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Development of SPECT imaging agents for the norepinephrine transporters: [^{123}I]INER☆

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Abstract—A series of reboxetine analogs was synthesized and evaluated for in vitro binding as racemic mixtures. The best candidate (INER) was synthesized as the optically pure (*S*,*S*) enantiomer, labeled with iodine-123 and its in vivo binding determined by SPECT imaging in baboons. The in vivo specificity, selectivity, and kinetics of [^{123}I]INER make it a promising agent for imaging NET in vivo by noninvasive SPECT imaging.

The norepinephrine transporter (NET) is a protein with 12 transmembrane-spanning domains, located at the pre-synaptic terminal of noradrenergic neurons. The principal physiological function of NET is to remove excess norepinephrine (NE) from the synaptic cleft to terminate the action of NE (avoiding over-stimulation) and to recycle NE into the pre-synaptic neuron (active reuptake) for later re-release. Brain structures known to be rich in NET include the locus coerulus (brainstem area); thalamus, hippocampus, and throughout the cerebral cortex, whereas low densities are found in cerebellum and striatum.[4] NET has been implicated in the pathophysiology of numerous neuropsychiatric and neurodegenerative disorders, including depression (reduced level of NE in the synapse appears to down-regulate the level of NET),[5,6] arousal,[7] anxiety,[8,9] attention-deficit/hyperactivity disorder (atomoxetine **1** is the first non-stimulant selective NET inhibitor for the treatment of ADHD[10]),[11] and Alzheimer's disease.[12] Therefore, the development of a specific radioligand to quantify the variation of NET density in vivo will be of great utility as a diagnostic tool to better understand the etiology and pathogenesis of these neuropsychiatric disorders, as well as a tool to evaluate potential new drugs targeting the NET.

Research on quantitative mapping of dopamine transporter (DAT) and serotonin transporter (SERT) related to various CNS disorders has benefited from the availability of suitable radioligands (e.g., β-CIT as DAT radioligand,[13,14] and DASB or ZIENT as SERT radioligands[15,16]). In contrast, brain imaging of NET has been hampered by lack of a suitable radioligand. However, in the past few years some selective NET radioligands have been prepared and evaluated in animals. [^{11}C]Nisoxetine[17] **2** showed significant binding in NET-rich tissue in mouse brain but also a high nonspecific binding and unfavorable slow kinetics, which excludes it as a practical NET imaging agent. Recently, several research groups have reported the evaluation of derivatives of reboxetine (**3**) with more promising properties than the nisoxetine series. [^{11}C]MeNER[18–20] **4** has shown the highest hypothalamus/striatum ratio of 2.5 at 60 min in rats, corresponding to the relative distribution of NET and SERT.[21] Displacement study

Keywords: SPECT; Imaging; Norepinephrine; Transporter; Reboxetine; Iodine-123.

☆ Preliminary results of these experiments have been reported at scientific conferences for imaging 1. See Refs. 1–3.

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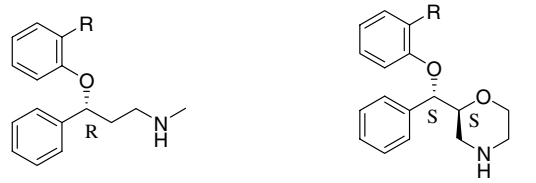
using reboxetine or desipramine as selective NET inhibitor pretreatment reduced this ratio to unity, whereas selective DAT and SERT inhibitors did not significantly affect the ratio.[18] However, the long time to achieve peak uptake (>90 min) makes this radioligand unsuitable for PET imaging.[19] From a statistical point of view it is not optimal to obtain the maximal specific binding at the end of the experiment when the signal to noise ratios are decreasing due to rapid decay of the carbon-11 (half-life = 20 min), although these issues are not as critical for SPECT imaging owing to the longer half-life of typical SPECT radionuclides (^{123}I, 13.2 h, ^{111}In, 2.8 days). In the nisoxetine series, (*R*)-[^{125}I]MIPP **5** showed good in vitro affinity for NET and moderate affinity for SERT, which, when combined with the significantly higher density of SERT compared to NET, decreased the selectivity for NET in vivo.



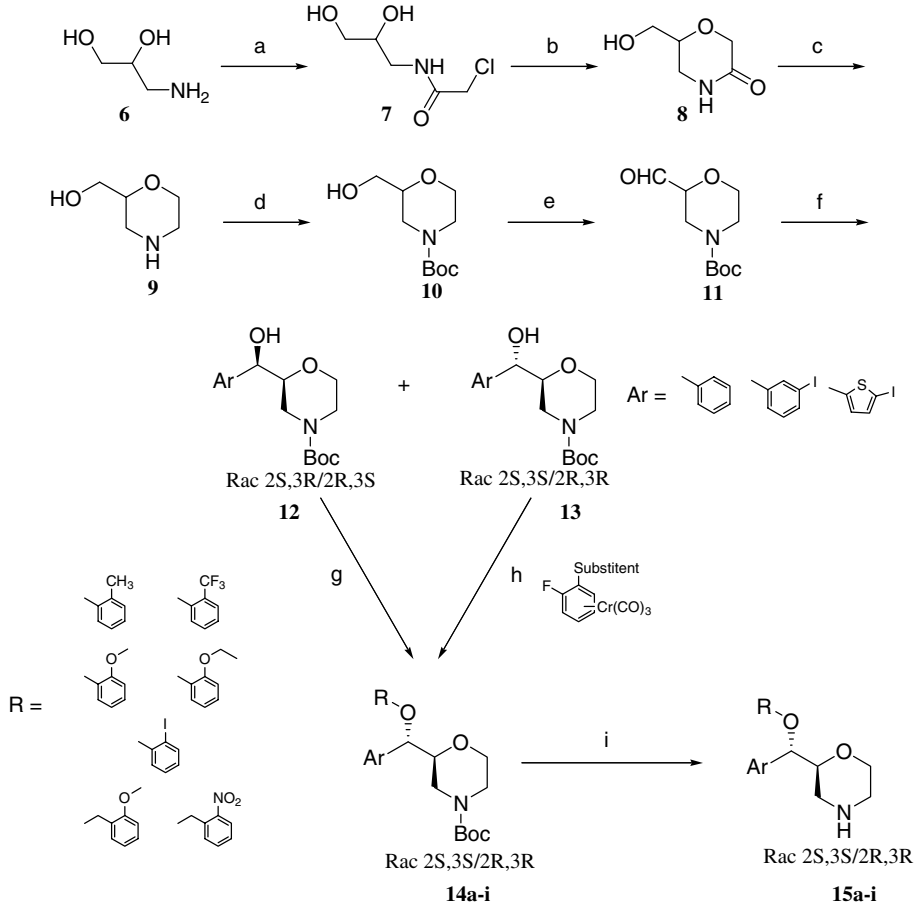
1 R = Me Atomoxetine
2 R = OMe Nisoxetine
5 R = I MIPP

3 R = OEt Reboxetine
4 R = Me MeNER

Figure 1. Structure of NET ligand.

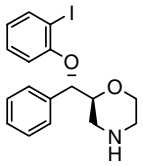
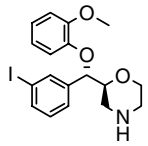
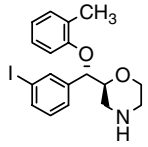
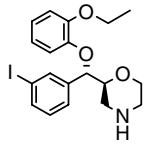
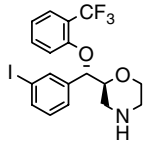
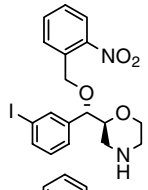
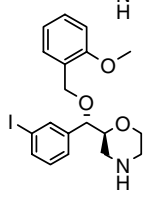
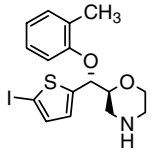
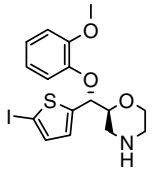
We recently reported a new asymmetric synthesis[2] which we used to synthesize new analogs of reboxetine. We report here the synthesis, in vitro evaluation of new reboxetine analogs as potential NET imaging agent as well as the in vivo evaluation of the most promising agent in this series (Fig. 1).

Since in the reboxetine series, the most active isomer always corresponds to the *S*,*S* isomer, in order to rapidly discriminate the future candidates we decided to first synthesize the racemic compounds (mixture *S*,*S*/*R*,*R*) and then to pursue the most promising candidates with the evaluation of the *S*,*S* enantiomer. The synthesis of the new compounds is summarized in Scheme 1. The key intermediate **11** was synthesized in good yield starting from the racemic aminopropanediol **6** in five steps (detail of the synthesis was described previously[2]). Subsequent condensation of substituted diaryl or diheteroaryl zinc provided a mixture of racemic diastereomers **12** and **13** easily separated by chromatography. The racemic (*S*,*S*/*R*,*R*) reboxetine analogs of N-Boc-protected **14a**–**i** were obtained from **12** and **13**, using either a Mitsunobu reaction (starting from **12**) or by a direct nucleo-



Scheme 1. Reagents and conditions: (a) $ClCH_2COCl/Et_3N$; (b) *t*-BuOK/*t*-AmOH; (c) red-Al/THF; (d) $(Boc)_2O$/NaOH; (e) trichloroisocyanuric acid/TEMPO/$NaHCO_3$; (f) Ar_2Zn/THF; (g) R-OH/DIAD/PPh_3/THF; (h) fluorobenzenetricarbonylchromium complex/NaH; (i) TFA/CH_2Cl_2.

Table 1. Affinity (K_i, nM) or SERT inhibition (% at 10 μM) of reboxetine analogs[a]

Compound	Structure[b]	NET	DAT	SERT	SERT inhibition (%)
15a		2.47	410		40
15b		15.4	>10,000		12
15c		27.6	740		24
15d		28.5	3139		44
15e		101	1871	486	
15f		70.4	14.2	252	
15g		73.8	716	351	
15h		71.9	186		31
15i		79.6	210	141	

[a] Assays of novel compounds employed cell membrane-containing homogenates of rat forebrain (frontoparietal cerebral cortex for SERT and NET, caudate-putamen for DAT), with [^{3}H]paroxetine (0.17 nM) for SERT (blank = 10mM *R,S*-fluoxetine, Sigma-RBI), [^{3}H]GBR-12935 (0.20 nM) for DAT (blank = 10 mM GBR-12909, Sigma-RBI), and [^{3}H]nisoxetine (0.40 nM) for NET (blank = 10 mM desipramine, Sigma-RBI); results are expressed as means of three separate determinations.

[b] All compounds are racemic mixtures; for clarity, just one enantiomer is represented.

Table 2. Binding affinity of INER (K_i, nM, means ± SEM)[a]

Compound	NET	DAT	SERT	Selectivity NET versus DAT	Selectivity NET versus SERT
INER	0.84 ± 0.12	228 ± 6.9	40.4 ± 10.4	270	51

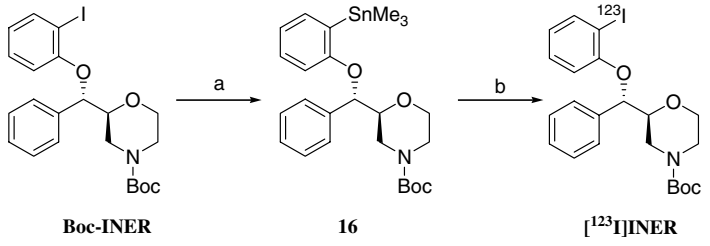
[a] Assays of INER employed cell membrane-containing homogenates of rat forebrain (frontoparietal cerebral cortex for SERT and NET, caudate-putamen for DAT), with [^{3}H]paroxetine (0.17 nM) for SERT (blank = 10 mM *R*,*S*-fluoxetine, Sigma-RBI), [^{3}H]GBR-12935 (0.20 μM) for DAT (blank = 10 μM GBR-12909, Sigma-RBI), and [^{3}H]nisoxetine (0.40 nM) for NET (blank = 10 μM desipramine, Sigma-RBI); results are expressed as means of three separate determinations.

philic substitution of the appropriate 2-fluoro benzene tricarbonylchromium complex (starting from **13**). Deprotection of compounds **14a–i** was achieved in quantitative yield using trifluoroacetic acid. Compounds **15a–i** were evaluated in vitro against all three monoamine transporters, using cell membrane-containing homogenates of rat forebrain (frontoparietal cerebral cortex for SERT and NET, caudate-putamen for DAT) as transporter sources (Table 1).

All compounds showed a better activity at NET than at the other monoamine transporters (DAT and SERT) except for compound **15f**, which was slightly more active at the DAT. In this series, compound **15a** is of particular interest, with a binding affinity of 2.5 nM for the racemic mixture. The introduction of an iodine at position 3 of the phenyl moiety (compounds **15b–e**) led to a decrease of the affinity. Replacement of the phenoxy by a benzyloxy moiety (**15f–g**) led to less active compounds (15.4 nM vs 73.8 nM for **15b** vs **15g**). Replacement of the phenyl ring by a thiophene reduced the activity of the compounds compared to the parent.

These results indicate that compound **15a** shows the most promise and thus we focused our effort on this structure. The enantiomeric pure synthesis was achieved using the same sequence illustrated in Scheme 1 but this time starting from the chiral (*S*)-(-)-3-amino-1,2-propanediol which led to INER (formal (2*S*,3*S*)-**15a**) with ee = 99%. INER was evaluated against all three monoamine transporters and shows binding value K_i = 0.84 nM for NET and selectivity versus DAT and SERT (270 and 51, respectively, Table 2).

Radiolabeled [^{123}I]INER was prepared via iododestannylation of the tin precursor **16**, easily synthesized by palladium-catalyzed stannylation of Boc-INER (formal (2*S*,3*S*)-**14a**). Removal of the Boc-protecting group with trifluoroacetic acid and HPLC purification led to [^{123}I]INER as described in Scheme 2. The lipophilicity of [^{123}I]INER was assessed by octanol-phosphate buffer (pH 7.4) partition. The partition coefficient log *D* of [^{123}I]INER was 2.6, which is high in the range of values considered acceptable for good blood–brain barrier penetration.[22]



Scheme 2. Reagents and conditions: (a) $(SnMe_3)_2$/$Pd(PPh_3)_4$/DME; (b) Na[^{123}I]/CH_3COOOH/H_3PO_4.

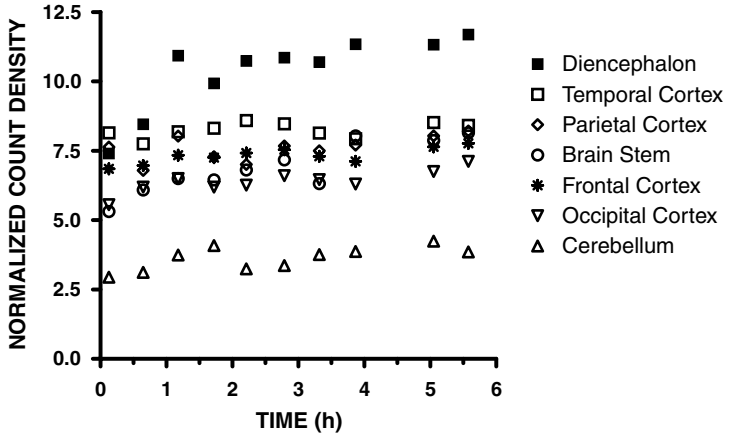


Figure 2. Decay-corrected time activity curve in a baboon undergoing constant infusion of [^{123}I]INER.

A series of SPECT imaging studies were conducted including both bolus-only and bolus plus continuous infusion of [^{123}I]INER in an ovariectomized female baboon (*Papio anubis*) to evaluate the regional brain uptake and washout of activity. Pharmacological specificity was assessed by blocking displacement experiments using reboxetine as selective NET antagonist and citalopram as SERT antagonist.

An initial study was performed by dynamic SPECT imaging after a single bolus injection of 7.89 mCi of [^{123}I]INER in an adult female olive baboon. The maximal radioactivity peak in the brain was obtained after 10 min, with 1% of the total injected dose, followed by a slow washout over more than 2 h. Regional brain tracer uptake is consistent with the distribution of NET in the baboon brain, with the highest uptake in locus coeruleus (brainstem) and thalamus (diencephalon), and lowest uptake in cerebellum and striatum. In the next study [^{123}I]INER was administered as a loading bolus injection followed by constant infusion using a bolus to infusion ratio of 1.3 h (Fig. 2), stable levels were obtained in less than 100 min. Regional distribution of radioactivity was identical to that obtained in the bolus experiment, which followed the established distribution of NET in the baboon brain.

To assess the pharmacological specificity, reboxetine was injected (1 mg/kg iv) at 210 min after administration of bolus-plus-constant infusion which resulted in a reduction of radioactivity in all brain regions. Reduction was more pronounced in the NET-rich regions, with up to 60% specific displacement. In contrast, displacement with citalopram (5 mg/kg) or methylphenidate (0.5 mg/kg) at 210 min pi did not show any displacement of [^{123}I]INER.

Recently, the same structure as compound **15a** was reported under the name 'IPBM' by Saji et al.[23] Results were comparable to those we have reported here.

In conclusion, we report the synthesis of nine racemic potential NET imaging agents. Of these, compound **15a** (INER) was the best candidate for further investigation and we developed an asymmetric synthesis leading to the enantiopure INER. INER showed high affinity for NET (K_i = 0.84 nM) and good selectivity versus DAT and SERT (270 and 51, respectively). We synthesized [^{123}I]INER and showed specific in vivo NET binding in baboon. Taken together, the in vivo specificity, selectivity, and kinetics suggest that [^{123}I]INER is a promising agent for imaging NET in vivo using SPECT or PET.

References and notes

1. Tamagnan, G. D.; Haile, C. N.; Brenner, E.; Koren, A. O.; Bois, F.; Amici, L.; Baldwin, R. M.; Seibyl, J. P.; Staley, J. K. *EJNM* **2005**, *32*, 287.
2. Brenner, E.; Baldwin, R. M.; Tamagnan, G. D. *Org. Lett.* **2005**, *7*, 937.
3. Brenner, E.; Baldwin, R. M.; Tarazi, F.; Baldessarini, R. J.; Tamagnan, G. D. *The 229th ACS National Meeting*, San Diego, CA, March 13–17, 2005.
4. Aston-Jones, G.; Foote, S.; Bloom, F. Anatomy and physiology of locus coeruleus neurons: functional implication. In *Norepinephrine (Frontiers of Clinical Neuroscience)*; Ziegler, M., Lake, C., Eds.; Williams and Wilkins: Baltimore, 1984; Vol. 2, pp 92–116.
5. Spillmann, M. K.; Van der Does, A. J. W.; Rankin, M. A.; Vuolo, R. D.; Alpert, J. E.; Nierenberg, A. A.; Rosenbaum, J. F.; Hayden, D.; Schoenfeld, D.; Fava, M. *Psychopharmacology* **2001**, *155*, 123.
6. Miller, H. L.; Delgado, P. L.; Salomon, R. M.; Berman, R.; Krystal, J. H.; Heninger, G. R.; Charney, D. S. *Arch. Gen. Psychiatry* **1996**, *53*, 117.
7. Kugaya, A.; Sanacora, G.; Staley, J. K.; Malison, R. T.; Bozkurt, A.; Khan, S.; Anand, A.; van Dyck, C. H.; Baldwin, R. M.; Seibyl, J. P. *Biol. Psychiatry* **2004**, *56*, 497.
8. Hisamichi, S.; Moon, A. Y.; Aoyagi, A.; Hara, T.; Abe, K.; Yamazaki, R.; Kumagae, Y.; Naruto, S.; Koyama, K.; Marumoto, S.; Tago, K.; Toda, N.; Takami, K.; Yamada, N.; Ori, M.; Kogen, H.; Kaneko, T. *J. Pharmacol. Sci.* **2003**, *93*, 95.
9. Allgulander, C.; Hackett, D.; Salinas, E. *Br. J. Psychiatry* **2001**, *179*, 15.
10. Bymaster, F. P.; Katner, J. S.; Nelson, D. L.; Hemrick-Luecke, S. K.; Threlkeld, P. G.; Heiligenstein, J. H.; Morin, S. M.; Gehlert, D. R.; Perry, K. W. *Neuropsychopharmacology* **2002**, *27*, 699.
11. Biederman, J.; Spencer, T. *Biol. Psychiatry* **1999**, *46*, 1234.
12. Tejani-Butt, S. M.; Yang, J.; Zaffar, H. *Brain Res.* **1993**, *631*, 147.
13. Scanley, B. E.; Al-Tikriti, M. S.; Gandelman, M. S.; Laruelle, M.; Zea-Ponce, Y.; Baldwin, R. M.; Zoghbi, S. S.; Hoffer, P. B.; Charney, D. S.; Wang, S.-Y.; Neumeyer, J. L.; Innis, R. B. *Eur. J. Nucl. Med.* **1995**, *22*, 4.
14. Malison, R. T.; McDougle, C. J.; van Dyck, C. H.; Scahill, L.; Baldwin, R. M.; Seibyl, J. P.; Price, L. H.; Leckman, J. F.; Innis, R. B. *Am. J. Psychiatry* **1995**, *152*, 1359.
15. Chen, P.; Kilts, C. D.; Galt, J. R.; Ely, T. D.; Camp, V. M.; Malveaux, E. J.; Goodman, M. M. *J. Nucl. Med.* **2000**, *41*, 39P.
16. Goodman, M. M.; Chen, P.; Plisson, C.; Martarello, L.; Galt, J.; Votaw, J. R.; Kilts, C. D.; Malveaux, G.; Camp, V. M.; Shi, B.; Ely, T. D.; Howell, L.; McConathy, J.; Nemeroff, C. B. *J. Med. Chem.* **2003**, *46*, 925.
17. Haka, M. S.; Kilbourn, M. R. *Nucl. Med. Biol.* **1989**, *16*, 771.
18. Wilson, A. A.; Patrick Johnson, D.; Mozley, D.; Hussey, D.; Ginovart, N.; Nobrega, J.; Garcia, A.; Meyer, J.; Houle, S. *Nucl. Med. Biol.* **2003**, *30*, 85.
19. Schou, M.; Halldin, C.; Sovago, J.; Pike, V. W.; Gulyas, B.; Mozley, P. D.; Johnson, D. P.; Hall, H.; Innis, R. B.; Farde, L. *Nucl. Med. Biol.* **2003**, *30*, 707.
20. Schou, M.; Halldin, C.; Sóvágó, J.; Pike, V. W.; Hall, H.; Gulyás, B.; Mozley, P. D.; Dobson, D.; Shchukin, E.; Innis, R. B.; Farde, L. *Synapse* **2004**, *53*, 57.
21. Smith, H. R.; Beveridge, T. J. R.; Porrino, L. J. *Neuroscience* **2006**, *138*, 703.
22. Laruelle, M.; Slifstein, M.; Huang, Y. *Mol. Imaging Biol.* **2003**, *5*, 363.
23. Kanegawa, N.; Kiyono, Y.; Kimura, H.; Sugita, T.; Kajiyama, S.; Kawashima, H.; Ueda, M.; Kuge, Y.; Saji, H. *EJNM* **2006**, *33*, 639.