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Identification of 1*S*,2*R*-milnacipran analogs as potent norepinephrine and serotonin transporter inhibitors

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Abstract—A series of milnacipran analogs were synthesized and studied as monoamine transporter inhibitors, and several potent compounds with moderate lipophilicity were identified from the 1*S*,2*R*-isomers. Thus, **15l** exhibited IC_{50} values of 1.7 nM at NET and 25 nM at SERT, which were, respectively, 20- and 13-fold more potent than 1*S*,2*R*-milnacipran **1**–**II**.

The antidepressant milnacipran (**1**, Fig. 1),[1] marketed as a racemic mixture, is a hydrophilic molecule ($\log D \sim 0$), and in this respect, differs from many CNS drugs such as atomoxetine **2** ($\log D = 0.8$).[2] Because of its low molecular weight and low lipophilicity, milnacipran exhibits almost ideal pharmacokinetics in humans, such as high oral bioavailability of ~85%, low inter-subject variability, limited liver enzyme interaction, moderate tissue distribution, and a reasonably long elimination half-life of ~8 h.[3] Its lack of potential for drug–drug interaction via cytochrome P450 enzymes is quite attractive because many CNS drugs are highly lipophilic and rely heavily on liver enzymes for elimination.[4]

The mechanism of action of milnacipran is believed to inhibit the monoamine uptake by the norepinephrine transporter (NET) and the serotonin transporter (SERT),[5] and milnacipran has a negligible activity at the dopamine transporters (DAT) and many monoamine receptors.[6] However, milnacipran has only a moderate potency at both human NET and SERT (Fig. 1), and its ratio at these two transporters is reported to be about 3:1.[7] Milnacipran is currently in phase III clinical trials for fibromyalgia, and recent reports have suggested a significant efficacy.[8] The SAR of milnacipran and its analogs based on in vivo efficacy was reported by Bonnaud and coworkers in 1987.[9] We have described the SAR of a series of *N*-alkyl and dialkyl amides, and potent analogs such as **3a** and **3b** were discovered.[10] Very recently, Roggen et al. reported the synthesis and SAR studies of a series of milnacipran analogs as single stereoisomers with a variation in the aromatic moiety.[11] Here, we report our continued efforts to discover potent 1*S*,2*R*-milnacipran analogs without a significant change in lipophilicity.

The milnacipran derivatives **6**–**8** were synthesized by a cyclization of the allyl esters **4**[12] to give the lactones **5**, which were elaborated to the desired products as a pair of enantiomers using a procedure similar to that for milnacipran (Scheme 1).[13]

The amide analogs of milnacipran **9**–**15** were prepared from phenylacetonitriles **16** and (*R*)-(−)-epichlorohydrin using a reported stereo-selective synthesis[11,13] or a modified procedure (NaHMDS/THF, Scheme 2).[14]

The target compounds **6**–**15** were tested in functional transport assays evaluating the inhibition of human

Keywords: Milnacipran; Stereoisomer; Monoamine transporter; Norepinephrine; Serotonin; Inhibitor; Structure–activity relationship; Lipophilicity; Metabolic stability; Permeability.
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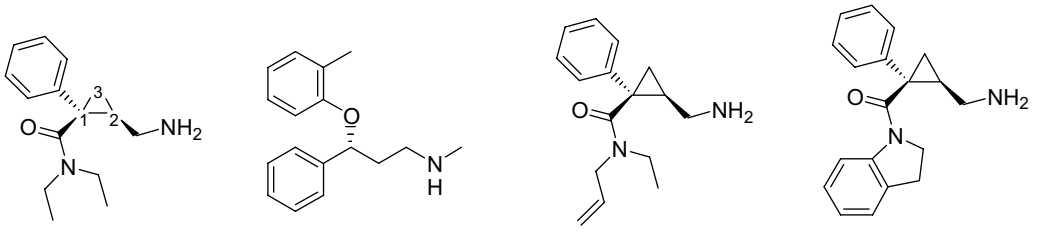
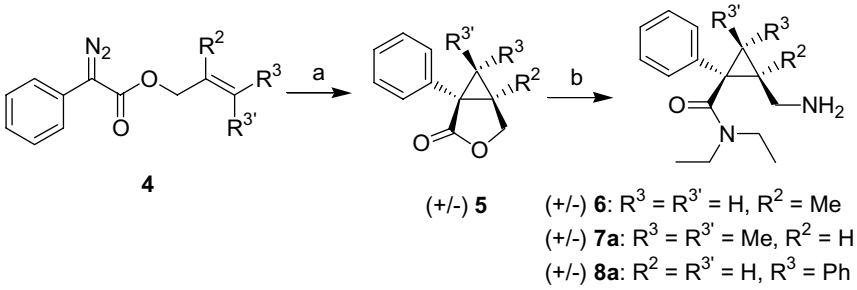
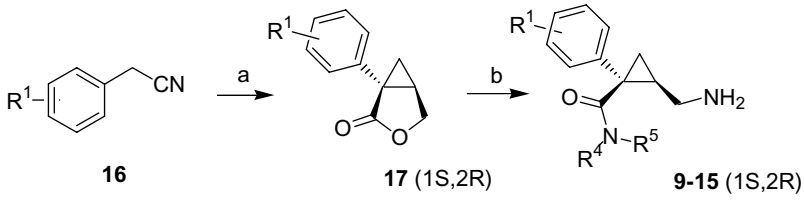


Figure 1. Chemical structures of atomoxetine, milnacipran and its potent analogs.

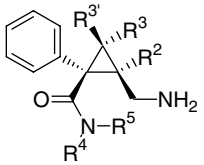


Scheme 1. Reagents and conditions: (a) $Rh(OAc)_2/CH_2Cl_2$/reflux, 3 h; (b) i—potassium phthalimide/DMF/140 °C, 16 h; ii—$SOCl_2/CH_2Cl_2$/rt, 2 h; iii—Et_2NH/CH_2Cl_2/0 °C to rt, 16 h; iv—NH_2NH_2/EtOH/rt, 16 h.



Scheme 2. Reagents and conditions: (a) i—$NaNH_2$/toluene or NaHMDS/THF/0 °C; ii—(*R*)-(−)-epichlorohydrin/0 °C to rt, 16 h; iii—KOH/EtOH/reflux, 8 h; iv—12N HCl/0 °C to rt, 2.5 h; (b) i—potassium phthalamide/DMF; ii—$SOCl_2$/reflux; iii—R^4R^5NH/CH_2Cl_2/0 °C to rt, 16 h; iv—NH_2NH_2/EtOH/rt, 16 h.

Table 1. Inhibition (IC_{50}, nM) of monoamine transporters by the cyclopropane-substituted milnacipran analogs **6**–**8**[a]



(+/-) **1**: $R^2 = R^3 = R^{3'} = H$
(+/-) **6**: $R^2 = Me$, $R^3 = R^{3'} = H$
(+/-) **7**: $R^2 = H$, $R^3 = R^{3'} = Me$
(+/-) **8**: $R^2 = R^{3'} = H$, $R^3 = Ph$

Compound	R^4NR^5	NET	SERT	DAT
1	NEt_2	77	420	6100
6	NEt_2	>10,000	6900	>10,000
7a	NEt_2	>10,000	>10,000	4500
7b	$EtNCH_2CH{=}CH_2$	6400	>10,000	>10,000
7c	Indolin-1-yl	>10,000	>10,000	>10,000
8a	NEt_2	>10,000	6400	>10,000
8b	$EtNCH_2CH{=}CH_2$	>10,000	3400	>10,000
8c	Indolin-1-yl	>10,000	560	4300

[a] Data are the average of two or more independent measurements.

NET, SERT, and DAT using a procedure similar to that described by Owens et al.[7,15] These results are summarized in Tables 1 and 2.

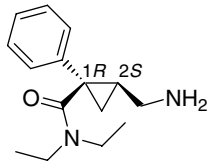
Since the conformation of milnacipran (**1**) is an important part of its pharmacophore, we first examined the substitution at the cyclopropane. Introducing a 2-methyl group (compound **6**) to milnacipran almost abolished its potency at both NET and SERT (Table 1). This result was somewhat of a surprise since the active pharmacophore based on our previous studies showed that the amino nitrogen is located under the cyclopropane ring,[10] and this additional methyl group might favor this conformation. One possible explanation is that this *cis*-2-methyl group limits the rotation of the 1-phenyl ring to a preferred orientation (Fig. 2). The 3,3-dimethyl derivative of milnacipran (**7a**) also possessed poor potency at the two transporters. In this case, the 3′-methyl group might prevent the *N*-alkyl group of **7** from getting close to the 1-phenyl

ring, which is a pharmacophore feature of this series of compounds.[10]

The *N*-allyl-*N*-ethyl amide **3a** is about 6-fold more potent at NET than milnacipran, and indoline **3b** has over 30-fold improvement at SERT. However, these functionalities as the amide side-chain of **7** had little effect on potency (compounds **7b** and **7c**), indicating that the conformation required by the pharmacophore of **3** is disrupted in **7**. Similarly, the 2-phenyl analogs **8a**–**c** were also poorly active.

Roggen et al. have shown that the NET activity of milnacipran resides in the 1*S*,2*R*-isomer,[11] and substitution at the phenyl group of the active 1*S*,2*R*-isomer has a negative impact in NET potency, although replacement of the phenyl group of the much less active 1*R*,2*S*-isomer with a lipophilic 3,4-dichlorophenyl

Table 2. Inhibition (IC_{50}, nM) of monoamine transport by the amides **9–15**[a]



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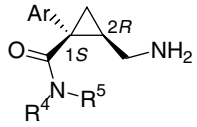

1-II, 9-15

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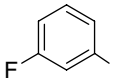

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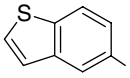

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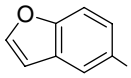

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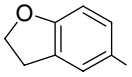

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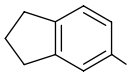

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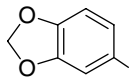

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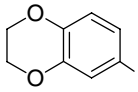

15

Compound	R^4NR^5	NET	SERT	DAT	$c\log P$[c]
1–I	NEt_2	5500	3500	>10,000	1.2
1–II	NEt_2	40	320	3200	1.2[d]
9	NEt_2	73	370	>10,000	1.3
10	NEt_2	47	1100	1300	3.4
11[b]	NEt_2	47	280	>10,000	1.7
12a[b]	NEt_2	160	2100	>10,000	1.2
12b	$EtNCH_2CH{=}CH_2$	18	430	>10,000	1.5
12c	$PrNCH_2CH{=}CH_2$	21	1700	>10,000	2.0
12d	$N(CH_2CH{=}CH_2)_2$	4.5	270	>10,000	1.9
12e	$CH_2{=}CHCH_2NCH_2C{\equiv}CH$	14	130	1>10,000	1.4
12f	$N(CH_2C{\equiv}CH)_2$	17	145	>10,000	0.9
12g	$CH_2{=}CHCH_2NCH_2C{\equiv}CMe$	40	210	>10,000	1.9
12h	$PrNCH_2C{\equiv}CMe$	80	460	>10,000	2.0
13	NEt_2	160	480	>10,000	2.4
14a[b]	NEt_2	12	480	>10,000	1.1[e]
14b	$EtNCH_2CH{=}CH_2$	7.1	150	>10,000	1.4
14c	$N(CH_2CH{=}CH_2)_2$	7.0	58	>10,000	1.8
14d	$N(CH_2C{\equiv}CH)_2$	7.6	49	4300	0.8
14e	$CH_2{=}CHCH_2NCH_2C{\equiv}CMe$	15	64	>10,000	1.8
14f	1-Indolinyl	4.2	28	640	2.5
14g	4-(1,4-Benzoxazinyl), 3,4-dihydro-2H	5.8	320	5100	2.5
14h	Isoindolin-1-yl	20	77	3600	1.9
14i	5-Thieno[3,2-*c*]pyridinyl, 4,5,6,7-tetrahydro	9.4	14	>10,000	2.1
14j	2-Isoquinolinyl, 1,2,3,4-tetrahydro	6.5	26	3600	2.4
15a[b]	NEt_2	6.3	140	>10,000	0.9
15b[b]	$EtNCH_2CH{=}CH_2$	2.9	65	>10,000	1.2
15c	$EtNCH_2CF{=}CH_2$	5.2	112	>10,000	1.9
15d	$EtNCH_2Pr$-c	7.1	540	>10,000	1.3
15e	$EtNCH_2C{\equiv}CH$	5.9	125	>10,000	0.8
15f	$cPrNCH_2C{\equiv}CH$	4.4	160	>10,000	0.6
15g	$cPrCH_2NCH_2CH{\equiv}CH$	3.9	210	>10,000	1.1
15h	$MeNCH_2CF{=}CH_2$	8.1	100	>10,000	1.4
15i	$CH_2{=}CHCH_2NCH_2CF{=}CH_2$	3.2	39	2,900	2.5
15j	$CH{\equiv}CCH_2NCH_2CF{=}CH_2$	4.0	54	>10,000	2.1
15k	$N(CH_2CH{=}CH_2)_2$	3.5	32	6300	1.7
15l	$N(CH_2C{\equiv}CH)_2$	1.7	25	>10,000	0.7
2		5.1	190	3100	3.3[f]

[a] Data are the average of two or more independent measurements.
[b] The ee value was determined using a chiral HPLC method: **11** (91.6%), **12a** (88.7%), **14a** (92.4%), **15a** (95.6%), and **15b** (96.4%).
[c] Calculated using ACD software.
[d] Measured log *P* was 1.6.
[e] Measured log*P* was 1.7.
[f] Measured log *P* was 3.5.

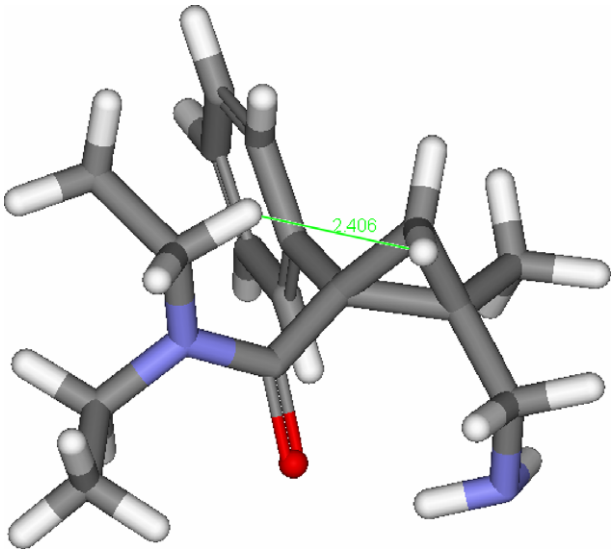

Figure 2. A low-energy conformation of **6**. If the phenyl ring rotates about 90°, its *ortho*-proton may clash with the 2-methyl group, preventing its free rotation.

or 2-naphthyl moiety increases its potency at all the three transporters up to 20-fold. We explored a set of oxygen-containing substituted phenyl groups of the active 1*S*,2*R*-milnacipran (**1–II**) in an effort to improve the potency while still maintaining the low lipophilicity of these analogs. In our assay, the 1*R*,2*S*-isomer (**1–I**) had a negligible activity on all the three transporters, and 1*S*,2*R*-milnacipran (**1–II**) exhibited IC_{50} values of 40 and 320 nM, respectively, at NET and SERT (Table 2). Compound **1–II** was only weakly active at DAT (IC_{50} = 3200 nM).[16] In comparison, the 3-fluorophenyl analog **9** slightly reduced the potency at both NET and SERT. The more lipophilic benzofuran **11** (*c*log *P* = 1.7) exhibited a similar pharmacological profile compared to **1–II** (*c*log *P* = 1.2), while benzothiophene **10** was less active at SERT, and showed some activity at DAT. Dihydrobenzofuran **12a** had a *c*log *P* value similar to **1–II**, but was much less potent at both NET and SERT. Benzocyclopentane **13** showed a moderate potency, while the 3,4-methylenedioxyphenyl analog (**14a**, NET IC_{50} = 12 nM) had improved NET activity, indicating an important role of the 3-oxygen in **14a** for NET. Finally, the 3,4-ethylenedioxyphenyl **15a** exhibited about 6-fold higher NET potency than **1–II**. More importantly, compounds **14a** and **15a** were not much more lipophilic than milnacipran. For example, **14a** and **1–II** had measured log *P* values of 1.7 and 1.6, respectively.

Previously we have found that replacing one of the ethyl groups of milnacipran with an allyl moiety increases its NET potency by about 5-fold (**3a**, NET IC_{50} = 14 nM).[10] We utilized this information to explore the SAR of three hydrophilic aryl analogs (**12**, **14**, and **15**), and the results are summarized in Table 3. Many potent NET inhibitors were discovered and their ratios to the SERT activity varies from 1- (**14i**) to almost 80-fold (**15d**). Their lipophilicities were not much higher than **1–II** but lower than that of atomoxetine **2** (*c*log *P* = 3.3). Tetrahydrothienopyridine **14i** exhibited almost a 1:1 ratio between NET and SERT, although it was slightly more lipophilic (*c*log *P* = 2.1) than milnacipran. None of the compounds showed significant activity at DAT.

Compounds **14a**, **15a**, **15b**, **15k**, and **15l** were also tested in an in vitro human liver microsomal assay[17] to compare their metabolic stability with that of milnacipran and atomoxetine. The scaled systemic clearance (CL_{sys}) of the 3,4-methylenedioxyphenyl compound **14a** was 7.9 mL/min kg which was slightly higher than **1–II** (CL_{sys} = 5.0 mL/min kg). In comparison, the 3,4-ethylenedioxyphenyl analog **15a** (CL_{sys} = 4.4 mL/min kg) was comparable to **1–II**. In contrast, atomoxetine **2**, which has high plasma protein binding[18] and is mainly eliminated as metabolites in humans,[19] exhibited high clearance (14.3 mL/min kg) in this assay. The potent analogs **15 k** and **15l** exhibited only slightly higher metabolic clearance than **1–II**, suggesting these compounds might still be, at least in part, eliminated by renal clearance in humans[20] since their lipophilic profiles were similar to milnacipran.

The renal clearance of milnacipran ($CL_R \sim 350$ mL/min)[20] in healthy human subjects is about 3-times higher than the glomerular filtration rate, indicative of an active secretion process possibly caused by P-glycoprotein (P-gp) activity.[21] In an in vitro Caco-2 assay, **1–II** displayed a moderate permeability (P_{app} = 51 nm/s) and a possible efflux mechanism reflected by its higher permeability from the basolateral to apical direction compared to the reverse direction [(*b* to *a*)/(*a* to *b*) = 7.4]. In comparison, the methylenedioxy and ethylenedioxy analogs **14** and **15** also showed high efflux ratios, possibly due to their high polar surface area (PSA). For example, **14a** (PSA = 65 Å^2) and **15l** (PSA = 65 Å^2) showed P_{app} values of 47 and 52 nm/s, respectively, and efflux ratios of 6 and 15, which were similar to that of **1–II** (PSA = 46 Å^2). The strong activity of these compounds as P-gp substrates should facilitate active tubular secretion for drug elimination, although the efflux mechanism might also limit brain penetration of its substrates[22].

In summary, a series of milnacipran analogs were studied for their structure–activity relationships as inhibitors of NET and SERT. Many potent compounds were iden-

Table 3. Metabolic stability of **14a, 15a, 15b, 15k** and **15l** in vitro[a]

Compound	CL_{sys} (mL/min kg)
1–II[b]	5.0
14a[c]	7.9
15a	4.4
15b	8.8
15k	8.1
15l	7.9
2[d]	14.3

[a] See Ref. 16 for assay conditions.
[b] Measured log *D* = −0.2 and pK_a = 9.6.
[c] Measured log *D* = 0.4
[d] Measured log *D* = 0.8 and pK_a = 10.1.

tified from the 1*S*,2*R*-isomers with a range of NET/SERT ratios and they possessed moderate lipophilicity similar to milnacipran. For example, **15l** exhibited an IC_{50} value of 1.7 nM at NET, which was 20-fold more potent than 1*S*,2*R*-milnacipran (**1–II**), and only slightly lower metabolic stability in human liver microsomes. Compound **15a** had a similar in vitro pharmacological profile to atomoxetine **2** but was more metabolically stable. These results indicate that compounds such as **15a** may have an elimination profile not much different than milnacipran in humans. Further studies on the pharmacokinetic characterization including brain penetration of this series of compounds will be published in due course.

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