring expansion provided the azepine **8d** which maintained some affinity at the transporters,

Table 4
Affinity at SERT[a], NET[a], DAT[a]



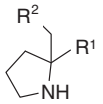
Compound	Ring A	SERT	NET	DAT
8b		6.1	6.8	6.1
8a		8.2	7.7	8.1
(−)-**51**[b]		5.9	8.1	7.4
8c		7.9	7.6	7.2
52		8.1	7.0	8.0
8d		7.1	6.6	6.6

[a] Monoamine reuptake pK_i values are the geometric mean of at least three experiments
[b] Racemate nor opposite enantiomer were synthesized.

but its inferior in vitro profile compared to the pyrrolidine and piperidine series led to its deprioritization. Perhaps the most exciting modification was the transposition of the basic nitrogen alpha to the carbonyl to generate the 2-ketopyrrolidine regioisomer (−)-**51** with a significantly reduced pK_a. While the SERT affinity of (−)-**51** was poor, the NET and DAT potency remained respectable. Data generated for other asymmetric scaffolds suggested that (+)-**51** should offer a more balanced profile. While (+)-**51** was not synthesized, the (+)-enantiomers of other members of this scaffold did provide more potent and balanced triple reuptake inhibitors than their antipodes (e.g., (+)-**55** vs **31**, Table 5).

Applying the SAR knowledge already developed (Table 2) to further improve this sub-series, we replaced the benzyl group with a propyl to generate **53** and were pleased with the high potency at SERT, NET, and DAT (Table 5). We next introduced a 7-F substituent to the indole (**54**), which improved HLM stability but showed strong CYP2D6 inhibition and increased affinity for the hERG channel (Tables 5 and 6). It has been proposed that the hERG channel prefers binding with lipophilic, basic substrates.[21] We had expected the reduction in calculated pK_a of the 2-ketopyrrolidines (cpK_a ~8) compared to the 3-ketopyrrolidines (cpK_a ~10) to reduce the relative hERG channel affinity, but this was not apparent. It appears that within this chemical series any positive effect gained by reducing pK_a was negated by the deleterious effect of the increased lipophilicity as measured by log *D* (Table 6). The increase in log *D* was also manifest in excellent permeability but sharp loss of stability towards HLMs. Replacing the indole with dichlorophenyl (**53** vs **55**) provided a further boost in lipophilicity which resulted in even stronger hERG channel binding and drastically reduced stability toward HLM. Ultimately a solution for this problem was found by introducing polarizing heteroatoms remote to the cyclic amine which reduced the overall log *D* of the molecule while maintaining the desired low pK_a. Thus, replacing the *para*-chloro moiety of **55**

Table 5
Affinity at SERT[a], NET[a], DAT[a], and CYP2D6, and human liver microsomal clearance



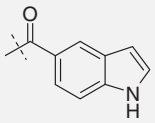
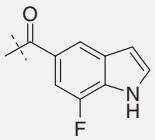
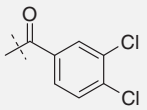
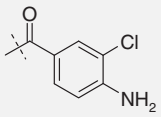
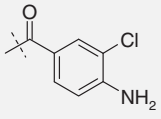
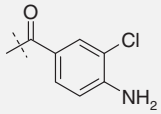
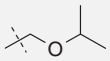
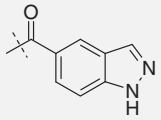
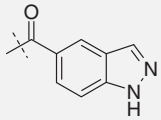
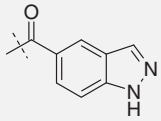
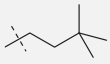
Compound	R^1	R^2	SERT	NET	DAT	CYP2D6 IC_{50} (μM)	HLM (μL/min/mg)
53			8.7	8.5	8.6	0.3	109
54			8.2	8.7	8.4	0.03	21
55			8.6	7.7	7.8	4	220
(+)-**55**			9.3	7.9	8.2	2	362
31			8.1	6.8	7.3	12	384
56			8.7	8.1	8.2	7	0
(+)-**57**			9.4	8.6	8.5	12	13

Table 5 (*continued*)

Compound	R^1	R^2	SERT	NET	DAT	CYP2D6 IC_{50} (μM)	HLM (μL/min/mg)
(+)-**58**			8.9	8.6	8.5	14	14
(+)-**59**			7.8	7.9	7.9	15	27
(+)-**60**			8.6	8.1	8.2	6	25
61			8.3	8.1	8.5	8	54

[a] Monoamine reuptake pK_i values are the geometric mean of at least three experiments.

Table 6
hERG, log *D*[a], Caco-2 permeability, and plasma protein binding for selected compounds

Compound	hERG IC_{50}/IC_{20} (μM)	Log *D*	BA/AB (ER) ($cm/s \times 10^{-6}$)	PPB h, r (%)
53	>10/7.3	1.45	10/41 (0.25)	63, 66
54	5.9/1.5	1.56	18/43 (0.41)	64, 65
55	3.1/1.2	2.77	8/19 (0.42)	86, 95
56	>10/6.1	0.86	19/37 (0.5)	42, 45
(+)-**58**	>10/3.1	0.84	18/30 (0.6)	61, 51
(+)-**59**	>10/7.9	1.0	16/29 (0.55)	2, 15
(+)-**60**	>10/4.2	1.0	20/32 (0.62)	20, 13
61	>10/4.0	2.03	12/20 (0.60)	50, 48

[a] Log *D* was measured using the shake flask method.

with an anilino-group (**56**) dramatically improved stability toward HLMs with a somewhat lessened affinity for hERG. Increasing the steric bulk of the side-chain (e.g., (+)-**57**) and inserting a second polarizing heteroatom (e.g., (+)-**58**) also provided molecules with good in vitro profiles. Likewise, replacing the indole in **53** with an indazole afforded additional drug-like molecules (see (+)-**59**, (+)-**60** and **61**). It should be noted that as the side-chain was extended and the log *D* approached 2 the HLM stability of the molecules again began to decrease ((+)-**60** vs **61**, Table 6). Compounds with lower measured log *D* also showed reduced plasma protein binding. These in vitro profiles translated into potent in vivo occupancies of the SERT and DAT transporters (Table 8) as well as behavioral paradigms (data not shown).

Pharmacokinetic properties were determined for representatives of several sub-series in the rat. As exemplified in Table 7, clearances in rat were generally high (a common characteristic of many marketed antidepressants) and oral bioavailability was low, however, microsomal and hepatocyte stability in human, dog and cynomolgus monkey was generally much better and this did not necessarily preclude compounds from undergoing additional in vivo assessment (data not shown). We were delighted when the improved in vitro profile obtained by replacing the indole of (+)-**38** with dichlorophenyl ((+)-**40**) translated into superior bioavailability, comparable to duloxetine (**4**). PK in higher species was also very good.

Table 7
Rat pharmacokinetic data for **4**, (+)-**38** and (+)-**40**

Compound	Species	$T_{1/2}$ (h)	CL (mL/min/kg)	*F* (%)	V_{ss} (L/kg)
4	Rat	1.5	178	22	20.9
(+)-**38**	Rat	1.6	153	5	14
(+)-**40**	Rat	1.2	125	57	14.3

Table 8
Receptor occupancy data for selected compounds in rats

Compound	$SERT_{Occ80}$ (mg/kg ip)	SERT:DAT Balance at $SERT_{Occ80}$
(+)-**33**	4	80:31
(+)-**38**	3.9	80:35
(+)-**40**	1.15	80:20
56	3.16	80:85
(+)-**57**	0.11	80:6
(+)-**60**	3.8	80:67

Several compounds were characterized in vivo to determine SERT and DAT receptor occupancies in rats.[22] High receptor occupancies are indicators of high brain penetration, and also allowed us to select compounds with diversified profiles for additional pharmacological testing to challenge the hypothesis that a TRI compound will have improved efficacy and faster onset of action than either an SSRI or SNRI. We established the dose that would provide 80% SERT occupancy and from this determined the corresponding DAT occupancy at the same dose as summarized in Table 8. We were pleased to find that we could obtain high occupancies at low ip doses for many of the preferred representatives, and that we could also cover a wide range of DAT occupancies. These compounds provide additional tools to help determine the optimal contribution of the dopamine transporter to best address the unmet clinical need for the treatment of major depressive disorder.

In summary, we have discovered several new series of novel and potent inhibitors of serotonin, norepinephrine and dopamine reuptake. The regioisomeric 2- and 3-ketopyrrolidinyl scaffolds have provided molecules with excellent in vitro properties as well as in vivo transporter occupancies at low doses. Molecules within these series possess therapeutic indices that permitted progression further towards the clinic. Further pharmacological studies for these compounds will be reported elsewhere.

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13. CL_{int} measured in HT format: total incubation volume 250 μL, 0.5 mg/mL microsomal protein, reaction started by adding NADPH and performed at 37 °C with no shaking. 40 μL aliquots at four time-points (0.1, 10, 20 and 30 min) were taken then the samples transferred to 384 well plates for LC/MS analysis.
14. hERG IC_{50}/IC_{20} values calculated using the PatchXpress 7000A (MDS, Inc. Toronto, Canada) to carry out electrical recording of K^+ currents in CHO cells expressing the human ERG channel. Recordings were made at 30 °C with test compounds at two concentrations (1 μM, 10 μM) or more.
15. Permeability was measured using Caco-2 cells at 21 days of culture. Efflux ratios are measured using the bi-directional (P_{app} A to B and B to A) values. Substrate concentration 10 μM.
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