MedChemComm

RESEARCH ARTICLE

View Article Online
View Journal

Cite this: DOI: 10.1039/c6md00245e

The biogenic amine transporter activity of vinylogous amphetamine analogs†‡

Ann M. Decker,[a] John S. Partilla,[b] Michael H. Baumann,[b] Richard B. Rothman§[b] and Bruce E. Blough*[a]

Received 30th April 2016,
Accepted 6th June 2016

DOI: 10.1039/c6md00245e

www.rsc.org/medchemcomm

A series of vinylogous amphetamine analogs was synthesized and examined for their activity at biogenic amine transporters and serotonin-2 receptor (5-HT_2) subtypes. (1*S*,3*E*)-1-Methyl-4-phenyl-but-3-enylamine (***S*-6**) is a potent dual dopamine/serotonin (DA/5-HT) releaser with no activity at 5-HT_2 receptors. This unique profile of actions suggests that analog ***S*-6** is a viable lead compound for identifying new structural classes of DA/5-HT releasers with therapeutic benefit and reduced abuse liability.

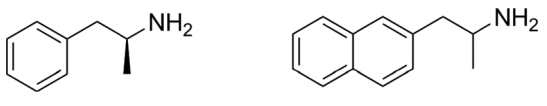
Introduction

Plasma membrane biogenic amine transporters (BATs) regulate neuronal signaling in the central nervous system by transporting previously released monoamine neurotransmitters – dopamine, norepinephrine, and serotonin (DA, NE, and 5-HT transported *via* DAT, NET, and SERT, respectively) – from the synapse back to the neuronal cytoplasm.[1] Ligands that interact with BATs are divided into two general classes: reuptake inhibitors and substrate-type releasers (for a review[2]). Both types of ligands elevate extracellular neurotransmitter concentrations but act *via* different mechanisms. Reuptake inhibitors bind to transporters and block transporter-mediated reuptake of neurotransmitters.[3] Substrate-type releasers bind to the substrate site on the transporters, are transported inside the neuron, and promote neurotransmitter efflux by carrier-mediated exchange.[4] Disruption of BAT function plays an important role in the pathophysiology of many neurological diseases such as depression, anxiety, Parkinson's disease, schizophrenia, and psychostimulant addiction.[5]

Psychostimulants, like cocaine and methamphetamine, are addictive drugs that target BATs in the central and peripheral nervous systems to cause a variety of harmful physiological effects in humans. One potential strategy to treat psychostimulant addiction is called agonist substitution therapy whereby patients are administered less potent and less addictive stimulant-like medications.[6] BAT releasers represent one class of compounds being evaluated as potential agonist medications (for a review[7]).

Several studies have demonstrated the ability of *S*(+)-amphetamine (**1**, Fig. 1), which has a high selectivity for releasing DA relative to 5-HT, to act as an agonist therapy for stimulant dependence. Chronic treatment with *S*(+)-amphetamine in rhesus monkeys results in a selective dose-dependent decrease in cocaine self-administration compared to food-maintained responding using progressive-ratio,[8] choice,[9] and second-order[10] schedules. In a double-blind, placebo-controlled clinical trial, treatment with *S*(+)-amphetamine results in a decrease in cocaine use,[11] which is consistent with other clinical trials testing agonist treatments (for a review[12]). However, a significant limitation of using *S*(+)-amphetamine as a medication is its abuse potential due to activation of mesolimbic dopamine neurons.[13]

Previous evidence suggests that deficits in both DA and 5-HT are associated with withdrawal symptoms and that elevations in extracellular 5-HT can counteract the stimulant and reinforcing effects of DA (dual deficit model of stimulant addiction).[14] One possible advantage of using dual DA/5-HT releasers as agonist medications is their combined ability to provide the necessary stimulant-like properties required for therapeutic efficacy (*i.e.*, DA release) while reducing abuse liability (5-HT release). As such, multiple lines of evidence show that 5-HT elevations can reduce drug seeking behavior. *In*



1 **2**

Fig. 1 *S*(+)-amphetamine and PAL-287.

[a] *Center for Drug Discovery, RTI International, 3040 E. Cornwallis Road, Research Triangle Park, NC 27709, USA. E-mail: beb@rti.org; Fax: +919 541 6499; Tel: +919 541 1244*

[b] *Medicinal Chemistry Section, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224, USA*

† The authors declare no competing interests.

‡ Electronic supplementary information (ESI) available. See DOI: 10.1039/c6md00245e

§ Present address. Department of Psychiatry, MedStar St. Mary's Hospital, 25500 Point Lookout Road, Leonardtown, MD 20650, USA.

vivo studies conducted in rats reveal that 5-HT release decreases the stimulant effects of amphetamine-type drugs[15] and that fenfluramine (5-HT releaser) dose-dependently attenuates cue-reinstated cocaine-seeking behavior.[16] The reduction in drug-seeking behavior observed in rats translates to humans in that fenfluramine significantly reduces cocaine craving in abstinent cocaine-dependent patients.[17] Further, in preliminary clinical trials, co-administration of the anorectics phentermine (DA releaser) and fenfluramine (Fen–Phen) shows promise in treating cocaine and alcohol dependence thus supporting the idea of using dual DA/5-HT releasers as therapeutics.[18]

Based on this evidence, our laboratory decided to focus our efforts on dual DA and 5-HT releasers. As part of this program, we identified 1-naphthyl-2-aminopropane (PAL-287¶ 2) as our lead compound (Fig. 1).[19] PAL-287 releases radiolabeled neurotransmitters from DAT, SERT, and NET with EC_{50} values of 12.6 nM, 3.4 nM, and 11.1 nM, respectively (Table 1). *In vivo* microdialysis experiments in rats corroborate the *in vitro* data by showing that PAL-287 (1–3 mg kg^{-1} i.v.) increases extracellular DA and 5-HT in the frontal cortex, with effects on 5-HT being larger (464% increase compared to 133% increase).[20] Furthermore, in rats, PAL-287 causes significantly less motor stimulation compared to *S*(+)-amphetamine, which has 71-fold greater potency to release DA compared to 5-HT; importantly high doses of PAL-287 do not cause depletion of cortical 5-HT.[20] In rhesus monkeys trained to self-administer cocaine, PAL-287 produces a dose-dependent decrease in cocaine self-administration and significantly decreases cocaine- *versus* food-maintained responding at 1.0 mg kg^{-1} h^{-1}.[21] Overall, the data collected with the non-amphetamine analog, PAL-287, support the hypothesis that dual DA/5-HT releasers possess the therapeutic effects of amphetamine-type releasers while being minimally reinforcing.

To begin to investigate the structure–activity requirements of the naphthyl moiety of PAL-287, a series of vinylogous PAL-287 analogs were synthesized and evaluated for transporter activity. BAT activity was measured as done previously using synaptosomes prepared from rat brain homogenates according to the protocol developed by Rothman and coworkers.[22] Compounds were first screened in uptake inhibition and release assays to determine the exact mode of drug action. Compounds active in both assays are releasers while compounds active only in the uptake inhibition assay are uptake inhibitors. Active compounds were then fully characterized by running 8-point concentration response curves in the assay corresponding to their mechanism of action. Substrate reversal experiments were conducted to validate substrate activity. The analogs were also tested for agonist activity at the serotonin-2 receptor subtypes (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}) using *in vitro* calcium mobilization assays in transfected HEK293 cells as previously described.[23] These receptors are associated with the pharmacology of abused drugs, as 5-HT_{2A} agonists are thought to be hallucinogenic[24,25] while 5-HT_{2B} agonists are associated with valvular heart disease and pulmonary hypertension;[26,27] activity at these receptors would be considered off-target liabilities. On the other hand, agonists at 5-HT_{2C} may be beneficial as potential pharmacotherapies for drug abuse and appetite suppression.[28,29]

¶ PAL = phenyl amine library.

Methods

Biological assays

DAT, NET, and SERT assays. All animal studies were conducted in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and experiments were performed in accordance with the Institutional Care and Use Committee (IACUC) of the National Institute on Drug Abuse Intramural Research Program (NIDA IRP). Rats were euthanized by CO_2 narcosis, and brains were processed to yield synaptosomes as previously described.[22] Synaptosomes were prepared from rat striatum for the DAT assays, whereas synaptosomes were prepared from whole brain minus striatum and cerebellum for the NET and SERT assays.

For uptake inhibition assays, 5 nM [^{3}H]DA, 10 nM [^{3}H]norepinephrine and 5 nM [^{3}H]5-HT were used to assess transport activity at DAT, NET, and SERT, respectively. The selectivity of uptake assays was optimized for a single transporter by including unlabeled blockers to prevent uptake of [^{3}H]transmitter by competing transporters. Uptake inhibition assays were initiated by adding 100 μl of tissue suspension to 900 μL Krebs-phosphate buffer (126 mM NaCl, 2.4 mM KCl, 0.83 mM $CaCl_2$, 0.8 mM $MgCl_2$, 0.5 mM KH_2PO_4, 0.5 mM Na_2SO_4, 11.1 mM glucose, 0.05 mM pargyline, 1 mg mL^{-1} bovine serum albumin, and 1 mg mL^{-1} ascorbic acid, pH 7.4) containing test drug and [^{3}H]transmitter. Uptake inhibition assays were terminated by rapid vacuum filtration through Whatman GF/B filters, and retained radioactivity was quantified by liquid scintillation counting. Concentration–response curves were generated to yield IC_{50} values.

For release assays, 9 nM [^{3}H]1-methyl-4-phenylpyridinium ([^{3}H]MPP+) was used as the radiolabeled substrate for DAT and NET, while 5 nM [^{3}H]5-HT was used as a substrate for SERT. All buffers used in the release assays contained 1 μM reserpine to block vesicular uptake of substrates. The selectivity of release assays was optimized for a single transporter by including unlabeled blockers to prevent the uptake of [^{3}H]MPP+ or [^{3}H]5-HT by competing transporters. Synaptosomes were preloaded with radiolabeled substrate in Krebs-phosphate buffer for 1 h (steady state). Release assays were initiated by adding 850 μL of preloaded synaptosomes to 150 μL of test drug. Release was terminated by vacuum filtration and retained radioactivity was quantified as described for uptake inhibition. Concentration–response curves were generated to yield EC_{50} values.

Substrate reversal experiments were conducted to validate substrate activity. The releasing ability of test compounds was tested at an EC_{80} concentration in the absence and

Table 1 Structure–activity of a series of vinylogous amphetamine analogs for releasing radiolabeled substrates from DAT, SERT and NET

			EC_{50} (nM ± SD)[a]					
PAL	#	Group	DAT	SERT	NET	5-HT_{2A}[b] EC_{50}, (nM ± SEM)	5-HT_{2B}[b] EC_{50}, (nM ± SEM)	5-HT_{2C}[b] EC_{50}, (nM ± SEM)
(±) AMP	**1**		24.8 ± 3.5	1770 ± 94	7.1 ± 1	IA	IA	IA
287	**2**		12.6 ± 0.4	3.4 ± 0.2	11.1 ± 0.9	466[c] nM	40[c] nM	23[c] nM (20%)
869	**4**	I	997 ± 220	384 ± 110	2980 ± 490	>10k	IA	IA
870	***S*-4**	I	443 ± 100	756 ± 79	784 ± 190	4950 ± 1400	IA	IA
871	***R*-4**	I	660 ± 190	288 + 84	496 ± 160	>10k	IA	IA
872	**6**	II	272 ± 44	54 ± 8	239 ± 59	5620 ± 1700	IA	IA
875	***S*-6**	II	206 ± 26	40 ± 9	138 ± 260	IA	IA	IA
873	***R*-6**	II	540 ± 87	109 ± 29	279 ± 24	>10k	IA	IA
904	**8**	III	900 ± 100	646 ± 70	621 ± 190	1860 ± 76	IA	>10k
905	***S*-8**	III	304 ± 110	663 ± 86	170 ± 32	1600 ± 320	IA	>10k
906	***R*-8**	III	1416 ± 180	1156 ± 160	211 ± 42	>10k	IA	IA
881	**10**	IV	666 ± 160	>10k	308 ± 100	IA	IA	IA
893	**11**	IV	1114 ± 150	>10k	301 ± 76	IA	IA	IA

[a] EC_{50} release values were determined as described in Methods. Each value is mean ± SD (n = 3). [b] Calcium mobilization EC_{50} values were determined as described in Methods. Each value is mean ± SEM (at least n = 3). [c] Data from ref. 19. IA = Inactive at 10 μM.

presence of an uptake inhibitor (250 nM GBR1209 for DAT, 166 nM desipramine for NET, 100 nM fluoxetine for SERT). If the test agent was a releaser, the uptake inhibitor reduced the effect of the test agent. If the test agent was an uptake inhibitor, the addition of a second uptake inhibitor led to either no change or an increased effect in the release assay.

Calcium mobilization assays. Three individual HEK293 cell lines stably expressing the human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors were used. The day before the assay, cells were plated into 96-well black-walled assay plates (2A – 40 000 cells per well; 2B and 2C – 35 000 cells per well) in DMEM-HG supplemented with 10% fetal bovine serum, 100 units of penicillin and streptomycin, and 15 mM HEPES. The cells were incubated overnight at 37 °C, 5% CO_2. Prior to the assay, Calcium 5 dye (Molecular Devices) was reconstituted according to the manufacturer instructions. The reconstituted dye was diluted 1 : 40 in pre-warmed (37 °C) assay buffer (1× HBSS, 20 mM HEPES, 2.5 mM probenecid, pH 7.4 at 37 °C). Growth medium was removed and the cells were gently washed with 100 μL of pre-warmed (37 °C) assay buffer. The cells were incubated for 45 minutes at 37 °C, 5% CO_2 in 200 μL of the diluted Calcium 5 dye. Serial dilutions of the test compounds were prepared in 1% DMSO/assay buffer, aliquoted into 96-well polypropylene plates, and warmed to 37 °C. After the dye-loading incubation period, the cells were pre-treated with 25 μL of 9% DMSO/assay buffer and incubated for 15 min at 37 °C. After the pre-treatment incubation period, the plate was read with a FlexStation II (Molecular Devices). Calcium-mediated changes in fluorescence were monitored every 1.52 seconds over a 60 second time period, with the FlexStation II adding 25 μL of test compound dilutions at the 19 second time point (excitation at 485 nm, detection at 525 nm). Peak kinetic reduction (SoftMax, Molecular Devices) relative fluorescent units (RFU) were plotted against compound concentration. Data were fit to the appropriate three-parameter logistic curve to generate EC_{50} values (GraphPad Prism 6.0, GraphPad Software, Inc., San Diego, CA).

Compound synthesis. The synthesis of all novel compounds is provided in the ESI.‡

Results & discussion

Four groups of PAL-287 vinylogous analogs were synthesized in which the naphthyl moiety was replaced with phenyl alkynyl, phenyl (*E*)-alkenyl, or phenyl (*Z*)-alkenyl moieties (Fig. 2). Group I consisted of the racemic alkynyl isostere **4**, as well as the two chiral isosteres, ***S*-4** and ***R*-4**. Group II consisted of the racemic and chiral (*E*)-alkenyl isosteres (**6**, ***S*-6**, ***R*-6**), while group III consisted of the corresponding (*Z*)-alkenyl isosteres (**8**, ***S*-8**, ***R*-8**). The analogs of group IV (**10**, **11**) have one less carbon between the phenyl ring and the amine group. All analogs were synthesized in three or four steps from commercially available materials. Scheme 1 shows the synthesis of the (*S*)-stereoisomers from groups I–III. To synthesize group I alkyne ***S*-4**, commercially available alcohol ***R*-3** was converted to the tosylate, which underwent displacement with inversion of configuration to the azide, which was reduced under Staudinger conditions to provide ***S*-4**. The same commercially available starting alcohol ***R*-3** was selectively reduced with lithium aluminum hydride (LAH) or Lindlar's catalyst to afford the corresponding (*E*)- or (*Z*)-olefins, ***R*-5** and ***R*-7**, respectively. These olefins were then converted to amines ***S*-6** and ***S*-8**, respectively, using the same three step tosylation/azide formation/Staudinger reduction steps. The (*R*)-stereoisomers and the racemates for groups I–III were synthesized using the same pathway starting with the corresponding commercially available (*S*)-alcohol and racemic alcohol, respectively. Scheme 2 shows the synthesis of group IV analogs (**10**, **11**) from commercially available alcohol **9** using the same pathway as the other analogs, except that mesylation was performed instead of tosylation due to stability issues.

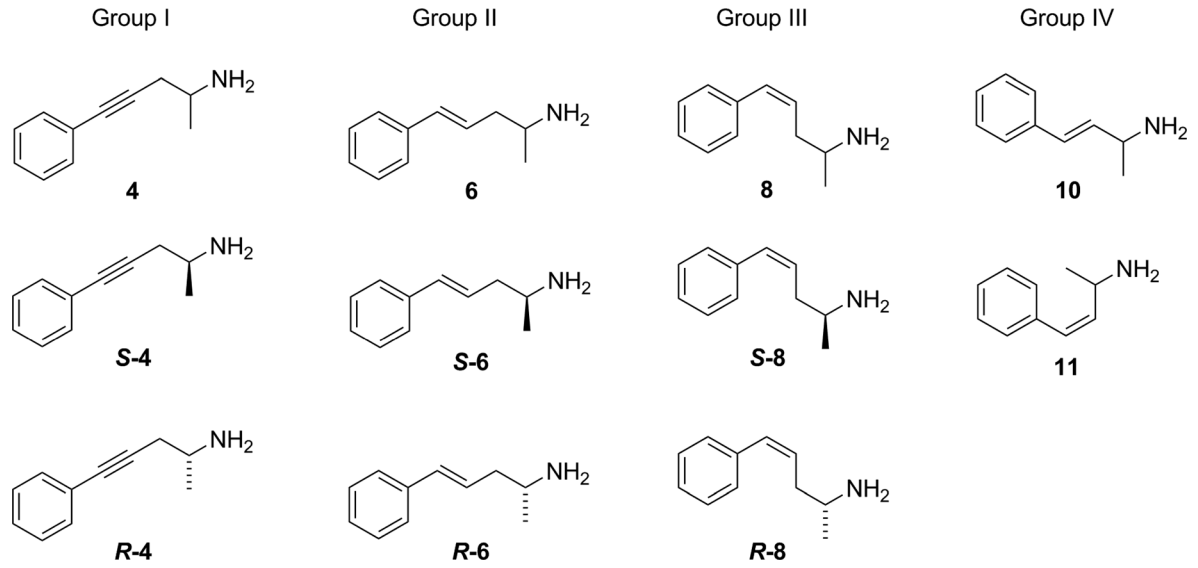
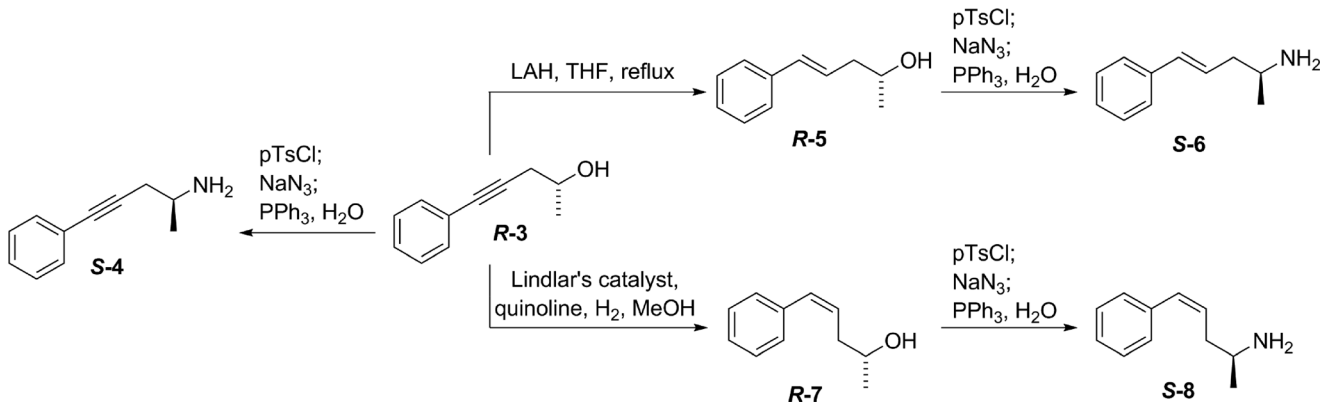
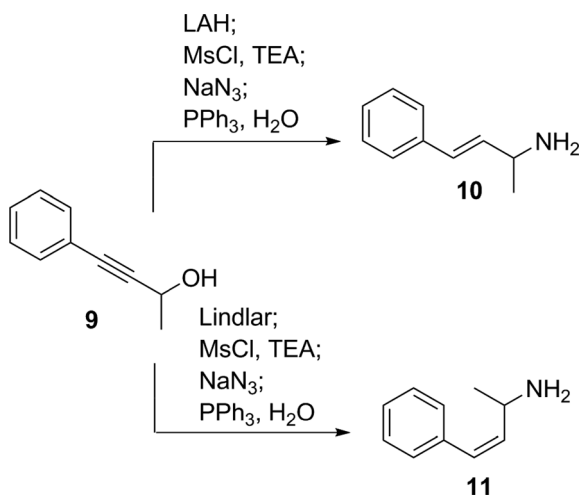


Fig. 2 Vinylogous amphetamine analogs synthesized for this study.



Scheme 1 Synthesis of group I alkynes, group II (*E*)-alkenes and group III (*Z*)-alkenes.

Table 1 shows the transporter data for the analogs. All compounds were active as DAT and NET releasers with varying potencies and all but two compounds, **10** and **11**, were active as SERT releasers. At the DAT, group I alkynes had similar potencies with ***S*-4** being the most potent with an EC_{50} value of 443 nM. At the SERT, the alkynes also had similar potencies with ***R*-4** being the most potent with an EC_{50} value of 288 nM. At the NET, alkyne ***R*-4** was the most potent (EC_{50} = 496 nM) and **4** was the least potent (EC_{50} = 2980 nM). The group II (*E*)-alkenes were potent at all three transporters, swith EC_{50} values less than 540 nM. At the DAT, group II (*E*)-alkenes had similar potencies with ***R*-6** being the least potent (EC_{50} = 540 nM) and ***S*-6** being the most potent with an EC_{50} value of 206 nM. At the SERT and NET, ***S*-6** was the most active compound in this group with EC_{50} values of 40 nM and 138 nM, respectively. At the DAT, group III (*Z*)-alkenes were less than 1500 nM, with ***S*-8** being the most potent at 304 nM. At the SERT, analogs **8** and ***S*-8** had similar potencies with **8** being slightly more potent (EC_{50} = 646 nM). At the NET, ***S*-8** was the most potent analog with an EC_{50} value of 170 nM. Group IV analogs were inactive at SERT and relatively weak releasers at DAT with **10** being the most potent (EC_{50} = 666 nM). However, both analogs had similar potencies at the NET (EC_{50} ~ 300 nM).



Scheme 2 Synthesis of group IV analogs.

From a structure–activity perspective, all compounds were substrates for the transporters, indicating that the transporters can translocate larger structures than previously believed.[30] All compounds, except group IV, were dual DA/5-HT releasers, but with varying degrees of transporter selectivity. The group I alkynes did not exhibit much selectivity for releasing 5-HT compared to DA as the racemic analog **4** and ***R*-4** were only 2.6-fold and 2.3-fold, respectively, more potent at the DAT. ***S*-4** was essentially equipotent at the DAT and SERT. The group II alkenes were all more selective at SERT relative to DAT with 5-fold greater potency at SERT. This group was interesting because the activities at the transporters were all very similar, indicating no differences between chiral isomers, ***R*-6** and ***S*-6**, and the racemate **6**. The group III (*Z*)-olefins were similar to the group I alkynes as the analogs did not show much SERT/DAT selectivity. The racemic analog **8** and ***R*-8** had similar potencies for releasing 5-HT relative to DA (1.4- and 1.2-fold, respectively) while ***S*-8** was slightly more potent for releasing DA relative to 5-HT (2.2-fold). Removing a carbon between the alkene and the amine (group IV) resulted in analogs that were selective at DAT and NET. These compounds were inactive at SERT indicating they could not bind to the site of translocation; this activity profile suggests the compounds may be weak stimulants.

As part of our program to develop neurotransmitter releasers as therapeutic agents, we have analyzed over 1000 small molecule phenethylamines for BAT activity and found that NE release almost always parallels DA release with slightly higher potency. Recently, we identified a group of tryptamines that are >10-fold selective for releasing DA relative to NE.[23] While most of the vinylogous analogs follow the DA/NE release trend, a few compounds show selectivity for DAT or NET. Group I racemic alkyne **4** was 3-fold more potent at releasing DA compared to NE with EC_{50} values of 997 nM and 2980 nM, respectively. Analog ***S*-4** followed the typical trend at DAT and NET with EC_{50} values of 660 nM and 496 nM, respectively, while activity at DAT and NET for analog ***R*-4** was reversed with EC_{50} values of 443 nM and 784 nM, respectively. All group II (*E*)-alkenes and two group III (*Z*)-alkenes followed the typical trend; however, group III (*Z*)-alkene ***R*-8** was 6.7-fold selective for releasing NE relative to DA with EC_{50} values of 211 nM and 1416 nM, respectively. Both group IV analogs, **10** and **11**, were more potent at NET compared to DAT, but with different selectivities (2.2-fold and 3.7-fold, respectively).

Group II (*E*)-alkenes were the most active compounds compared to the other three groups. The most potent analog at the DAT, SERT, and NET was (*E*)-alkene ***S*-6** with EC_{50} values of 206 nM, 40 nM, and 138 nM, respectively. This analog retains the same configuration as *S*(+)-amphetamine, has the same number of carbons between the phenyl and amine groups as PAL-287, and has a similar steric conformation as PAL-287, compared to the more sterically hindered (*Z*)-olefins. While PAL-287 is 10-fold more potent than ***S*-6** at all three transporters, the compounds share some activity characteristics. ***S*-6** has 5-fold 5-HT/DA release potency, similar to PAL-287, which has 3.7-fold selectivity. ***S*-6** has 3.5-fold higher potency for 5-HT release compared to NE release, which is similar to PAL-287's 3.3-fold selectivity, and both compounds have almost equal DA/NE release potencies.

The vinylogous analogs were also evaluated for agonist activity at 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors using *in vitro* calcium mobilization assays (Table 1). Overall, the analogs had varying degrees of weak activity at all three receptors, making them much more like *S*(+)-amphetamine (inactive in all three assays) than PAL-287 (Table 1). Previous functional studies reveal that PAL-287 is a full agonist at 5-HT_{2A} and 5-HT_{2B} receptors (EC_{50} = 466 nM and 40 nM, respectively) and a partial agonist (EC_{50} = 2.3 nM, E_{MAX} = 20%) at 5-HT_{2C}.[19] At 5-HT_{2A}, the vinylogous analogs were all less potent than PAL-287 as most of them were inactive (***S*-6**, **10**, **11**) or had EC_{50} values > 10 μM (**4**, ***R*-4**, ***R*-6**, ***R*-8**). The remaining analogs had potencies in the micromolar range. Analog ***S*-8**, which had an EC_{50} value of 1600 nM and E_{MAX} of 102%, was the most potent and efficacious analog. The racemic analog **8** had a similar potency (EC_{50} = 1860 nM) and efficacy (E_{MAX} = 90%). The only other compounds that were active were alkyne ***S*-4** and racemic alkene **6**, which had a 2.7-fold and 3-fold reduction in potency, respectively, compared to **8**. These compounds were also not as efficacious and had E_{MAX} values in the lower 80% range. At 5-HT_{2B}, all the analogs were inactive. This was interesting because PAL-287 was active at 5-HT_{2B} as an agonist with an EC_{50} value of 40 nM. At 5-HT_{2C}, only group III (*Z*)-alkenes **8** and ***S*-8** were weak agonists with activity less than 50% of the control 5-HT E_{MAX} at 10 μM. The most active transporter compound (***S*-6**) was inactive at all three receptors, indicating that this compound may not produce the typical effects associated with agonist activity at the 5-HT_2 receptors; however, the full *in vivo* effects of ***S*-6** would need to be evaluated in appropriate assays.

As noted, elevations in extracellular 5-HT can counteract the stimulant and reinforcing effects of DA release[14] in a dual DA/5-HT releaser leading to non-stimulant, non-addictive therapeutics, but the potential psychedelic effects observed with many 5-HT releasers remains an issue. Neurotransmitter releasers such as PAL-287 and ***S*-6** possess transporter profiles using rat brain synaptosomes that are similar to psychedelic compounds such as 3,4-methylenedioxymethamphetamine (MDMA)[31] and the "bath salt" mephedrone.[32] Similar relative profiles are also observed in engineered cell-based transporter assays containing over-expressed transporter proteins.[33] While the biogenic amine transporters play a role in the psychoactivity of these psychedelics, a complete rationale for these effects remains unclear. The 5-HT selective releaser fenfluramine was not euphoric at therapeutic doses (20 mg) but hallucinogenic episodes were observed at high doses (240 mg),[34] implying that hallucinogenic effects are not primarily transporter related. While similar studies have not yet been conducted on PAL-287, anecdotal internet reports suggest that PAL-287 does not possess strong psychoactive effects.

Regardless, further therapeutic development of dual DA/5-HT releasers would need to include behavioral assessment including drug discrimination to avoid such side-effects, especially drug candidates that are 5-HT selective.

Another important development issue is serotonin depletion and neurotoxicity. Many serotonin releasers deplete stores of 5-HT potentially leading to neurotoxicity with chronic use. The 5-HT releasers fenfluramine, MDMA, and *p*-chloroamphetamine (PCA) all cause long-term 5-HT depletion;[35] PCA is even used as a behavioral tool to help unmask the effects of 5-HT on behavior.[36] However, not all 5-HT releasers cause depletion and neurotoxicity. *Meta*-chlorophenylpiperazine (*m*CPP), while equipotent with fenfluramine, was found to lack the 5-HT depleting effects of fenfluramine[37] as was our laboratory's main lead compound, PAL-287.[38] As part of the devlopment process, dual DA/5-HT releaser drug candidates will need to be assessed for 5-HT depletion in order to determine any potential neurotoxicity associated with chronic use.

Conclusions

In conclusion, eleven vinylogous PAL-287 analogs with less conformational restriction were studied, resulting in the discovery of a new series of amphetamine analogs that have transporter activity. All of the analogs were substrates for the BATs, lending support to the notion that transporters are able to translocate larger structures than previously believed. Of the eleven analogs, three compounds (**6**, ***S*-6**, ***R*-6**) were potent releasers of DA, 5-HT, and NE, with ***S*-6** being the most potent with EC_{50} values of 206 nM, 40 nM, and 138 nM, respectively. This analog has the same configuration as *S*(+)-amphetamine, and has a similar steric conformation as PAL-287 due to the same number of carbons between the phenyl and amine groups. Although ***S*-6** is 10-fold less potent than PAL-287, the two compounds share similar transporter selectivities. Analog ***S*-6** was also inactive in *in vitro* 5-HT_2 calcium mobilization assays, indicating no potential *in vivo* effects. The unique activity profile for ***S*-6** suggests that this compound represents a new lead for identifying neurotransmitter releasers with therapeutic potential.

Acknowledgements

This research was supported by the National Institute on Drug Abuse Project DA12970 (BEB) and the Intramural Research Program, National Institute on Drug Abuse, NIH.

References

1 J. Masson, C. Sagné, M. Hamon and S. El Mestikawy, *Pharmacol. Rev.*, 1999, **51**, 439.
2 R. B. Rothman, B. E. Blough and M. H. Baumann, *Prog. Brain Res.*, 2008, **172**, 385.
3 G. Rudnick and J. Clark, *Biochim. Biophys. Acta*, 1993, **1144**, 249.
4 A. E. Fleckenstein, T. J. Volz, E. L. Riddle, J. W. Gibb and G. R. Hanson, *Annu. Rev. Pharmacol. Toxicol.*, 2007, **47**, 681.
5 S. G. Amara and M. S. Sonders, *Drug Alcohol Depend.*, 1998, **51**, 87.
6 D. V. Herin, C. R. Rush and J. Grabowski, *Ann. N. Y. Acad. Sci.*, 2010, **1187**, 76.
7 L. L. Howell and S. S. Negus, *Adv. Pharmacol.*, 2014, **69**, 129.
8 S. S. Negus and N. K. Mello, *Psychopharmacology*, 2003, **167**, 324.
9 S. S. Negus, *Neuropsychopharmacology*, 2003, **28**, 919.
10 S. S. Negus and N. K. Mello, *Drug Alcohol Depend.*, 2003, **70**, 39.
11 J. Grabowski, H. Rhoades, J. Schmitz, A. Stotts, L. A. Daruzska, D. Creson and F. G. Moeller, *J. Clin. Psychopharmacol.*, 2001, **21**, 522.
12 J. Grabowski, J. Shearer, J. Merrill and S. S. Negus, *Addict Behav.*, 2004, **29**, 1439.
13 L. L. Howell and H. L. Kimmel, *Biochem. Pharmacol.*, 2008, **75**, 196.
14 R. B. Rothman and M. H. Baumann, *Eur. J. Pharmacol.*, 2003, **479**, 23.
15 M. H. Baumann, R. D. Clark, W. L. Woolverton, S. Wee, B. E. Blough and R. B. Rothman, *J. Pharmacol. Exp. Ther.*, 2011, **337**, 218.
16 J. J. Burmeister, E. M. Lungren and J. L. Neisewander, *Psychopharmacology*, 2003, **168**, 146.
17 L. Buydens-Branchey, M. Branchey, J. Hudson, M. Rothman, P. Fergeson and C. McKernin, *Am J Addict.*, 1998, **7**, 142.
18 R. B. Rothman, T. Gendron and P. Hitzig, *J. Subst. Abuse Treat.*, 1994, **11**, 273.
19 R. B. Rothman, B. E. Blough and M. H. Baumann, *Trends Pharmacol. Sci.*, 2006, **27**, 612.
20 R. B. Rothman, B. E. Blough, W. L. Woolverton, K. G. Anderson, S. S. Negus, N. K. Mello, B. L. Roth and M. H. Baumann, *J. Pharmacol. Exp. Ther.*, 2005, **313**, 1361.
21 S. S. Negus, N. K. Mello, B. E. Blough, M. H. Baumann and R. B. Rothman, *J. Pharmacol. Exp. Ther.*, 2007, **320**, 627.
22 R. B. Rothman, M. Katsnelson, N. Vu, J. S. Partilla, C. M. Dersch, B. E. Blough and M. H. Baumann, *Eur. J. Pharmacol.*, 2002, **447**, 51.
23 B. E. Blough, A. Landavazo, J. S. Partilla, A. M. Decker, K. M. Page, M. H. Baumann and R. B. Rothman, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 4754.
24 D. E. Nichols, *Pharmacol. Ther.*, 2004, **101**, 131.
25 J. González-Maeso, N. V. Weisstaub, M. Zhou, P. Chan, L. Ivic, R. Ang, A. Lira, M. Bradley-Moore, Y. Ge, Q. Zhou, S. C. Sealfon and J. A. Gingrich, *Neuron*, 2007, **53**, 439.
26 R. B. Rothman and M. H. Baumann, *Expert Opin. Drug Saf.*, 2009, **8**, 317.
27 J. M. Launay, P. Hervé, K. Peoc'h, C. Tournois, J. Callebert, C. G. Nebigil, N. Etienne, L. Drouet, M. Humbert, G. Simonneau and L. Maroteaux, *Nat. Med.*, 2002, **8**, 1129.
28 G. A. Higgins and P. J. Fletcher, *Eur. J. Pharmacol.*, 2003, **480**, 151.
29 G. A. Higgins, E. M. Sellers and P. J. Fletcher, *Trends Pharmacol. Sci.*, 2013, **34**, 560.

30 M. E. Reith, B. E. Blough, W. C. Hong, K. T. Jones, K. C. Schmitt, M. H. Baumann, J. S. Partilla, R. B. Rothman and J. L. Katz, *Drug Alcohol Depend.*, 2015, **147C**, 1.

31 M. H. Baumann, R. D. Clark, A. G. Budzynski, J. S. Partilla, B. E. Blough and R. B. Rothman, *Ann. N. Y. Acad. Sci.*, 2004, **1025**, 189.

32 M. H. Baumann, M. A. Ayestas Jr, J. S. Partilla, J. R. Sink, A. T. Shulgin, P. F. Daley, S. D. Brandt, R. B. Rothman, A. E. Ruoho and N. V. Cozzi, *Neuropsychopharmacology*, 2012, **37**, 1192.

33 (*a*) L. D. Simmler, T. A. Buser, M. Donzelli, Y. Schramm, L. H. Dieu, J. Huwyler, S. Chaboz, M. C. Hoener and M. E. Liechti, *Br. J. Pharmacol.*, 2013, **168**, 458; (*b*) L. D. Simmler, A. Rickli, Y. Schramm, M. C. Hoener and M. E. Liechti, *Biochem. Pharmacol.*, 2014, **88**, 237.

34 J. D. Griffith, J. G. Nutt and D. R. Jasinski, *Clin. Pharmacol. Ther.*, 1975, **18**, 563.

35 (*a*) X. Wang, M. H. Baumann, H. Xu and R. B. Rothman, *Synapse*, 2004, **53**, 240; (*b*) M. H. Baumann, M. A. Ayestas, C. M. Dersch, J. S. Partilla and R. B. Rothman, *Ann. N. Y. Acad. Sci.*, 2000, **914**, 172; (*c*) R. B. Rothman, S. Jayanthi, J. L. Cadet, X. Wang, C. M. Dersch and M. H. Baumann, *Ann. N. Y. Acad. Sci.*, 2004, **1025**, 151.

36 R. W. Fuller, *Neurochem. Res.*, 1992, **17**, 449.

37 M. H. Baumann, M. A. Ayestas, C. M. Dersch and R. B. Rothman, *Neuropsychopharmacology*, 2001, **24**, 492.

38 R. B. Rothman, B. E. Blough, W. L. Woolverton, K. G. Anderson, S. S. Negus, N. K. Mello, B. L. Roth and M. H. Baumann, *J. Pharmacol. Exp. Ther.*, 2005, **313**, 1361.