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Bioorganic & Medicinal Chemistry Letters 18 (2008) 3230–3235
Bioorganic & Medicinal Chemistry Letters

Studies on a series of milnacipran analogs containing a heteroaromatic group as potent norepinephrine and serotonin transporter inhibitors

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Received 27 February 2008; revised 18 April 2008; accepted 22 April 2008
Available online 25 April 2008

Abstract—A series of milnacipran analogs containing a heteroaromatic group were synthesized and studied as monoamine transporter inhibitors. Many compounds exhibited higher potency than milnacipran at NET and NET/SERT with no significant change in lipophilicity. For example, compound ***R*-26f** was about 10-fold more potent than milnacipran with IC_{50} values of 8.7 and 26 nM at NET and SERT, respectively.
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The cell membrane monoamine transporters, norepinephrine (NET), serotonin (SERT), and dopamine (DAT), are important targets for CNS drugs.[1] Both norepinephrine and serotonin selective reuptake inhibitors (sNRI and sSRI) are effective against major depression and a range of other psychiatric illnesses, while the dopamine transporter is a key target for amphetamine and methylphenidate used in the treatment of attention deficit hyperactivity disorder. Recently, duloxetine (**3**) was introduced as a dual NET/SERT inhibitor (NSRI) for the treatment of both depression and neuropathic pain.[2]

Studies have suggested that compounds with greater NET activity should be more effective for the treatment of pain than compounds having only SERT activity and that the potency and effectiveness of NET-selective inhibitors might be enhanced by the presence of SERT activity.[3] However, data from reboxetine (**4**)[4] indicate that this NET-selective antidepressant may also have efficacy in the treatment of chronic pain in patients with depression.[5] Therefore, a potent NET inhibitor with SERT activity could be useful in treating some types of pain.[6]

Milnacipran (**1**, Fig. 1), as a mixture of 1*R*,2*S*- and 1*S*,2*R*-isomers, is known as a dual NET/SERT reuptake inhibitor available in many countries as an antidepressant.[7] Milnacipran inhibits human NET and SERT in a 3:1 ratio.[8] This compound is in phase III clinical trials for fibromyalgia, and recent reports have suggested its effectiveness.[9,10]

As a small molecule (molecular weight of 246), milnacipran differs from many monoamine transporter inhibitors, such as duloxetine, by having low lipophilicity (Log $D \sim 0$).[11] Because of its chemical properties, milnacipran exhibits an almost ideal pharmacokinetic profile in humans.[12,13] It has low human plasma protein binding (~13%) and is mainly excreted in the urine as the parent (50–60%) and glucuronide (~20%). Only a small fraction (<10%) of milnacipran is metabolized via N-deethylation by the CYP3A4 enzyme.[14] This lack of potential for drug-drug interaction via liver enzymes is quite

Keywords: Milnacipran; Monoamine transporter inhibitor; Potency; Lipophilicity; Metabolic stability; Clearance.
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doi:10.1016/j.bmcl.2008.04.045

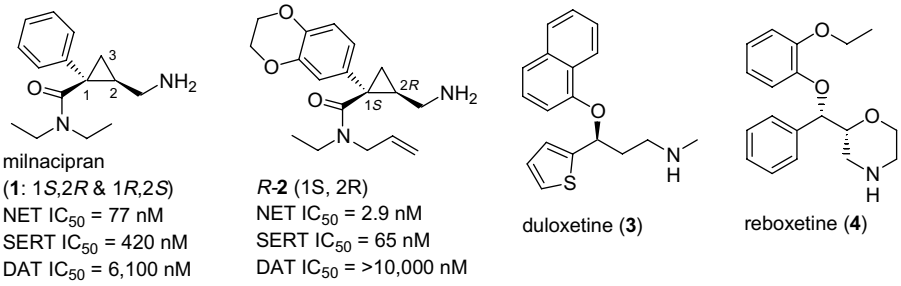
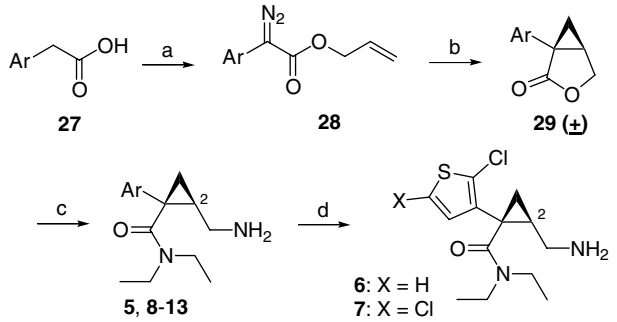


Figure 1. Chemical structures of some monoamine transporter inhibitors.

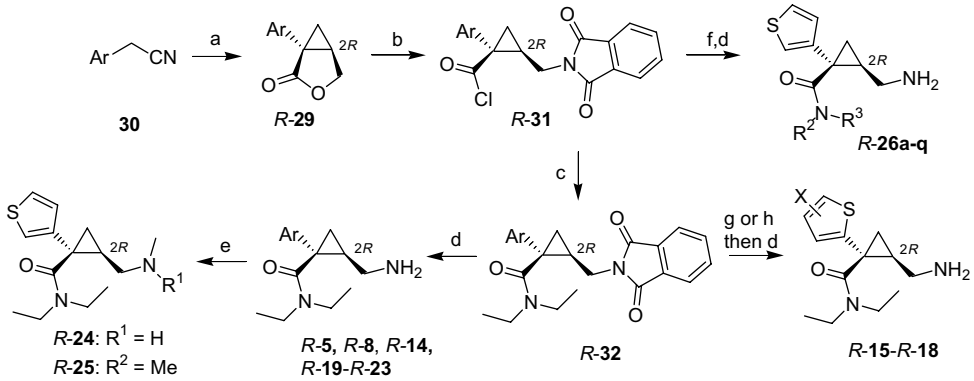
an attractive feature for CNS drugs because many are highly lipophilic and rely heavily on liver enzymes for drug elimination.[12] The structure-activity relationship (SAR) of milnacipran and its analogs based on in vivo efficacy was reported by Bonnaud and coworkers in 1987.[15] The potency of milnacipran at the transporter level is only moderate. Vaishnavi et al. have reported IC_{50} values of 68, 151, and >100,000 nM, respectively, for inhibition of monoamine uptake at human NET, SERT, and DAT.[16] Recently, Roggen et al. showed in a rat synaptosomal uptake inhibition assay that the 1*S*,2*R*-isomer of milnacipran (*R*-**1**) was much more potent than its 1*R*,2*S*-isomer (*S*-**1**) at NET and replacement of the phenyl group of *R*-**1** with a variation of aromatic groups had no improvement on its NET activity.[17] We have described the SAR of a series of *N*-alkyl and *N*-dialkyl amide derivatives of milnacipran and shown that NET potency was improved when a small alkene group was incorporated.[18] Recently, we also found that introducing an oxygen-containing group to the phenyl ring of *R*-**1** also increased NET potency.[19] Thus, the 3,4-ethylenedioxyphenyl compound *R*-**2** was over 25-fold more potent than milnacipran at NET (Fig. 1). Here, we report our continued efforts using the milnacipran template to discover potent NET-selective and dual NET/SERT inhibitors without a significant change in lipophilicity and metabolic stability.

The milnacipran analogs (**5**–**13**) as a 2*R* and 2*S* mixture were synthesized by intra-molecular cyclizations of the allyl esters **28**[20] to give the lactones **29**, which were elaborated to the desired products using a procedure similar to that for milnacipran (Scheme 1).[15] Chlorination of the 3-thienyl compound **5** with sulfuryl chloride gave **6** (with 1 equiv SO_2Cl_2) or **7** (with 2 equiv SO_2Cl_2).



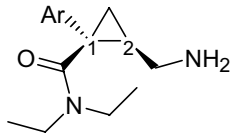
Scheme 1. Reagents and conditions: (a) i—$Na_2CO_3/BrCH_2CH{=}CH_2$/DMF/rt, 16 h; ii—4-$AcNHC_6H_4SO_2N_3$/DBU/CAN/0 °C to rt, 16 h; (b) $Rh(OAc)_2/CH_2Cl_2$/rt to reflux, 3 h; (c) i—potassium phthalamide/DMF/140 °C, 16 h; ii—$SOCl_2/CH_2Cl_2$/rt, 2 h; iii—Et_2NH/CH_2Cl_2/0 °C to rt, 16 h; iv—NH_2NH_2/EtOH/rt, 16 h; (d) SO_2Cl_2 (1 equiv for **6** and 2 equiv for **7**)/CH_2Cl_2/rt, 1 h.

It is known that the 1*S*,2*R*-milnacipran (*R*-**1**) is much more potent at NET than the 1*R*,2*S*-isomer (*S*-**1**).[17,19] Therefore, the 1*S*,2*R*-milnacipran analogs *R*-**5**,*R*-**8**,*R*-**14**-*R*-**23**, and *R*-**26a–q** were then synthesized from arylacetonitriles **30** and *R*-(−)-epichlorohydrin using



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—12 N HCl/0 °C to rt, 2.5 h; (b) i—potassium phthalamide/DMF/140 °C, 16 h; ii—$SOCl_2$/reflux; (c) Et_2NH/CH_2Cl_2/0 °C to rt, 16 h; (d) NH_2NH_2/EtOH/rt, 16 h; (e) $CH_2O/NaBH(OAc)_3/CH_2Cl_2$/rt, 2 h; (f) i—$R^2R^3NH/CH_2Cl_2$/0 °C to rt, 16 h; (g) NBS/DMF/rt, 16 h; (h) SO_2Cl_2 (1–3 equiv)/CH_2Cl_2/rt, 1 h.

Table 1. SAR of the aromatic substitutions **5**–**23**[a] (IC_{50}, nM)



Compound	Stereo	Ar	NET	SERT	DAT	*c*Log*P*
1[b]	1*S*,2*R*&1*R*,2*S*	Ph	77	420	6100	1.2
5	1*S*,2*R*&1*R*,2*S*	3-Thienyl	150	2100	>10,000	0.9
6	1*S*,2*R*&1*R*,2*S*	3-Thienyl, 2-chloro	3300	5200	>10,000	1.6
7	1*S*,2*R*&1*R*,2*S*	3-Thienyl, 2,5-dichloro	470	330	>10,000	2.2
8[c]	1*R*,2*R*&1*S*,2*S*	2-Benzothienyl	18	96	>10,000	3.4
9[c]	1*R*,2*R*&1*S*,2*S*	2-Benzothienyl, 3-methyl	30	280	>10,000	3.9
10[c]	1*R*,2*R*&1*S*,2*S*	2-Benzothienyl, 3-methyl-5-chloro	26	8	>10,000	4.5
11	1*S*,2*R*&1*R*,2*S*	2-Indenyl	190	3200	6100	2.2
12	1*S*,2*R*&1*R*,2*S*	3-Indolyl	7700	4600	>10,000	1.2
13	1*S*,2*R*&1*R*,2*S*	3-Indolyl, 1-Boc-	4800	>10,000	>10,000	2.9
S-**1**	1*R*,2*S*	Ph[d]	5500	3500	>10,000	1.2
R-**1**	1*S*,2*R*	Ph[d]	40	320	3200	1.2
R-**5**[e]	1*S*,2*R*	3-Thienyl[f]	49	830	>10,000	0.9
R-**14**[c,e]	1*R*,2*R*	2-Thienyl[g]	76	520	>10,000	0.9
R-**15**[c]	1*R*,2*R*	2-Thienyl, 5-bromo	29	350	6300	1.8
R-**16**[c]	1*R*,2*R*	2-Thienyl, 5-chloro	40	520	>10,000	1.6
R-**17**[c]	1*R*,2*R*	2-Thienyl, 4,5-dichloro	560	2200	>10,000	2.1
R-**18**[c]	1*R*,2*R*	2-Thienyl, 3,4,5-trichloro	270	2000	>10,000	2.6
R-**19**[c,e]	1*R*,2*R*	2-Pyridinyl	1500	3500	>10,000	−0.3
R-**20**	1*S*,2*R*	3-Pyridinyl	1500	4000	>10,000	−0.3
R-**8**[c]	1*R*,2*R*	2-Benzothienyl	65	620	2400	3.4
R-**21**[c]	1*R*,2*R*	2-Benzothienyl, 5-fluoro	150	290	>10,000	3.4
R-**22**[c]	1*R*,2*R*	2-Benzothiazolyl	2200	9600	>10,000	1.6
R-**23**	1*S*,2*R*	3-Benzothienyl	170	3100	>10,000	3.4

[a] IC_{50} values for the cell uptake inhibition of norepinephrine, serotonin, and dopamine via the respective human transporter stably expressed in HEK293 cells. Data are average of two or more independent measurements.
[b] Vaishnavi et al. reported IC_{50} values of 61, 151, and >100,000, respectively, at human SERT, NET, and DAT for milnacipran, see Ref. 16.
[c] Nomenclature is changed due to sulfur taking priority.
[d] IC_{50} values for the synaptosomal uptake inhibition of two enantiomers at NET, SERT, and DAT are 7, 120, and 10,000 nM, respectively, for *R*-**1**, and 200, 420, and 10,000 nM, respectively, for *S*-**1**, see Roggen et al. in Ref. 17.
[e] The ee values were 92% for *R*-**5**, 98% for *R*-**14**, and 95% for *R*-**15** based on chiral HPLC analyses.
[f] IC_{50} values reported by Roggen et al. (Ref. 17) for the synaptosomal uptake inhibition of *R*-**5** at NET, SERT and DAT are 19, 190, and 10,000 nM, respectively.
[g] IC_{50} values reported by Roggen et al. (Ref. 17) for the synaptosomal uptake inhibition of *R*-**14** at NET, SERT, and DAT are 65, 250, and 10,000 nM, respectively.

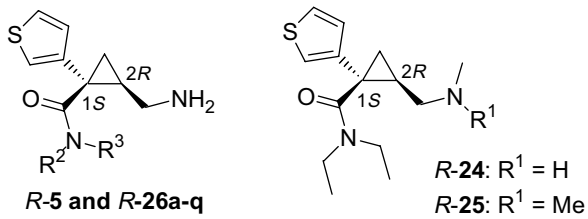
a stereo-selective procedure as described previously (Scheme 2).[21]

The target compounds were tested in inhibitory assays at human NET, SERT, and DAT using procedures similar to those described by Owen,[22] and the results are summarized in Tables 1 and 2.

Compared to milnacipran **1**, the 3-thienyl analog **5** as a 1*R*,2*S*- and 1*S*,2*R*-mixture was slightly less potent at both NET and SERT (Table 1). Chlorination of **5** at the 2-position of the 3-thienyl group resulted in the lipophilic derivative **6** with significantly reduced activity, suggesting an unfavored steric effect by the bulky 2-chlorine. Introducing an additional 5-chlorine to **6** increased its NET and SERT potencies by 7-fold (**7**). The larger 2-benzo[*b*]thiophene **8** was over 4-fold more potent than **1** at both NET and SERT, while its 3-methyl derivative **9** was slightly less active than **8**, implying an unfavored steric effect. An additional chlorine in **10** resulted in a significant increase in SERT potency (IC_{50} = 8 nM) compared to **9**, indicating that a lipophilic aromatic group is favored by SERT. In contrast, the indene **11** and indoles **12** and **13** were only moderately to weakly active, indicative of the specificity of the 2-benzo[*b*]thiophene group for the transporters.

The single isomeric 1*S*,2*R*-analog *R*-**5** displayed 2- to 3-fold higher potency at NET and SERT than the mixture **5**, confirming that this isomer is more potent than its 1*R*,2*S*-isomer.[23] This result was parallel to the single isomers of milnacipran *R*-**1** and *S*-**1**. Bromination or chlorination of the 2-thienyl compound *R*-**14** provided slightly improved analogs *R*-**15** and *R*-**16**. In contrast, the lipophilic dichloro *R*-**17** and trichloro *R*-**18** were only weakly active. Interestingly, the single isomeric *R*-**8** (1*R*,2*R*-isomer), which has the same absolute configuration as the 1*S*,2*R*-isomer *R*-**1** but different nomenclatures due to the priority of the sulfur atom, was significantly less active than the mixture **8**, suggesting that the activity of **8** mainly resides in the 1*S*,2*S*-isomer. Roggen et al. have demonstrated that replacement of the phe-

Table 2. SAR of the amides *R*-**26a–q**[a] (IC_{50}, nM)



Compound	R^2NR^3	NET	SERT	DAT	*c*Log*P*
R-**5**[b]	NEt_2	49	830	>10,000	0.9
R-**24**[b]		3700	4400	>10,000	1.1
R-**25**[b]		>10,000	>10,000	>10,000	1.6
R-**26a**[b]	$EtNCH_2CH{=}CH_2$	5.6	140	>10,000	1.2
R-**26b**	$MeNCH_2CH{=}CH_2$	100	60	>10,000	0.7
R-**26c**	$N(CH_2CH{=}CH_2)_2$	2.7	76	8300	1.7
R-**26d**	$HC{\equiv}CCH_2NCH_2CH{=}CH_2$	2.8	71	>10,000	1.2
R-**26e**	$MeOCH_2CH_2NCH_2CH{=}CH_2$	20	47	>10,000	1.1
R-**26f**	$cPrNCH_2CH{=}CH_2$	8.7	26	>10,000	1.1
R-**26g**	$MeNCH_2CH_2OMe$	1100	590	>10,000	0.2
R-**26h**	$MeOCH_2CH_2NCH_2C{\equiv}CH$	64	47	>10,000	0.7
R-**26i**	$MeNCH_2C{\equiv}CH$	110	34	>10,000	0.3
R-**26j**	$N(CH_2C{\equiv}CH)_2$	8.2	84	>10,000	0.7
R-**26k**	$MeNCH_2CF{=}CH_2$	44	60	>10,000	1.4
R-**26l**	$MeOCH_2CH_2NCH_2CF{=}CH_2$	3.9	40	>10,000	1.8
R-**26m**	Pyridine-1-yl, 1,2,3,6-tetrahydro	200	1800	>10,000	1.1
R-**26n**[b]	Isoquinolin-2-yl, 1,2,3,4-tetrahydro	8	8.3	>10,000	2.2
R-**26o**	Isoindolin-1-yl	37	76	6900	1.7
R-**26p**	Quinolin-1-yl, 1,2,3,4-tetrahydro	20	420	>10,000	2.9
R-**26q**	1,4-Benzoxazin-4-yl, 2,3-dihydro	12	340	>10,000	2.3

[a] Data are average of two or more independent measurements.
[b] The ee value was 92% based on chiral HPLC analysis.

nyl group of 1*R*,2*S*-milnacipran *S*-**1** with a lipophilic 2-naphthyl moiety increases its NET potency by 40-fold and SERT potency by over 20-fold, while the 1*S*,2*R*-configured 2-naphthyl isomer was less potent than *R*-**1**.[17] These results might suggest that a large aromatic ring such as benzo[*b*]thiophene or naphthalene changes the binding mode of milnacipran at both NET and SERT. Therefore, we predict the 1*S*,2*S*-isomer *S*-**10** would be a potent NET/SERT dual inhibitor. However, we were not interested in further investigating these molecules due to their high lipophilicities (*c*Log*P* values above 3).

The 5-fluoro2-benzo[*b*]thiophene *R*-**21** had similar potency to the 3-benzo[*b*]thiophene *R*-**23** at NET, while the hydrophilic 2-benzothiazole *R*-**22** and the two pyridines *R*-**19** and *R*-**20** were only weakly active, suggesting that a lipophilic aromatic group such as a phenyl ring is required for high NET potency.

The N-methylation of *R*-**5** had a significant reduction in NET potency (*R*-**24**), while the *N*,*N*-dimethyl analog *R*-**25** was inactive at all three transporters (Table 2). These results indicate that the amino group of *R*-**5** needs to be close to the *trans*-phenyl ring possibly via intramolecular hydrogen-bonding with the carbonyl moiety,[18] and a steric bulky group at the amino nitrogen of *R*-**5** decreases this possibility.

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 with little change in lipophilicity.[18] We then utilized this information to explore the SAR of the moderately lipophilic 3-thienyl analog (*R*-**5**), and the results are summarized in Table 2. Many potent NET inhibitors were discovered. The NET/SERT ratios of these compounds (*R*-**26a–q**) varied from 1- (*R*-**26n**) to almost 30-fold (*R*-**26c**), and their lipophilicities and molecular weights were not much different from milnacipran **1**.

The NET and SERT potencies of *R*-**26a** increased by 9- and 6-fold, respectively, when one of the two ethyl groups in *R*-**5** was replaced with an allyl moiety. In contrast, *R*-**26b** with an *N*-methyl-*N*-ethyl group had an almost 20-fold reduction at NET but a 2-fold increase at SERT compared with *R*-**5**, indicating that a small lipophilic group such as ethyl is required by NET. These results might also suggest that the π-electron of the allyl group of *R*-**26a** is important for forming a preferred conformation required for the pharmacophore of both NET and SERT.[16] Introducing an additional allyl group to *R*-**26a** increased potency by 2-fold at both NET and SERT (*R*-**26c**), and this allyl moiety could be replaced by a propargyl group (*R*-**26d**). The polar methoxyethyl compound *R*-**26e** displayed reduced potency at NET compared to the ethyl analog *R*-**26a**, while the cyclopropyl group of *R*-**26f** offered the lipophilicity required by NET for this group but did not disturb the conformation favored by SERT, resulting in improved SERT activity. The low lipophilic *R*-**26g** (*c*Log*P* = 0.2) was

Table 3. Drug elimination profiles of *R*-**26** in vitro

Compound	CL_{sys}[a] (mL/min kg)		Caco-2[b]	
	HLM	RLM	P_{app} (nm/s)	(b to a)/(a to b)
1	3.1	24	89	4.5
R-**1**	5.0	24	51	7.4
S-**1**	<2.6	18	n.d.	n.d.
R-**5**	7.1	34	47	4.8
R-**26a**	6.8	57	64	8.9
R-**26c**	4.7	59	95	9.8
R-**26d**	<2.6	44	96	6.0
R-**26e**	<2.6	52	24	22
R-**26f**	<2.6	42	53	7.3
R-**26h**	<2.6	18	32	18
R-**26j**	<2.6	42	164	5.6
R-**26k**	<2.6	32	138	3.0
R-**26l**	<2.6	54	34	12
R-**26o**	10	66	47	23
R-**26q**	13	67	200	4.1

[a] HLM—human liver microsomes; RLM—rat liver microsomes; for liver microsomal assay conditions, see Ref. 24.
[b] Digoxin was used in assay as a positive control. Its P_{app} value was an average of 10 nm/s, and its efflux ratio was about 25.

10-fold less active than *R*-**26b**, demonstrating that the polar methoxyethyl group can not replace the allyl moiety of *R*-**26b**. However, the allyl group of *R*-**26b** could be replaced by a propargyl (*R*-**26i**) or 2-fluoroallyl functionality (*R*-**26k**). The di(propargyl) compound *R*-**26j** showed slightly reduced NET activity compared to the di(allyl) analog *R*-**26c**. Using the cyclic 1,2,3,6-tetrahydropyridine to replace the *N*-ethyl-*N*-allyl group of *R*-**26a** resulted in a significant reduction in both NET and SERT potencies for *R*-**26m**. The tetrahydroisoquinoline *R*-**26n** (cLog P = 2.2) exhibited potent NET and SERT activities with a 1:1 ratio, but this compound was significantly more lipophilic than **1** (cLog P = 1.2). The other lipophilic compounds *R*-**26o–q** also displayed good NET activity.

Several compounds such as *R*-**26c–f**, *R*-**26h**, and *R*-**26j–l** were also tested for their metabolic stability using an in vitro liver microsomal assay to predict their pharmacokinetic properties in humans. Many of these compounds were highly stable in human liver microsomes (Table 3), most likely due to their low lipophilicities. For example, *R*-**26f** had a predicted systemic clearance (CL_{sys}) of less than 2.6 mL/min kg, which was lower than that of 1*S*,2*R*-milnacipran *R*-**1** (CL_{sys} = 5.0 mL/min kg). In comparison, *R*-**26a** was slightly less stable than *R*-**1**, possibly because of its allyl functionality. In comparison, the 3,4-ethylenedioxyphenyl compound *R*-**2** has a CL_{sys} of 8.8 mL/min kg, which was higher than *R*-**1** despite their equal cLog P values (1.2). The two lipophilic compounds *R*-**26o** and *R*-**26q** displayed moderate stability as expected.

Since rats are the primary species used for efficacy, pharmacokinetic, and toxicity studies, we also profiled these compounds in rat liver microsomes. Despite their high resistance to metabolism in human liver microsomes, these compounds displayed poor metabolic stability in rats as shown in Table 3. For example, *R*-**26a** had a rat CL_{sys} of 57 mL/min kg, which was substantially higher than that of *R*-**1**. The most stable compound in rats was *R*-**26h** (CL_{sys}18 mL/min kg), which had a low cLog P value of 0.7. The diethyl amide *R*-**5** was much more stable in rat liver microsomes than many other analogs, such as *R*-**26a**, indicating that the allyl group could be one of the sites for metabolism.

While milnacipran **1** is mainly eliminated via the kidney due to its low plasma protein binding and high metabolic stability, its renal clearance of ~350 mL/min in healthy human subjects is about 3-times higher than the glomerular filtration rate (~125 mL/min),[8] indicative of an active secretion mechanism probably caused by its P-glycoprotein (P-gp) activity. In an in vitro Caco-2 assay, **1** displayed moderate permeability (P_{app} = 89 nm/s) and a possible efflux mechanism reflected by its higher permeability from the basolateral to apical (a to b) direction compared to the reverse direction [(b to a)/(a to b) = 4.5]. While some of the tested compounds (*R*-**26a**, *R*-**26c–d**, and **26f**) showed similar permeability from the apical to basolateral direction compared to *R*-**1**, others displayed higher efflux ratios (*R*-**26e**, *R*-**26h**, and *R*-**26o**), suggesting strong activity of these compounds as P-gp substrates which might facilitate active tubular secretion.

The efflux mechanism by P-gp might also limit brain penetration of its substrates.[25] Compound *R*-**26a**[26] was studied for its brain penetration after oral administration to rats. While its plasma exposure was much lower than that of *R*-**1** (Table 4), most likely caused by the first-pass effect due to the high liver extraction in rats, the brain penetration of *R*-**26a** based on the brain/plasma ratio at 1- and 4-h time points postdosing was only slightly lower than that of *R*-**1**, most likely due to the similarity in their molecular structures and lipophilicity (measured Log P of 1.6 for *R*-**1** and 1.5 for *R*-**26a**).

R-**26a** was also briefly studied for its metabolic profile. In human liver microsomes, only one minor hydroxyl-

Table 4. Plasma and brain exposure of ***R*-1** and *R*-**26a** in rats after oral administration[a]

Compound	p.o. Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC (ng/mL h)	C_{brain} (ng/g) at 1 and 4 h	b/p ratio at 1 and 4 h
R-**1**[b]	10	232	0.3	566	103, 55	0.42, 0.86
R-**26a**[c]	30	201	0.5	502	39, 15	0.30, 0.40

[a] Average of three animals.
[b] Measured Log D = −0.2, pK_a = 9.6.
[c] Measured Log D = 0.1, pK_a = 9.5.

ated metabolite (M+16) was detected after incubation.[27] In human hepatocytes, an additional minor oxidative (M+16) metabolite with higher polarity was detected. The structures of these metabolites were not determined.

In summary, a series of milnacipran analogs were studied for their structure-activity relationship as NET and SERT inhibitors. Many potent compounds were discovered which possessed moderate lipophilicity similar to milnacipran. For example, compound *R*-**26f** exhibited an IC_{50} value of 8.7 nM at NET, which was 5-fold more potent than the active isomer of milnacipran (*R*-**1**). Its potency at SERT (IC_{50} = 26 nM) was 10-fold better than *R*-**1**. This compound exhibited high metabolic stability in human liver microsomes, and its P-gp profile was similar to that of *R*-**1**. These results indicate that compounds such as *R*-**26f** might have a drug elimination profile similar to milnacipran in humans but their higher potency could be beneficial for efficacy.

References and notes

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26. Characterization of *R*-**26a** hydrochloride: white powder; HPLC purity: 100% (220 and 254 nm); ^{1}H NMR ($CDCl_3$, mixture of E/Z amides, 59:41): 0.92 & 1.09 (t, J = 6.3 Hz, 3H), 1.21 (m, 1H), 1.64 & 1.75 (m, 1H), 1.82 (m, 1H), 2.41 (m, 1H), 3.04–3.65 (m, 2H), 3.73 (m, 1H), 3.82 & 3.87 (m, 1H), 3.96–4.16 (m, 1H), 5.02–5.10 (m, 2H), 5.47 & 5.72 (m, 1H), 6.84–7.02 (m, 3H), 8.76 (brs, 3H); MS: 265 (MH^+); Anal. for ($C_{14}H_{20}N_2OS{\cdot}HCl$), calcd: C 55.89%, H 7.04%, N, 9.31%, S 10.66%; found: C 55.90%, H 7.21%, N 9.18%, S 10.59%.
27. The compound (50 μM) was incubated at 37 °C for 2 h in human liver microsomes (0.5 mg/mL P450 proteins) or human hepatocytes (2.0 million cells/mL).