Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1346–1349
Bioorganic & Medicinal Chemistry Letters

Studies on the SAR and pharmacophore of milnacipran derivatives as monoamine transporter inhibitors

Chen Chen,[a,*] Brian Dyck,[a] Beth A. Fleck,[b] Alan C. Foster,[c] Jonathan Grey,[a] Florence Jovic,[a] Michael Mesleh,[a] Kasey Phan,[a] Junko Tamiya,[a] Troy Vickers[a] and Mingzhu Zhang[a]

[a]*Department of Medicinal Chemistry, Neurocrine Bioscience, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[b]*Department of Pharmacology, Neurocrine Bioscience, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[c]*Department of Neuroscience, Neurocrine Bioscience, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*

Received 3 December 2007; revised 27 December 2007; accepted 4 January 2008
Available online 9 January 2008

Abstract—Derivatives of milnacipran were synthesized and studied as monoamine transporter inhibitors. Potent analogs were discovered at NET (**9k**) and at both NET and SERT (**9s** and **9u**). A pharmacophore model was established based on the conformational analysis of milnacipran in aqueous solution using NMR techniques and was consistent with the SAR results.

The synaptic actions of the monoamine neurotransmitters norepinephrine (NE), serotonin (SER), and dopamine (DA) are terminated by reuptake into the nerve endings from which they are released and by uptake into adjacent cells. Reuptake is achieved by cell membrane transporters specific for each monoamine (NET, SERT, and DAT) which have been successfully targeted for the development of CNS drugs.[1] Both selective serotonin reuptake inhibitors (sSRI) and inhibitors of both NET and SERT (NSRI) are effective against major depression and a range of other psychiatric illnesses. The dopamine transporter (DAT) is a key target for amphetamine and methylphenidate, used in the treatment of attention deficit hyperactivity disorder.[2]

Fluoxetine (**1**, Prozac®) is an sSRI (marketed as a racemic mixture) and has been used as an antidepressant since 1988.[3] Atomoxetine (**2**) was introduced into the market as a selective NET inhibitor (sNRI) for ADHD.[4] Recently, duloxetine (**3**) has been approved by the FDA for the treatment of depression as well as diabetic neuropathy.[5] Duloxetine is an NSRI, and two other drugs usually considered in this class are venlafaxine (**4**)[6] and milnacipran (**5**, Fig. 1).[7] Available in many countries as an antidepressant, including Japan and France, milnacipran inhibits NE and SER reuptake in a 3:1 ratio.[8] While the SERT inhibition is likely to improve depression,[9] the NET reuptake blockade is thought to improve chronic pain.[10] Milnacipran is currently in phase III clinical trials for fibromyalgia, and recent results suggested efficacy in this indication.[11]

Milnacipran, marketed as a racemic mixture of two enantiomers, is a hydrophilic molecule that differs from the other more hydrophobic monoamine transporter inhibitors such as duloxetine.[12] Milnacipran is mainly excreted in the urine as the parent and glucoronide (>80%), and only a small fraction (<10%) is metabolized via *N*-de-ethylation by the CYP3A4 enzyme.[13] This lack of potential for drug–drug interaction via the CYP450 enzymes is quite an attractive feature for a CNS drug because many are highly lipophilic and rely heavily on liver enzymes for elimination. The SAR of milnacipran and its analogs based on in vivo efficacy was reported by Bonnaud and coworkers in 1987.[14] Recently, Roggen et al. reported a series of milnacipran analogs as single stereoisomers with variation in the aromatic moiety and their activity as NET and SERT inhibitors.[15] However, the SAR and pharmacophore of milnacipran

Keywords: Structure–activity relationship; Pharmacophore; Milnacipran; Norepinephrine; Serotonin; Monoamine transporter; Inhibitor; Synthesis.
* Corresponding author. Tel.: +1 858 617 7600; fax: +1 858 617 7602; e-mail: cchen@neurocrine.com

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.01.011

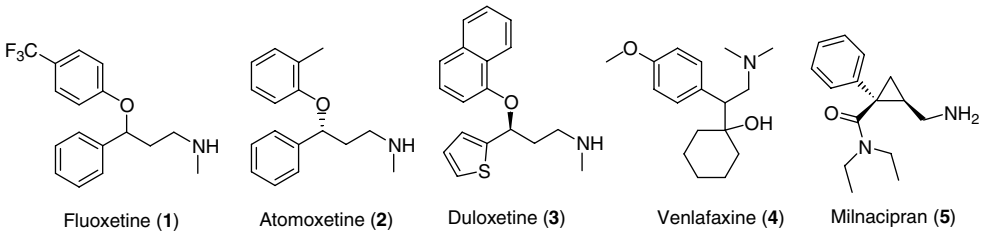


Figure 1. Chemical structures of some monoamine transporter inhibitors.

derivatives at the transporter level are still largely unclear. Here we report the synthesis of milnacipran analogs and their structure–activity relationships at NET and SERT.

N-Alkyl derivatives of milnacipran **6** were synthesized by reductive alkylation of milnacipran with various aldehydes in the presence of sodium triacetoxyborohydride as shown in Scheme 1. *N*,*N*-Dimethyl milnacipran **7** was obtained from the corresponding aldehyde[16] by a reductive amination with dimethylamine in the presence of sodium triacetoxyborohydride.

Amide analogs of milnacipran were prepared from the known acid intermediate **8**[14] as depicted in Scheme 2. Selected primary amines **9** were also converted to the aminoacetamides **10** using a coupling reaction with *N*-Boc-glycine, followed by a deprotection with trifluoroacetic acid.

The target compounds **6**–**7** and **9**–**10** as racemic mixtures were tested for inhibition of human NET, SERT, and DAT using a procedure similar to that described by Owens et al.[17] These results are summarized in Tables 1–3.

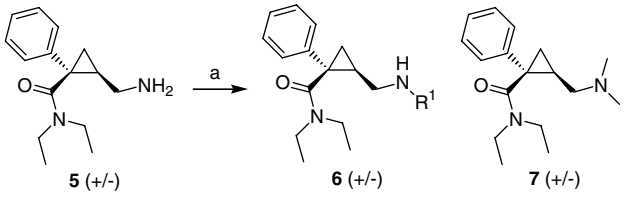
Milnacipran (**5**) as a pair of enantiomers exhibited moderate potencies at NET (IC_{50} = 77 nM) and SERT (IC_{50} = 420 nM) and was weakly active at DAT (IC_{50} = 6100 nM). *N*-Methylation of milnacipran reduced its NET activity by almost 100-fold (**6a**, NET IC_{50} = 6500 nM) and SERT activity by about 10-fold. All *N*-alkyl derivatives (**6b**–**e**) were weakly active at NET and SERT and devoid of activity at DAT (**6k** was very weakly active). Among them, *N*-(2-oxazolemethyl) milnacipran (**6g**, NET IC_{50} = 450 nM) displayed the best potency at NET but was still 6-fold less potent than the parent. Therefore, it could be concluded that *N*-alkylation of milnacipran, including the *N*,*N*-dimethyl analog **7**, detrimentally decreased its interaction with all three transporters (Table 1).

Table 1. SAR of the *N*-alkyl milnacipran derivatives **6** and **7**[a]

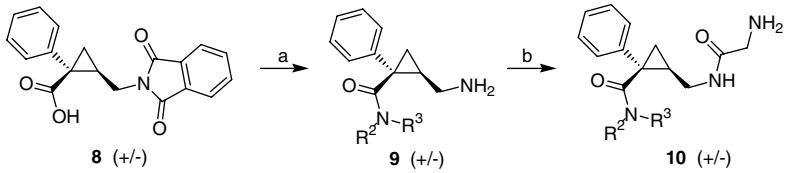
Compound	R^1	NET	SERT	DAT
5	H	77	420	6,100
6a	Me	6500	5400	>10,000
6b	Et	860	1300	>10,000
6c	^{n}Bu	6100	>10,000	>10,000
6d	^{i}Bu	>10,000	>10,000	>10,000
6e	CyclopentaneCH_2	8400	>10,000	>10,000
6f	$(MeO)_2CHCH_2$	960	1100	>10,000
6g	2-OxazoleCH_2	450	1600	>10,000
6h	5-OxazoleCH_2	2900	4800	>10,000
6i	2-FuranCH_2	>10,000	>10,000	>10,000
6j	2-PyrroleCH_2	2600	4400	>10,000
6k	2-PyridineCH_2	8100	3500	6200
6l	3-PyridineCH_2	>10,000	>10,000	>10,000
6m	4-PyridineCH_2	>10,000	>10,000	>10,000
7		>10,000	>10,000	4500

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

Among the secondary amides **9a**–**d**, the *N*-phenyl compound **9c** showed some activity at NET, and the *N*-benzyl **9d** showed activity at SERT (Table 2), suggesting a probable role of π-electrons for the interaction between the transporters and ligands. *N*-Methyl-*N*-ethyl amide **9e** was much less potent at NET than milnacipran **5**. In comparison, the *N*-ethyl-*N*-propylamide **9f** possessed slightly higher NET activity but lower SERT potency than **5**, while *N*-ethyl-*N*-butyl analog **9g** exhibited high IC_{50} values at both NET and SERT, indicating milnacipran was the most balanced dual inhibitor among these dialkyl amides. Both **9h** and **9i** displayed poor



Scheme 1. Reagents and conditions: (a) aldehyde/THF/Et_3N/rt; then $NaBH(OAc)_3$/EtOAc/rt.



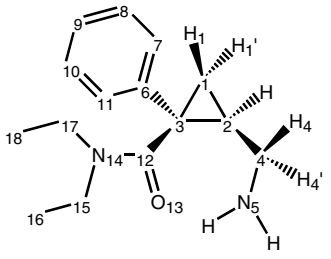
Scheme 2. Reagents and conditions: (a) i—$SOCl_2$/reflux; ii—R^2R^3NH/CH_2Cl_2; iii—NH_2NH_2/MeOH; (b) i—$BocNHCH_2COOH$/EDC/HOBt/Et_3N/CH_2Cl_2/rt; ii—TFA/CH_2Cl_2/rt, 1 h.

Table 2. SAR of the amides **9**[a]

Compound	R^2NR^3	NET	SERT	DAT
9a	NHEt	>10,000	>10,000	>10,000
9b	NH(^{c}Pr)	>10,000	>10,000	>10,000
9c	NHPh	570	>10,000	>10,000
9d	NHBn	>10,000	3200	>10,000
9e	EtNMe	1400	830	2400
5	NEt_2	77	420	6100
9f	EtNPr	41	1700	>10,000
9g	EtN(^{n}Bu)	360	2000	>10,000
9h	EtN(^{t}Bu)	8000	>10,000	>10,000
9i	EtN(^{c}Hx)	4900	7500	>10,000
9j	$EtNCH_2CH_2OMe$	180	1300	>10,000
9k	$EtNCH_2CH{=}CH_2$	14	280	>10,000
9l	EtNPh	63	4400	>10,000
9m	EtNBn	200	160	>10,000
9n	$N(^i Pr)_2$	2,800	>10,000	>10,000
9o	Pyrrolidin-1-yl	3700	3100	>10,000
9p	Morpholin-1-yl	2300	1700	7400
9q	Piperidin-1-yl	170	1200	4600
9r	Homopiperidin-1-yl	130	640	>10,000
9s	Indolin-1-yl	4.4	13	3900
9t	THQ[b]	21	100	>10,000
9u	THIQ[c]	8.4	8.3	>10,000
2		5.1	190	3100
3		8.9	6.6	660

[a] Data are average of two or more independent measurements.
[b] THQ, tetrahydroquinoline.
[c] THIQ, tetrahydroisoquinoline.

Table 3. Summary of NMR studies of milnacipran



Proton	Chemical shift	NOE
1	1.48 (m, 1H)	18,1′,4,4′,17,17′
1′	1.79 (m, 1H)	1,2,7, or 11
2	1.79 (m, 1H)	1′,4, or 4′
4,4′	3.12 (m, 2H)	1,2
7,11	7.33 (m, 2H)	18,1′,17,17′
8,10	7.44 (m, 2H)	
9	7.34 (m, 1H)	
15,15′	3.35 and 3.42 (m, 1H)	16
16	1.13 (t, 3H)	15,15′
17,17′	3.39 and 3.55 (m, 1H)	18,1,7,11
18	0.83 (t, 3H)	1,17,17′,7,11

potencies at all three transporters. While the methoxyethyl **9j** was slightly better than the butyl **9g** at both NET and SERT, the *N*-allyl **9k** showed significant improvement over the *N*-propyl **9f** for the two transporters, indicating an important role of the π-electrons of the allylic double bond.

The *N*-ethyl-*N*-phenyl amide **9l** was about 10-fold better than the secondary amide **9c**. Its NET activity also matched that of milnacipran **5**, but its SERT potency was 10-fold lower. In contrast, the *N*-ethyl-*N*-benzyl **9m** exhibited 20-fold better SERT potency than the secondary **9d**, and **9m** was the best SERT inhibitor among the acyclic amides **9a**–**n**. The diisopropyl **9n** was only weakly active at NET, indicative of a significant difference in conformations from **5** due to the bulkiness of its amide side chain. Compared to the pyrrolidine and morpholine analogs (**9o**–**p**), the piperidine **9q** and homopiperidine **9r** were much more potent at NET, suggesting a requirement for a relative lipophilic group with a limited size at this site.

The indoline **9s** displayed high potencies at both NET and SERT, much better than the acyclic **9l**. The tetrahydroquinoline (THQ) **9t** exhibited much reduced potency from **9s**, indicative of certain geometrical requirements. The tetrahydroisoquinoline (THIQ) **9u** exhibited equal potency at both NET and SERT and was much better than its acyclic **9m**. Compound **9u** had a similar profile to duloxetine **3** at NET and SERT but was less potent at DAT.

Since milnacipran is a relatively rigid molecule, NMR is a good tool to study its conformation in solution. Kazuta et al. have investigated the conformation of an ethyl milnacipran analog in aqueous solution based on NOE data.[18] Their results indicate that the amino nitrogen is away from the carbonyl oxygen due to the steric clash caused by the 4-ethyl group, and this compound is devoid of SERT activity. In our NMR experiments,[19] it was observed that the proton 1 and the proton 4 of milnacipran **5** (see numbering in Table 3) in water had NOE correlation, suggesting their close proximity. The two methyl groups of **5** had different chemical shifts, indicating their different chemical environments. One of the two methyl groups was located close to the phenyl ring since NOE was observed between the methyl and the *ortho*-proton (Table 3). Its associated ethylene was nearby to the cyclopropane because NOE between this ethylene and one of the protons at position-1 was observed. These results provide evidence for a possible 3D structure of milnacipran in aqueous solution, and a model was constructed based on these data using computational simulation (Fig. 2). In this conformer,

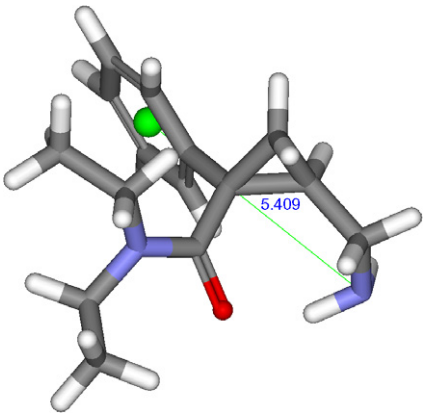


Figure 2. Computational simulation of milnacipran conformation required for NET inhibition.

Table 4. SAR of aminoacetamides **10a–d**[a]

Compound	R^2NR^3	NET	SERT	DAT
10a	NEt_2	700	2200	>10,000
10b	$N(^iPr)_2$	>10,000	>10,000	4600
10c	Indolin-1-yl	12	77	>10,000
10d	THIQ	150	150	>10,000

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

the distance between the amine nitrogen and the benzene centroid is about 5.6 $Å^2$. One of the ethyl groups is closely located to the phenyl and cyclopropane, and the aminomethyl protons are in a close range with one of the methylene protons of the cyclopropane. This conformation puts the NH_2 proton near the carbonyl oxygen atom, which may form hydrogen bonding.

This conformation might be the essential active pharmacophore required at least for NET, and the SAR data support this model. Thus, *N*-methylation of **5** decreases its chance of forming this conformation due to a steric effect, resulting in a much less potent inhibitor at both NET and SERT. Any *N*-alkyl group basically reduces the possibility of this conformation. It is also possible that the allyl group of **9k** contributes an additional π–π interaction with the phenyl group compared to the ethyl group of **5**, resulting in further stabilization of this favored conformation. For high SERT potency, an additional phenyl group is required at the amide side chain to interact with the transporter. The fact that the cyclic THIQ **9u** exhibited much higher potency at NET and SERT than its acyclic *N*-ethyl-*N*-benzyl analog **9m** also indicates that there is a limited space available in the transporters and so key binding features of an inhibitor are optimally contained in a more compact molecule.

To test this model, we synthesized several aminoacetamides of milnacipran analogs and the results are summarized in Table 4. The aminoacetamide of milnacipran **10a** displayed 9- and 5-fold reduction in potency at NET and SERT, respectively, compared to **5**, and these values were similar to those of the *N*-ethyl analog **6b**. In comparison, the indoline derivative **10c** only reduced NET activity less than 3-fold from its parent **9s**. These results might suggest the terminal amine of **10** is able to replace the function of the basic nitrogen in **9** through a different conformation.

In summary, a series of milnacipran derivatives were synthesized, their SAR of NET and SERT inhibition was studied, and potent compounds were discovered. Thus, compound **9k** exhibited a similar pharmacological profile to atomoxetine but with lower lipophilicity (c log *D* = 1.5 vs 3.3 for atomoxetine), while the pharmacological profiles of compounds **9s** (c log *P* = 2.7) and **9u** (c log *P* = 2.6) matched with that of duloxetine (c log *P* = 3.7) at the transporters. These compounds possessed lower lipophilicity than many of the marketed drugs in this class. A pharmacophore model for the milnacipran analogs was also established based on the conformational analysis in aqueous solution, which could be useful for designing novel potent monoamine transporter inhibitors with ideal pharmacokinetic properties.

References and notes

1. Iversen, L. *Br. J. Pharmacol.* **2006**, *147*, S82.
2. Runyon, S. P.; Carroll, F. I. *Curr. Top. Med. Chem.* **2006**, *6*, 1825.
3. Stokes, P. E.; Holtz, A. *Clin. Therapeut.* **1997**, *19*, 1135.
4. Gibson, A. P.; Bettinger, T. L.; Patel, N. C.; Crismon, M. L. *Ann. Pharmacother.* **2006**, *40*, 1134.
5. Frampton, J. E.; Plosker, G. L. *CNS Drugs* **2007**, *21*, 581.
6. Dierick, M. *Eur. Psychiatry* **1997**, *12*, 307s.
7. Bisserbe, J. C. *Int. Clin. Psychopharmacol.* **2002**, *17*, S43.
8. Puozzo, C.; Panconi, E.; Deprez, D. *Int. Clin. Psychopharmacol.* **2002**, *17*, S25.
9. Stahl, S. M.; Grady, M. M.; Moret, C.; Briley, M. *CNS Spectr.* **2005**, *10*, 732.
10. Mochizucki, D. *Hum. Psychopharmacol.* **2004**, *19*, S15-9.
11. (a) Rooks, D. S. *Curr. Opin. Rheumatol.* **2007**, *19*, 111; (b) Leo, R. J.; Brooks, V. L. *Curr. Opin. Investig. Drugs* **2006**, *7*, 637.
12. The calculated log *D* values are 1.2 for milnacipran and 3.3 for duloxetine using ACD software.
13. Puozzo, C.; Lens, S.; Reh, C.; Michaelis, K.; Rosillon, D.; Deroubaix, X.; Deprez, D. *Clin. Pharmacokinet.* **2005**, *44*, 977.
14. Bonnaud, B.; Cousse, H.; Mouzin, G.; Briley, M.; Stenger, A.; Fauran, F.; Couzinier, J. P. *J. Med. Chem.* **1987**, *30*, 318.
15. Roggen, H.; Kehler, J.; Stensbol, T. B.; Hansen, T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2834.
16. Shuto, S.; Ono, S.; Imoto, H.; Yoshii, K.; Matsuda, A. *J. Med. Chem.* **1998**, *41*, 3507.
17. Owens, M. J.; Morgan, W. N.; Plott, S. J.; Nemeroff, C. B. *J. Pharmacol. Exp. Ther.* **1997**, *283*, 1305.
18. Kazuta, Y.; Tsujita, R.; Yamashita, K.; Uchino, S.; Kohsaka, S.; Matsuda, A.; Shuto, S. *Bioorg. Med. Chem.* **2002**, *10*, 3829.
19. The NMR experiments were performed on a Varian 500 MHz spectrometer at 27 °C. Milnacipran (5 mg) was dissolved in 25 mM TRIS-d_{11} buffer (pH = 7.0), and water suppression was accomplished using Excitation Sculpting with Gradients. Protons 16 and 18 were assigned from chemical shifts and multiplicities. Protons 15 and 17 were assigned based on correlations in COSY. Protons 7–11 were assigned from absolute value COSY. Protons 1, 2, and 4 were assigned from chemical shift and COSY correlations. The overlap of one proton from 1 and the proton 2 is confirmed via the HSQC experiment, which clearly shows two different carbons with the similar proton chemical shift.