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Synthesis and Pharmacological Evaluation of (*Z*)-9-(Heteroarylmethylene)-7-azatricyclo[4.3.1.0^{3,7}]decanes: Thiophene Analogues as Potent Norepinephrine Transporter Inhibitors

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Abstract—To further explore the structure–activity relationships (SARs) of certain tropanes, and to gain insights into the structural features required for high activity and selectivity at norepinephrine transporters (NET), we have introduced both five- and six-membered heteroaromatic moieties such as substituted pyridyl, pyrazinyl, pyrimidyl, thiazolyl, and mono- or disubstituted thienyl groups into conformationally constrained, tricyclic tropane analogues. A number of (*Z*)-9-(heteroarylmethylene)-7-azatricyclo[4.3.1.0^{3,7}]decanes were synthesized, and their abilities to block dopamine, serotonin, and norepinephrine reuptake by their respective transporters were evaluated. It was found that the five- or six-membered *N*-containing aromatics are too basic to display high NET activity, while some of the thiophene analogues were identified as potent and selective NET inhibitors.

Introduction

The norepinephrine transporter (NET) is located in the plasma membrane of noradrenergic neurons, where it functions to take up synaptically released norepinephrine (NE). The NET thus serves as the primary mechanism for the inactivation of noradrenergic signaling.[1–3] Accumulating evidence supports the view that norepinephrine plays an important role in the central nervous system and, although its exact mechanism still remains unknown, it is likely to play a role in a number of psychiatric disorders such as depression and attention-deficit/hyperactivity disorder (ADHD).[4–12] Reuptake of NE is competitive with a variety of naturally occurring amines and drugs. Drugs of abuse such as cocaine, and antidepressants (e.g., desipramine, imipramine, venlafaxine, mirtazapine, reboxetine, bupropion), block the transport of NE, and result in an elevation of the synaptic concentrations of NE and potentiation of the activation of postsynaptic receptors.[13–15] Several NET-selective ligands such as the antidepressant desipramine are quite effective in certain patient populations; however, their use is often limited by side effects, particularly those thought to be mediated by their anticholinergic properties.[3,16] It is possible that other selective ligands for the norepinephrine transporter may find possible applications in the treatment of depression. However, currently there are a limited number of NET-selective ligands available. Recently, some of our research efforts directed toward the discovery of cocaine medications have led us to undertake the synthesis and pharmacological evaluation of conformationally constrained tricyclic tropane analogues. These efforts have resulted in the elucidation of structural factors relevant to achieving varying degrees of monoamine transporter selectivity. As reported herein, we have now identified NET-selective ligands. As such, these ligands serve as lead candidates for the generation of molecules that may find use as therapeutics in the treatment of depression as well as ADHD. Moreover, appropriately radiolabeled ligands of high affinity can be used as tools in obtaining a better understanding of psychiatric diseases through the use of positron emission tomography (PET).

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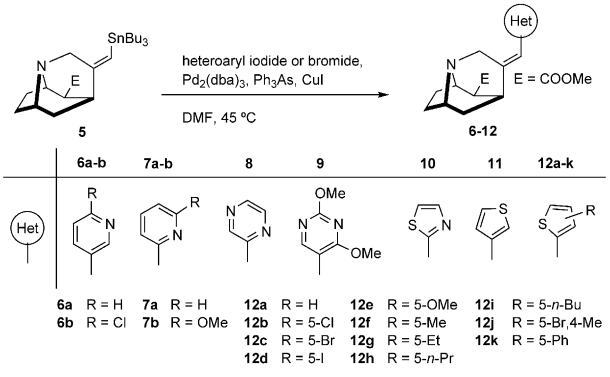
Most of the potent tropane-based monoamine transporter inhibitors, including cocaine (**1**, Chart 1), are believed to have at least three major interactions with the transporter binding sites: one ionic or H-bonding interaction of the basic nitrogen, one dipole–dipole or H-bonding interaction of the ester group of the ligand, and an interaction of the aryl group of the ligand with a lipophilic binding pocket.[17–21] Based upon our preliminary SAR studies on conformationally constrained tricyclic tropanes (**2**) the lipophilic aromatic substituent at the 9-position appears to be a crucial determinant of selectivity.[22–25] The different shape and size of the lipophilic recognition pocket that encompasses the aryl ring(s) of these tropanes are major determinants of a ligand's transporter activity at either the SERT or the NET, and these studies have led to the discovery of analogues selective for the SERT such as compound **3**[23] as well as analogues selective for the NET such as compound **4**.[25]

It is well-established that some heteroaromatics can be effective bioisosteres of the benzene ring, and that their introduction into an active molecule may result in the discovery of new pharmacological agents.[26–28] Previously, we reported that a series of thiophene derivatives of the parent structure, (*Z*)-9-benzylidene-7-azatricyclo[4.3.1.0^{3,7}]decane, act as potent norepinephrine and serotonin reuptake inhibitors.[22,24] To further explore the structure–activity relationship (SAR) and to gain insight into the structural features required for high activity and selectivity at norepinephrine transporters, we have introduced both five- and six-membered heteroaromatic moieties such as substituted pyridyl, pyrazinyl, pyrimidyl, thiazolyl, and mono- or disubstituted thienyl groups into our conformationally constrained tricyclic tropane analogues in continuation of our previous work.[22–25] In this paper, the synthesis and pharmacological activity of a number of (*Z*)-9-(heteroarylmethylene)-7-azatricyclo[4.3.1.0^{3,7}]decanes will be discussed.



Scheme 1.

Chemistry

As illustrated in Scheme 1, analogues **6–12** were synthesized by Stille coupling of vinylstannane **5**[22] with the corresponding heteroaryl iodides (except for compound **7b**, for which the bromide was used) in the presence of $Pd_2(dba)_3$ and Ph_3As in moderate yields.[29] It is noteworthy that the yields were significantly improved utilizing the catalyst system with the ligand Ph_3As instead of $(o\text{-}CH_3C_6H_4)_3P$ that was previously used.[22–24] 2-Iodothiazole was prepared by the iododestannylation reaction of 2-(trimethylstannyl)thiazole, which was generated by quenching 2-lithiothiazole with trimethyltin chloride.[30] Mono- or disubstituted thienyl iodides were prepared by iodination of the corresponding thiophenes using yellow mercuric oxide and iodine[31] except for 2- and 3-iodothiophene, 2,5-diiodothiophene, and 2-iodo-5-methylthiophene that were commercially available. Thiophene analogues **13a–d** were obtained by Suzuki coupling of the iodide **12d** with arylboronic acids in the presence of $Pd(PPh_3)_4$ or $Pd(OAc)_2$ as the catalyst in moderate yields (Scheme 2).

Pharmacological Results

All final compounds were tested as the free base for their ability to inhibit high-affinity reuptake of DA, 5-HT and NE into nerve endings (synaptosomes) prepared from brain regions enriched in transporters for these biogenic amine neurotransmitters.[32] The effect of candidate compounds in antagonizing biogenic amine high-affinity uptake was determined using a method similar to that previously employed for [^{3}H]DA uptake.[33] Rat striatum, midbrain, and parietal/occipital

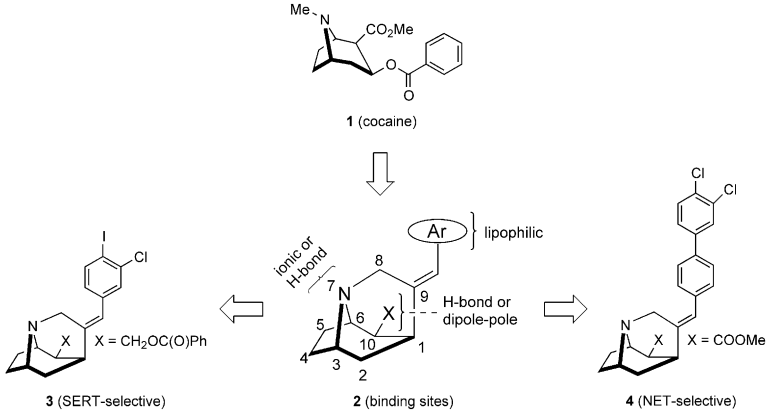
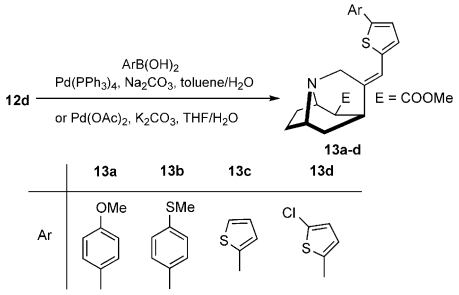


Chart 1.

cortex were dissected and used as a source of DAT, SERT, and NET, respectively. The Cheng–Prusoff equation for classic, competitive inhibition was used for calculating K_i from IC_{50} values in uptake experiments. The K_m values used were about 67 nM for [^{3}H]DA, 53 nM for [^{3}H]5-HT, and 54 nM for [^{3}H]NE. The uptake data and selectivity profiles (based on the K_i values) of all the new compounds prepared are listed in Table 1. For comparison purposes, data for compounds **12a**, **12d**, **12f**, **12k**, and **13a** from our previous papers[22,24,25] and for (−)-cocaine, the antidepressant desipramine as well as the drug atomoxetine for ADHD are also included. All data are mean values ± SEM from two to four independent experiments, each consisting of six drug concentrations in triplicate.



Scheme 2.

The present SAR data demonstrate that the modification of the aromatic ring at the 9-position with different heterocycles results in significant effects on the transporter inhibitory activity of the ligands. Most of the substituted pyridyl, pyrazinyl, pyrimidyl, and thiazolyl analogues, that is, compounds containing one or two nitrogen atoms in the six- or five-membered aromatic ring, display rather low activities except compound **7a**, which exhibits a moderate NET activity similar to that of cocaine. These heteroaromatics are too basic and thus disfavor the interaction of the ligand with the lipophilic binding pocket of the NE transporter.

However, most of the thienyl analogues exhibit a moderate to high activity at the NET. The 3- and 2-thienyl analogues **11** and **12a** display similar activities and selectivities with a K_i around 30 nM at the NET, a 100-fold selectivity versus the SERT, and a 10-fold selectivity versus the DAT, respectively. All of the halogenated derivatives **12b–d** exhibit a good to high potency at the NET, with the 5-iodo analogue **12d** having the best activity and selectivity among them. The 5-methoxy analogue **12e** displays a rather poor activity whereas the 5-methyl analogue **12f** has the same remarkable potency ($K_i = 5$ nM) as **12d** at the NET, a 100-fold selectivity versus the DAT, and a 33-fold selectivity versus the SERT. In comparison to **12f**, a dramatic loss in activity was observed for compounds **12g–i** containing bulkier

Table 1. Inhibition of reuptake at monoamine transporters [K_i ± SEM (nM)][a]

Compd	[^{3}H]DA uptake K_i (nM)	[^{3}H]5-HT uptake K_i (nM)	[^{3}H]NE uptake K_i (nM)	Uptake ratio (based on K_i)		
				5-HT/DA	NE/DA	NE/5-HT
Cocaine	423 ± 147	155 ± 0.4	108 ± 3.5	0.37	0.26	0.7
Desipramine[b]	> 10,000	163[c]	7.36			
Atomoxetine[d]	1451	77	5			
6a	> 10,000	4580 ± 58	12,700 ± 1180	N/d	N/d	2.77
6b	> 10,000	810 ± 29	6510 ± 78	N/d	N/d	8.03
7a	1060 ± 64	3150 ± 293	115 ± 9	2.97	0.11	0.04
7b	14,000 ± 750	477 ± 80	308 ± 97	0.03	0.02	0.65
8	6570 ± 642	7140 ± 543	1930 ± 9	1.09	0.29	0.27
9	> 10,000	5140 ± 868	9450 ± 2600	N/d	N/d	1.84
10	5090 ± 821	> 10,000	855 ± 189	N/d	0.17	N/d
11	485 ± 80	3140 ± 651	33 ± 4.5	6.47	0.07	0.01
12a[e]	268 ± 17	2050 ± 42	26 ± 1.9	7.63	0.10	0.01
12b	130 ± 27	53 ± 2	9.7 ± 2.8	0.41	0.07	0.18
12c	147 ± 4	36 ± 7	20 ± 5.2	0.24	0.14	0.57
12d[f]	368 ± 2	29 ± 1.6	5.0 ± 1.3	0.08	0.01	0.17
12e	> 10,000	544 ± 103	2250 ± 410	N/d	N/d	4.12
12f[f]	403 ± 20	179 ± 38	4.9 ± 0.2	0.44	0.01	0.03
12g	> 10,000	346 ± 26	2130 ± 46	N/d	N/d	6.16
12h	1600 ± 361	269 ± 39	624 ± 122	0.17	0.39	2.32
12i	4700 ± 815	665 ± 43	364 ± 51	0.14	0.08	0.55
12j	1280 ± 35	6.75 ± 1.15	36.8 ± 7.5	0.01	0.03	5.45
12k[g]	179 ± 19.4	71 ± 5	13.5 ± 2.0	0.40	0.08	0.19
13a[g]	371 ± 11	531 ± 51	10.3 ± 0.5	1.43	0.03	0.02
13b	1650 ± 47	3320 ± 525	273 ± 37	2.01	0.16	0.08
13c	298 ± 36	48 ± 1	98 ± 4	0.16	0.33	2.04
13d	242 ± 48	93 ± 2	58 ± 13	0.38	0.24	0.62

[a] K_i values are mean ± SEM from two to four independent experiments, each consisting of six drug concentrations (in triplicate) that were selected on the basis of preliminary screening experiments to bracket the approximate IC_{50} value.
[b] Data taken from PDSP K_i Database (http://kidb.bioc.cwru.edu/pdsp.php).
[c] Data for cloned human receptors.
[d] Data taken from ref 34 and for cloned human receptors.
[e] Data taken from ref 22.
[f] Data taken from ref 24.
[g] Data taken from ref 25.

5-substituents such as ethyl, *n*-propyl, and *n*-butyl. Interestingly, the disubstituted thienyl analogue **12j**, which differs from **12c** by the presence of an additional 4-methyl group, displays an increased potency ($K_i = 6.75$ nM) at the SERT but a decreased potency ($K_i = 36.8$ nM) at the NET. We also investigated the biaryl derivatives **12k** and **13a–d** with an 'outer' aromatic substituent at the 5-position of the 'inner' thiophene ring, most of which exhibit a good to high activity at the NET. Ligand **13a** which contains a 4-methoxy group on the phenyl moiety displays a better NET activity ($K_i = 10.3$ nM) and selectivity in comparison to the unsubstituted phenyl analogue **12k**. However, introduction of the more bulky 4-methylthio group results in a dramatic loss in activity. Compound **13d** resulting from introduction of a 5-chloro group into the 'outer' thiophene ring exhibits a slightly higher NET potency ($K_i = 58$ nM) and selectivity in comparison to the bithienyl analogue **13c**. The above SAR indicates that the binding pocket that encompasses the thienyl group is highly sensitive to the substituent attached to this ring, and that further modification of this ring may therefore result in the discovery of even more potent NET-selective inhibitors.

The ClogPs of selected analogues with a moderate to high potency are given in Table 2. Most of the (*Z*)-9-(heteroarylmethylene) - 7 - azatricyclo[4.3.1.0^{3,7}]decanes except the biaryl analogues have a ClogP value in the range of 2-4 and can therefore be expected to readily penetrate the blood–brain barrier. In comparison to the biphenyl-type ligands,[25] the 5-aryl-2-thienyl analogues generally retain NET activity and also have an improved ClogP even though they are still somewhat too lipophilic (ClogP in the range of 4–5). From this SAR study, it is anticipated that modification of the biaryl series by the replacement of the 'inner' aromatic ring with proper bioisosteres will result in new ligands with both a good ClogP (in the range of 2–3) and retained NET activity. Related work is underway and will be reported in due course.

In conclusion, a number of new (*Z*)-9-(heteroarylmethylene)-7-azatricyclo[4.3.1.0^{3,7}]decanes, conformationally constrained tricyclic tropane analogues, containing various heteroaromatic moieties were synthesized, and their abilities to block dopamine, serotonin, and norepinephrine reuptake by their respective transporters were evaluated. This SAR study demonstrates that compounds substituted with five- or six-membered *N*-containing aromatics are likely too basic to display high NET activity, while some of the thiophene analogues such as compounds **12b**, **12d**, **12f**, and **13a** were identified as potent and selective NET inhibitors. The binding pocket that encompasses the thienyl group is highly sensitive to the substituent attached to this ring. Bulky substituents on the aromatic ring reduce the activity of the ligands. Because of their good NET activity and ClogP, compounds **12d** and **12f** are currently being examined for use as positron emission tomography (PET) imaging agents. Further extensions of the present work are likely to afford the opportunity to discover ligands of picomolar affinity for the NET.

Table 2. ClogP of selected analogues with a moderate to high potency[a]

Compd	ClogP 'Daylight'	ClogP 'KowWin'
11	2.54	3.07
12a	2.54	3.07
12b	3.29	3.72
12c	3.44	3.96
12d	3.70	4.24
12f	3.04	3.62
12j	3.94	4.51
12k	4.63	4.83
13a	4.57	4.92
13d	5.25	5.30

[a]ClogP calculated by using web sites: (1) http://www.daylight.com/daycgi/clogp; and (2) http://esc.syrres.com/interkow/kowdemo.htm.

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29. Typical experimental procedure: (1*S*,3*S*,6*R*,10*S*)-(*Z*)-9-[2-(5-chlorothienyl)methylene]-7-azatricyclo[4.3.1.$0^{3,7}$]decane-10-carboxylic acid methyl ester (**12b**): 2-chloro-5-iodothiophene (73.9 mg, 0.30 mmol), triphenylarsine (24.7 mg, 0.081 mmol), $Pd_2(dba)_3$ (9.2 mg, 10.0 μmol), and CuI (15.3 mg, 0.08 mmol) were dissolved in anhydrous DMF (2 mL) and stirred at room temperature for 20 min under nitrogen. Then the stannane **5** (50.0 mg, 0.10 mmol) in DMF (2 mL) was added, and the mixture was stirred at 45–50 °C for 24 h. The solvent was removed under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate/triethylamine 98/2 as the eluent to give a crude product, which was further chromatographed on silica gel with dichloromethane/methanol/HOAc 95/4.5/0.5 as the eluent to provide the desired compound **12b** as a white solid (18.6 mg, 57%): $[\alpha]_D^{25}$ +62.3° (*c* 0.91, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.44–1.58 (m, 3H), 1.96–2.08 (m, 1H), 2.10–2.30 (m, 2H), 2.40 (t, *J* = 3.3 Hz, 1H), 2.68 (dd, *J* = 3.3 and 5.6 Hz, 1H), 3.25–3.32 (m, 1H), 3.63 (s, 3H), 3.70–3.78 (m, 1H), 3.76 and 3.88 (ABq, *J* = 18.6 Hz, both d with *J* = 2.4 Hz, 2H), 6.22 (t, *J* = 2.7 Hz, 1H), 6.62 (d, *J* = 3.9 Hz, 1H), 6.81 (d, *J* = 3.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 32.0, 32.5, 36.3, 36.5, 48.2, 51.9, 52.2, 53.7, 56.3, 114.6, 124.9, 126.1, 129.1, 139.6, 174.1; MS *m*/*z* (%) 323 (M^+, 29), 287 (22), 264 (26), 228 (16), 195 (9), 147 (14), 131 (18), 114 (33), 83 (100), 68 (73), 41 (62). Anal. calcd for $C_{16}H_{18}ClNO_2S$: C 59.34, H 5.60, N 4.33; found: C 59.19, H 5.57, N 4.43.
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