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# Synthesis of enantiomerically pure milnacipran analogs and inhibition of dopamine, serotonin, and norepinephrine transporters

Heidi Roggen,[a] Jan Kehler,[b] Tine Bryan Stensbøl[b] and Tore Hansen[a,*]

[a]*University of Oslo, Department of Chemistry, Sem Sælands vei 26, 0315 Oslo, Norway*
[b]*H. Lundbeck A/S, Ottiliavej 9, 2500 Copenhagen-Valby, Denmark*

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**Abstract**—A series of Milnacipran analogs with variation in the aromatic moiety were prepared in high enantiomeric excess. Structure–activity relationships for two parallel enantiomeric series are described. The (−)-(1*R*,2*S*)-naphthyl analog (**8h**) showed the highest potency in the two series and is a triple reuptake inhibitor of the SERT, NET, and DAT.


Monoamine transporters, including the dopamine transporter (DAT), the serotonin transporter (SERT), and the norepinephrine transporter (NET), play an important role in maintaining the concentration of biogenic amine neurotransmitters in the central nervous system (CNS).[1] These transporters are involved in different pathological processes leading to several neurological disorders.[2–7] While cocaine's reinforcing and behavioral effects are believed to be mediated via the DAT,[8,9] it is thought that dysfunctional SERT and NET systems in the CNS play an important role in depression.[10,11] Virtually all effective antidepressants increase the synaptic concentration of serotonin (5-HT) and/or norepinephrine (NE) by blocking the reuptake of one or both of the neurotransmitters. This common property of antidepressants was discovered initially with tricyclic antidepressants (TCAs).[12,13] However, the additional interactions of TCAs at a variety of neurotransmitter receptors often result in poor tolerability and toxicity in overdose.[12,14] Thus, the newer antidepressant agents such as SSRIs (for example fluoxetine **1**, Fig. 1) have become popular due to less severe adverse effects.[14] However, they do not represent an improvement over older antidepressants in terms of efficacy and latency of onset. As new approaches to antidepressant therapy continue to be a significant area of CNS research, a new strategy to address some of these issues has been the development of dual reuptake inhibitors of serotonin and norepinephrine. The class of serotonin and norepinephrine reuptake inhibitors (SNRIs) now comprises three medications: venlafaxine (**2**), milnacipran (**3**), and duloxetine (**4**) (Fig. 1). These three drugs block the reuptake of 5-HT and NE with differing selectivity. Whereas milnacipran blocks 5-HT and NE reuptake with approximately equal potency, duloxetine has a 10-fold selectivity for 5-HT and venlafaxine displays a 30-fold selectivity for 5-HT.[15]

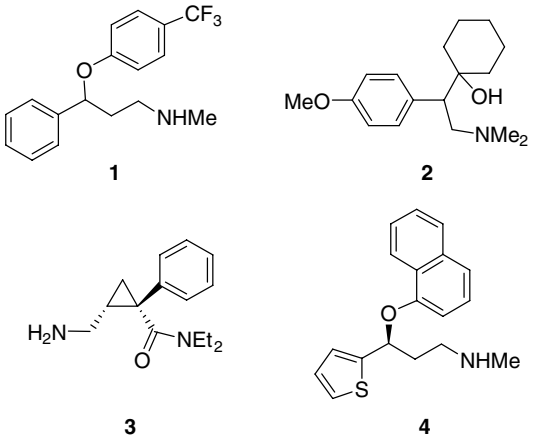


**Figure 1.** Molecular structures for selected SSRI and SNRIs.

To the best of our knowledge there are no studies published that describe the structure–activity relationship for enantiomerically pure milnacipran[16] and derivatives

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* Corresponding author. Tel.: +47 228 55386; fax: +47 228 55441; e-mail: torehans@kjemi.uio.no

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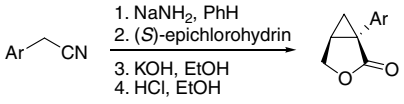
thereof with respect to their potency at the SERT, NET, and DAT. It has, however, been demonstrated that the aromatic group in milnacipran is essential for binding to the serotonin transporter.[17] Alkyl substitution on the nitrogen atom leads to a decrease in the binding affinity to the SERT. In addition to being a 5-HT and NE reuptake inhibitor, milnacipran is also a weak NMDA antagonist.[17] The binding affinity for the NMDA receptor is not very high, but milnacipran has served as a lead in the search for more potent NMDA receptor antagonist. Several analogs of milnacipran have been synthesized and tested in vitro.[18–22] However, the main aims of the published studies have been to optimize the structure with respect to the NMDA activity and not monoamine transporter inhibition. Based on that information we set out to synthesize both enantiomers of a series of milnacipran analogs varying the aromatic moiety and to examine their structure–activity relationships with respect to SNRIs. In this letter, we describe the asymmetric synthesis of a range of milnacipran analogs and their inhibition of the SERT, NET, and DAT.

The general synthetic strategy for the synthesis of enantiomerically enriched milnacipran analogs was based on published procedures.[17,18,21] The one-pot reaction of various aryl acetonitriles with sodium amide and (+)- or (−)-epichlorohydrin resulted in the synthesis of a series of highly enantioenriched 1-aryl-3-oxa-bicyclo-[3.1.0]hexane-2-ones as illustrated in Table 1. Both enantiomeric series of the building blocks **6a**–**j** were synthesized.

Although the yields are moderate, the method has several advantages. It is a one-pot reaction. Both enantiomers of epichlorohydrin and many aryl-acetonitriles are commercially available. The building blocks are obtained in >90% enantiomeric excess.

**Table 1.** Yields and enantiomeric excess for the synthesis of 1-aryl-3-oxa-bicyclo[3.1.0]hexane-2-ones (**6**) from arylacetonitriles (**5**)




**5a-j** **6a-j**

| Compound | Ar | Yield[a](%) | Ee[b](%) |
|---|---|---|---|
| **6a** | Ph | 43 | >99 |
| **6b** | 3-Cl-Ph | 39 | 98 |
| **6c** | 4-Cl-Ph | 44 | 94 |
| **6d** | 3,4-di-Cl-Ph | 37 | 95 |
| **6e** | 4-F-Ph | 33 | 95 |
| **6f** | 4-MeO-Ph | 45 | 91 |
| **6g** | 1-Naphthyl | 36 | >99 |
| **6h** | 2-Naphthyl | 50 | 98 |
| **6i** | 2-Thiophene | 25 | 98 |
| **6j** | 3-Thiophene | 40 | >90 |

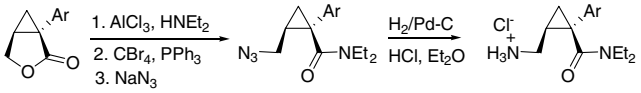
Only one enantiomeric series is shown.
[a] Isolated yields after flash chromatography.
[b] Measured by chiral HPLC.

Synthesis and yields of the target 2-(aminomethyl)-1-aryl-*N*,*N*-diethylcyclopropanecarboxamide hydrochlorides **8** are outlined in Table 2. The bicyclic lactones **6a**–**j** were converted by aminolysis with diethylamine in DCM to give the corresponding hydroxyl amides. Reaction of the hydroxyl amides with $CBr_4$ and $PPh_3$ followed by reaction with sodium azide gave azides **7a**–**j** in good yields. Azides **7a**–**j** were reduced to the corresponding amines with $H_2$/Pd/C and the desired target compounds were isolated by precipitation with HCl to give the HCl salts **8a**–**j**.

Table 3 summarizes the potency of inhibition of SERT, NET, and DAT of the two enantiomeric series of milnacipran analogs **8**.

There was a significant difference in potency between the two enantiomers of milnacipran **8a**. The (+)-(1*S*,2*R*)-isomer showed 20 times higher potency in the inhibition of NE uptake compared to its enantiomer. The difference in potency of inhibition of 5-HT uptake between the two enantiomers was only 3-4-fold, but still favoring the (+)-(1*S*,2*R*)-isomer. None of the two enantiomers were active at the DAT. For the isomer B series, a consistent trend was observed. The order of potency in transporter inhibition was NET > SERT > DAT. The inhibition of the DAT was very low throughout this series. The inhibition of the NET varied only slightly with different aromatic substituents. The only exception was the 1-naphthyl derivative (+)-**8g** which experienced a 10-fold drop in potency compared to the rest of the series. The 1-naphthyl derivative showed very low affinity in SERT-inhibition as well. For the rest of the isomer B series the $IC_{50}$ values for the SERT-inhibition ranged from 86 to 790 nM and no apparent systematic trend was observed.

**Table 2.** Yields for the synthesis of azides (**7**) from lactones (**6**), and 2-(aminomethyl)-1-aryl-*N*,*N*-diethylcyclopropanecarboxamide hydrochlorides (**8**) from azides (**7**)

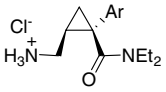



**6a-j** **7a-j** **8a-j**

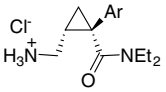
| Ar | Compound | Yield[a](%) | Compound | Yield[b](%) |
|---|---|---|---|---|
| Ph | **7a** | 47 | **8a** | 97 |
| 3-Cl-Ph | **7b** | 80 | **8b** | 11[c] |
| 4-Cl-Ph | **7c** | 90 | **8c** | 75[c] |
| 3,4-di-Cl-Ph | **7d** | 97 | **8d** | 78 |
| 4-F-Ph | **7e** | 89 | **8e** | 65 |
| 4-MeO-Ph | **7f** | 94 | **8f** | 58 |
| 1-Naphthyl | **7g** | 83 | **8g** | 78 |
| 2-Naphthyl | **7h** | 86 | **8h** | 81 |
| 2-Thiophene | **7i** | >95 | **8i** | 60 |
| 3-Thiophene | **7j** | 72 | **8j** | 93 |

Only one enantiomeric series is shown.
[a] Isolated yield after column chromatography.
[b] Isolated yield after precipitation as HCl salts.
[c] Reduction of azide was accomplished with $PPh_3$ in wet MeOH due to hydrodechlorination with $H_2$/Pd/C.

**Table 3.** Effects of selected compounds on 5-HT, NE, and DA uptake inhibition




(-)-(1*R*, 2*S*)-isomer A




(+)-(1*S*, 2*R*)-isomer B

| Compound | Ar | 5-HT[a] | NE[a] | DA[a] | 5-HT[a] | NE[a] | DA[a] |
|---|---|---|---|---|---|---|---|
| **8a** | Ph | 420[d] | 200[d] | 10,000[d] | 120[d] | 7[d] | 10,000[d] |
| **8b** | 3-Cl-Ph | 170 | 38 | 10,000 | 86 | 33 | 9000 |
| **8c** | 4-Cl-Ph | 190 | 140 | 3700 | 790 | 44 | 10,000 |
| **8d** | 3,4-di-Cl-Ph | 84 | 10 | 540 | 440 | 26 | 1700 |
| **8e** | 4-F-Ph | 320 | 500 | 10,000 | 210 | 63 | 10,000 |
| **8f** | 4-MeO-Ph | 230 | 140 | 10,000 | 510 | 35 | 10,000 |
| **8g** | 1-Naphthyl[b] | 1200 | 200 | 5300 | 7100 | 370 | 10,000 |
| **8h** | 2-Naphthyl | 18 | 5 | 140 | 130 | 29 | 1400 |
| **8i** | 2-Thiophene[c] | 520 | 1500 | 970 | 250 | 65 | 10,000 |
| **8j** | 3-Thiophene[b] | 250 | 410 | 10,000 | 190 | 19 | 10,000 |

$IC_{50}$ values for the synaptosomal uptake inhibition of two enantiomeric series of 2-(aminomethyl)-1-aryl-*N*,*N*-diethylcyclopropanecarboxamide hydrochlorides (**8**) with the 5-HT, NE, and DA transporters. Values are given in nM. Data shown are means of a minimum of two values. $IC_{50}$ values were determined using drug concentrations covering three decades.

[a] The $IC_{50}$ values in the biological assays were measured using the procedures described in Ref. 23.

[b] The sign of optical rotation is reversed compared to the rest of the series.

[c] Absolute stereochemistry is (1*R*,2*R*) for isomer A and (1*S*,2*S*) for isomer B.

[d] Owen et al. have reported $IC_{50}$ values of 151, 61, and >100,000, respectively, on SERT, NET, and DAT for (±)-milnacipran. See Ref. 24.

The inhibition of DAT uptake was also consistently lower than uptake inhibition of SERT and NET in the isomer A series. Most of the compounds in the isomer A series showed very low inhibition of the DAT. The selectivity for the NET over the SERT was not consistent throughout the series. Both thiophene analogs (**8i** and **8j**) and the 4-fluoro derivative (**8e**) displayed higher inhibition of the SERT over the NET. Two compounds displayed significantly higher inhibition of both the SERT and NET over the others. The 3,4-dichloro-phenyl derivative (−)-**8d** had $IC_{50}$ values of 84 and 10 nM to the SERT and NET respectively, which were similar to the $IC_{50}$ values for (+)-milnacipran. The major difference in binding profile between the two was the difference in inhibition of the DAT. Compound (−)-**8d** was moderately potent, while (+)-milnacipran displayed no uptake inhibition of the DAT. The naphthyl derivative (−)-(1*R*,2*S*)-**8h** was the most potent analog of those tested in both enantiomeric series with $IC_{50}$ values of 5, 18, and 140 nM to the NET, SERT, and DAT, respectively. The inhibition potency of (−)-**8h** to the SERT was similar to (+)-**8a**. Compound (−)-**8h** displayed slightly higher potency to the SERT compared to (+)-**8a**, giving a NET/SERT selectivity ratio which was slightly lower for (−)-**8h** compared to (+)-**8a**. To the limit of our awareness, compound (−)-**8h** is the first example of a milnacipran analog which displays significant inhibition of DAT as well as to the SERT and NET. Thus, compound (−)-**8h** can be considered a triple reuptake inhibitor. The development of triple reuptake inhibitors for the treatment of depression has only recently been pursued.[25] Combination studies have indicated that addition of some inhibition of the DA uptake site may have some clinical benefits.[26–28] The serendipitous discovery that compounds within this series can be triple reuptake inhibitors merits further study.

In conclusion, these studies describe novel milnacipran analogs with potent in vitro dual reuptake inhibition. The (−)-(1*R*,2*S*)-isomer of the 1-naphthyl derivative **8h** is a novel example of a triple reuptake inhibitor. Further SAR studies on the development of triple reuptake inhibitors will be reported in due course.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.02.054.

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