Identification of N-[(5-[(4-Methylphenyl)sulfonyl]amino)-3-(trifluoroacetyl)-1H-indol-1-yl)acetyl]-l-leucine (NTRC-824), a Neurotensin-like Nonpeptide Compound Selective for the Neurotensin Receptor Type 2

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Supporting Information

ABSTRACT: Compounds acting via the neurotensin receptor type 2 (NTS2) are known to be active in animal models of acute and chronic pain. To identify novel NTS2 selective analgesics, we searched for NTS2 selective nonpeptide compounds using a FLIPR assay and identified the title compound (NTRC-824, $) that, to our knowledge, is the first nonpeptide that is selective for NTS2 versus NTS1 and behaves like the endogenous ligand neurotensin in the functional assay.

INTRODUCTION

The discovery of novel analgesics has been a major pursuit of medicinal chemists for more than a hundred years. Although opiates and opioids remain the treatment of choice for severe acute pain, they produce serious side effects including addiction, respiratory depression, and tolerance. Furthermore, opioids often fail to provide relief from chronic pain, a persistent form of pain resulting from nerve injury. Thus, identification of nonopioid analgesics remains a key challenge of discovery science.

The tridecapeptide neurotensin (NT, Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu), acting via two G-protein coupled receptors NTS1 and NTS2 (NTR1, NTR2, etc.) and the multiligand type-1 receptor sortilin, mediates many biological functions including nonopioid analgesia.1–5 Compounds acting via neurotensin receptor types 1 and 2 have been reported to be active in animal models of both acute and chronic pain.6–11 NT mediated analgesia is also synergistic, with opioid analgesia suggesting that NT-based compounds could function alone or as adjuncts to opioids in the management of pain.12 Together, these findings underpin the rational for identification of NT-based analgesics.

The search for such compounds is decades old, yet to date, nearly all of the NT compounds reported to be active in animal models of pain are peptides, variants of the terminal hexapeptide fragment of NT (NT(8–13), 1a, Chart 1).12–16 The vast majority of these compounds produce analgesia that is accompanied by hypothermia and hypotension, a feature ascribed to interaction with the NTS1 receptor.17,18 Reports from the study of the NTS2 selective peptide NT79 (1b) support this notion as it possessed activity against visceral pain but lacked the side effects described above.12,19

Surprisingly, only one nonpeptide compound has so far been described to possess NT-based analgesic properties, the NTS2 versus NTS1 selective compound levocabastine (2), which shows activity in both visceral and chronic pain models.10,20,21 Coupled with our desire to identify nonpeptide compounds, the lower side effect profile demonstrated by the NTS2 selective peptide 1b prompted us to develop methods of identifying NTS2 selective compounds.22

Literature reports with a CHO cell line stably expressing rNTS2 indicated that the pyrazole compound 3a was an agonist in the FLIPR assay and that NT was an antagonist of the calcium release mediated by 3a.23 This suggested that we could identify NT-like (agonist) compounds by first activating NTS2 with 3a and then screening for compounds that would block this activity. The binding assay versus [125I]NT could then be used as a secondary screen for active compounds to verify interaction with NTS2.

We tested this idea using the CHO cell line above and a FLIPR Tetra and studied the peptide NT, the nonpeptide levocabastine (2), and the two well-known nonpeptide pyrazole-based ligands SR48692 (3a) and SR142948a (3b), all of which are known to bind NTS2.24,25 In line with literature precedent, we found that both pyrazole compounds (3a,b)
behaved as full agonists with 3b being more potent than 3a but equally efficacious. Levocabastine showed potent partial agonist activity (14–16% percent of 3b). NT, on the other hand, did not induce calcium release but was an antagonist of 3b in the FLIPR assay.

While it seemed counterintuitive, our data showed clearly that NT was an antagonist and levocabastine (2) a very low efficacy potent partial agonist in the FLIPR assay, although both are known to be antinociceptive in animal models of pain. The two pyrazole compounds (3a, 3b), on the other hand, were found to be agonists even though it is well documented that both antagonize the analgesic action of NT-based compounds in a variety of animal models. Overall, this pilot study suggested that our search for novel NTS2-based analgesics should begin in a variety of animal models. Overall, this pilot study suggested that our search for novel NTS2-based analgesics should begin in a variety of animal models.

We recently reported using this assay to drive an SAR study that led to the identification of the NTS2 selective, low efficacy, potent partial agonist 4 (NTRC-739).22 In this article, we report a parallel study that identified the NTS2 selective nonpeptide compound 5 that, like NT, is an antagonist of 3b in the FLIPR assay. The details of this work are presented herein.

## CHEMISTRY

The chemistry used to prepare target compounds 5 and 13 is illustrated in Scheme 1. Thus, 5-nitroindole (7) was alkylated with methyl bromoacetate to give ester 8 that was subsequently hydrolyzed to the corresponding acid intermediate 9 by treatment with LiOH in dioxanes and water. Compound 9 was then coupled to leucine tert-butyl ester using O-benzotriazol-1-yl-N,N,N′,N″-tetramethyluronium hexafluorophosphate (HBTU) and triethylamine to give intermediate 10. This was reduced using hydrogen and palladium on carbon to give intermediate 11 and then coupled to tosyl chloride to give intermediate 12. Compound 13 was obtained from 12 by treatment with trifluoroacetic acid (TFA) in CH2Cl2. Compound 5 was obtained from 12 by treatment with trifluoroacetic anhydride (TFAA) to give the acylated indole derivative 14, which was subsequently deprotected with TFA to give 5.

## BIOLOGY

The binding affinities of the test compounds for the rNTS1 and rNTS2 receptors, listed in Table 1, were determined using

Table 1. Data for Reference Compounds NT and 3b and Test Compounds 5 and 13 in the FLIPR Assay (NTS2) and Binding Assays (NTS1 and NTS2 Receptors)

| FLIPR assay | binding assays
|-------------|-------------|
|             | NTS2        | NTS1        | NTS2        |
|             | EC50        | Emax        | IC50        | Kt  | Kt  |
| NT          | NA          | 114 ± 24    | 1.1 ± 0.3   | 28 ± 6 |
| 3b          | 22 ± 5      | 100 ± 6     | 1.4 ± 0.4   | 5.8 ± 1.1 |
| 5           | NA          | 38 ± 11     | >30 μM      | 202 ± 44 |
| 13          | NA          | 3322 ± 556  | >30 μM      | 1504 ± 244 |

*[^[125I]NT. EEC50, IC50 and Kt values are nM ± SEM. Emax value is %.
[^3b]Not active.*
LIBRARY SCREENING

Fan and co-workers reported that the nonpeptide compound 6a, Chart 1, possessed partial agonist activity at the NTS1 receptor.26 With the hope that some variant of this compound might also be active at NTS2, we made a library of 66 compounds with general structure 6b using commercially available sulfonyl chlorides and amino acid esters by following the chemistry route described for compound 13 in Scheme 1. We screened these for agonist and antagonist activity in parallel at a single concentration (10 μM) using 3b as our NTS2 agonist standard and NT as our antagonist standard. Only one compound (13) showed >50% inhibition of 3b induced calcium mobilization in the NTS2 screen. Compound 13 was also devoid of NTS2 agonist activity and showed no activity at NTS1 (data not shown).

RESULTS AND DISCUSSION

In confirming the hit 13, an impurity was discovered that represented 25% of the material in the well. Mass spectral and 19F NMR analysis suggested that the desired compound (13) had incorporated a trifluoroacetyl group to give byproduct 5. We postulated that this would have occurred at the indole 3-position during deprotection with TFA and tested this by synthesizing 5 from a different direction as described in Scheme 1. Comparison of the 1H NMR and mass spectral data of 5 to that of the impurity confirmed that they were identical. Both compounds 5 and 13 were carried forward into dose response testing.

In Table 1, we provide the FLIPR assay (NTS2) and binding assay (NTS1 and NTS2) data collected for the reference compounds NT and 3b as well as the test compounds 5 and 13. In the FLIPR assay, the reference compounds performed as expected. NT showed no agonist activity up to 100 nM, but as previously reported, the Kᵦ experiment with NT at NTS2 versus 3b demonstrated insurmountable antagonist activity.22,27,28 For this reason, we determined and report here its IC₅₀ value of 114 nM. In the binding assay at NTS2, NT provided a Kᵦ value of 28 nM. Reference compound 3b gave an IC₅₀ of 22 nM (100% Eₘₐₓ) in the FLIPR assay and a Kᵦ of 5.8 nM in the binding assay.

Neither of the test compounds (5 or 13) showed activity in the NTS1 FLIPR assay (data not shown), but in the NTS2 FLIPR assay, they both behaved like NT. Each compound antagonized the action of 3b in the Kᵦ experiment in an insurmountable manner. The graph of the Kᵦ experiment for 5 is illustrated in Figure 1. Both 5 and 13 were tested in the IC₅₀ experiment and gave IC₅₀ values of 38 and 3322 nM, respectively. The results from the IC₅₀ experiment of NT and 5 versus 3b are illustrated in Figure 2.

In the binding assays at NTS2, 5 and 13 showed Kᵦ values of 202 and 1504 nM, respectively, versus 28 nM for NT. Testing of compounds 5 and 13 at the NTS1 receptor showed no binding activity in the NTS1 assay up to 30 μM, making 5 selective for NTS2 in both the binding and functional assays.

A comparison of FLIPR data for compounds 5 and 13 revealed that the impurity 5 was not only 90-fold more active than the target compound 13 but also 3-fold more active than the endogenous ligand NT. This result was unexpected, as the impurity was found to be far more interesting than the intended compound. Indeed, it is unlikely that this discovery would have been made if not by accident as substitution with the trifluoroacetyl group is not a common practice in medicinal chemistry. Overall, however, we have identified a molecular region of 5 that is very important to antagonism of calcium mobilization at NTS2.

The activity of both 5 and 13 in the NTS2 FLIPR and binding assays provides additional evidence that the calcium release mediated by 3b is an NTS2 mediated event. Also, a comparison of their binding data at NTS2 revealed that the trifluoroacetyl substitution of 5 did not increase the binding affinity to the same degree observed for the FLIPR assay. We found a 90-fold boost to antagonist activity for 5 versus 13 in the FLIPR assay and a 7.5-fold boost to binding affinity. Although these need not necessarily be equivalent, we suspect that this could be a consequence of measuring the two end points using two different reference compounds. In the FLIPR assay, 5 is believed to compete directly with 3b, while in the binding assay 5 is competing against NT. In most assay systems, the endogenous ligand (or another more active compound) serves as both the reference compound in the functional assay and the radioligand in the binding assay. In this case, however, the endogenous ligand (NT) does not mobilize calcium. Thus, we would require radioactive 3b in order to
achieve parity. While this is possible, radioactive NT is commercially available and far more convenient to obtain.29

Empirically, however, we observe that every compound so far identified that inhibits 3b in the FLIPR assay also competes with NT in the binding assay, suggesting that the difference in magnitude of effect does not prevent the detection of NTS2 active compounds.

As noted above, we do not know that 5 is competing directly with 3b for its binding site, this is only a working assumption. There is, however, empirical evidence that supports the assumption that the binding sites for 3b and NT are, at least partially, overlapping. The most important observation is that NT, 3a, 3b, and 5 all rely heavily upon a free carboxylic acid group for binding to NTS2 (and NTS1). In studies of the NTS1 receptor, the ionic interaction established by the binding of this group to an arginine residue in the receptor, formed the principle ligand to receptor stabilizing interaction.30 Along with this, a partially overlapping scenario has been postulated for NT and 3a binding sites based on evidence obtained from molecular modeling and point mutation studies at the NTS1 receptor.31 Although not conclusive, these observations do offer support to the notion that these compounds all share partially overlapping binding sites.

**SUMMARY**

The work presented herein provides additional evidence that 3b can be used as an agonist in the NTS2 FLIPR assay to identify novel NTS2 active compounds. Because NT is an antagonist of 3b, novel NT-like antagonist compounds can be identified. To our knowledge, 5 is the first nonpeptide compound that behaves like, and is more active than, the endogenous ligand in this functional assay. This work compliments our discovery of compound 4, providing an additional nonpeptide compound whose in vitro properties align with those of compounds that are known to be active in models of pain. Together, 4 and 5 should be useful in additional studies related to the biology of the NT receptors. We are evaluating these compounds in animal models of pain and additional studies related to the biology of the NT receptors. Additional SAR studies with 5 are in progress and will be reported in the near future.

**EXPERIMENTAL SECTION**

Reactions were conducted under a nitrogen atmosphere using oven-dried glassware as required. All solvents and chemicals used were reagent grade. S-Nitroindole (7) was purchased from Combi-blocks, Inc. Flash column chromatography was carried out using a Teledyne ISCO CombiFlash RF system and Redtex RF gold prepaticed HP silica columns. Flow rates were automatically determined by column size. Fractions were collected as “peaks only” over 30 min. The purity of target compounds was determined to be ≥95% by combustion analysis. NMR spectra were recorded on a Bruker Avance DPX-300 (300 MHz). Low-resolution mass spectra were obtained using a Waters Alliance HT/Micromass ZQ system (ESI) in both positive and negative mode. Optical rotations were measured on an Auto Pol III polarimeter at the sodium D line. Thin layer chromatography (TLC) was performed on EMD precoated silica gel 60 F254 plates, and spots were visualized with UV light and I2 or phosphomolybdic acid stain. CHO-k1 cells were from the American Type Culture Collection, and [11C]NT was from PerkinElmer. Calcium 5 dye was from Molecular Devices. Cell culture reagents were from Life Technologies.

**N-(5-[[(4-Methylphenyl)sulfonyl]amino]-3-[(trifluoroacetyl)-1H-indol-1-yl]acetetyl]]-leucinate (5).** Into a 250 mL RBF with a magnetic stir bar are placed S-nitroindole (7, 4.864 g, 0.030 mol) and K2CO3 (4.561 g, 0.033 mol) and 90 mL of acetone. The methyl bromoacetate (5.048 g, 0.033 mol, 3.10 mL) was added over a minute via syringe and then stirred 4 days at RT. The reaction was then concentrated, poured into water, and extracted with EtOAc (2×), and the combined organics were washed (H2O, brine) and dried (Na2SO4) and concentrated to give a yellow oil. This was chromatographed on an ISCO silica gel column (80 g) using a 0–60% EtOAc/hexanes gradient and then crystallized from ethyl acetate to give the desired product as a yellow solid (4.38 g, 65% yield).1 H NMR (CDCl3) δ 8.60 (d, J = 2.3 Hz, 1 H), 8.14 (d, J = 2.3 Hz, 1 H), 7.26 (d, J = 9.1 Hz, 1 H), 7.25 (d, J = 3.3 Hz, 1 H), 6.75 (d, J = 3.2 Hz, 1 H), 4.92 (s, 2 H), 3.76 (s, 3 H).

**5-Nitro-1H-indol-1-ylacacet Acid (9).** To a solution of 8 (10.10 g, 0.045 mol) in 50 mL of 1,4-dioxane was added 50 mL of 1 N LiOH, and then stirred for 14 h at RT. The reaction was then concentrated, poured into water, and extracted with EtOAc (2×), and then dried under vacuum to provide a yellow oil. This was chromatographed on a silica gel column (100 g) using a 0–80% MeOH/EtOAc/hexanes gradient and then crystallized from ethyl acetate to give the desired product as a yellow solid (10.10 g, 65% yield).1 H NMR (CDCl3) δ 7.14 (d, J = 8.5 Hz, 1 H), 6.98 (d, J = 3.0 Hz, 1 H), 6.76–7.58 (m, 2 H), 6.76 (d, J = 3.2 Hz, 1 H), 5.16 (s, 2 H). MS (ESI): 219.3 [M–H]–.

**tert-Butyl N-[[5-Nitro-1H-indol-1-yl]acetetyl]-leucinate (10).** Into a 100 mL RBF with stir bar containing 9 (2.202 g, 0.010 mol), (S)-(+)leucine tert-butyl ester HCl salt (2.461 g, 0.011 mol), and HBTU (4.172g, 0.011 mol) was added CH3Cl (50 mL) and then Et3N (3.036 g, 0.030 mol, 4.18 mL), and this was stirred at RT overnight. The reaction mixture was then concentrated and chromatographed on an ISCO silica gel column using a 10–60% gradient of EtOAc in hexanes to give 10 (4.07 g) as a yellow oil which crystallized when dried under vacuum to give a yellow–orange solid (3.859 g, 99%).1 H NMR (CDCl3) δ 8.59 (d, J = 2.3 Hz, 1 H), 8.13 (d, J = 2.2, 9.0 Hz, 1 H), 7.35 (d, J = 9.0 Hz, 1 H), 7.31 (d, J = 3.3 Hz, 1 H), 6.80 (d, J = 0.5, 3.2 Hz, 1 H), 5.96 (d, J = 8.3 Hz, 1 H), 4.88 (s, 2 H), 4.50 (dt, J = 5.2, 8.5 Hz, 1 H), 1.59–1.42 (m, 2 H), 1.40 (s, 9 H), 0.85 (dd, J = 3.3, 6.2 Hz, 6 H). MS (ESI): 360.5 [M+H]+.

**tert-Butyl N-[[5-Amino-1H-indol-1-yl]acetetyl]-leucinate (11).** 10 (3.894 g, 0.010 mol) was dissolved in ethanol (200 mL) and reduced using a Parr apparatus with 10% Pd/C (400 mg) under 40 psig of H2. The reaction mixture was filtered through Celite, the cake was washed with EtOH, and the filtrate was concentrated under vacuum. Drying under HVAC gave 11 (3.620 g, 100%) as a red oil. [NOTE: This material darkens with exposure to air.]1 H NMR (CDCl3) δ 7.09 (d, J = 8.6 Hz, 1 H), 7.01 (d, J = 3.1 Hz, 1 H), 6.96 (d, J = 2.1 Hz, 1 H), 6.72 (d, J = 2.2, 8.6 Hz, 1 H), 6.44 (d, J = 2.7 Hz, 1 H), 5.62 (d, J = 8.5 Hz, 1 H), 4.74 (s, 2 H), 4.45 (dt, J = 5.1, 8.7 Hz, 1 H), 1.50–1.41 (m, 2 H), 1.36 (s, 9 H), 1.31–1.20 (m, 1 H), 0.81 (dd, J = 6.9, 7.8 Hz, 6 H). MS (ESI): 358.5 [M–H]–, 360.3 [M+H]+.

**N-[5-[[4-Methylphenylsulfonyl]amino]-1H-indol-1-yl]acetetyl]-leucinate (12).** 11 (3.595 g, 0.010 mol) was dissolved in CH2Cl2 (50 mL) in a 250 mL RBF equipped with a stir bar. DMAP (1.344 g, 0.011 mol) was added, followed by a solution of tosyl chloride (2.002 g, 0.011 mol) in CH2Cl2 (20 mL). The reaction was
stirred overnight and then concentrated and chromatographed on an 80 g silica gel cartridge with a 0–80% EtOAc/hexanes gradient. The product crystallized from the fractions upon standing to give 12 (4.65g, 91%) as colorless crystals. 1H NMR (CDCl3) δ 7.61 (d, J = 8.2 Hz, 2 H), 7.39 (d, J = 1.9 Hz, 1 H), 7.18 (s, 2 H), 7.16 (s, 1 H), 7.12–7.09 (m, 1 H), 6.95 (dd, J = 1.9, 8.8 Hz, 1 H), 6.52 (d, J = 3.2 Hz, 1 H), 5.75 (d, J = 8.5 Hz, 1 H), 4.77 (s, 2 H), 4.46 (d, J = 5.1, 8.5 Hz, 1 H), 3.35 (s, 3 H), 1.53–1.41 (m, 3 H), 1.37 (s, 9 H), 0.79 (t, J = 6.2 Hz, 6 H). MS (ESI): 514.3 [M + H]+.

N-[(5-[(4-Methylphenyl)sulfonyl]amino)-1H-indol-1-yl]-acetyl]-L-leucine (13). 12 (51.4 mg, 0.1 mmol) was dissolved in dry CH2Cl2 (2 mL), and then TFA was added (2 mL) and the reaction stirred at RT for 30 min. This was then concentrated to dryness and the residue dissolved in a minimal amount of CH2Cl2/MeOH and then triturated with 2 mL of ethyl ether and hexanes to precipitate the acid as an off-white powder. This was collected by filtration and washed with hexanes, and then the solids were dried under vacuum. This was then purified on a 4 g ISCO silica gel column using a gradient of 0–10% MeOH/NH4OH (10:1) in CH2Cl2 to provide the 13 (11.7 mg, 26%) as a colorless solid.

1H NMR (CD3OD) δ 7.55 (d, J = 8.3 Hz, 1 H), 7.41–7.31 (m, 1 H), 7.16–7.23 (m, 1 H), 7.02–7.15 (m, 2 H), 6.98 (t, J = 8.5 Hz, 1 H), 6.89 (d, J = 2.0, 8.8 Hz, 1 H), 6.73 (dd, J = 2.0, 8.8 Hz, 1 H), 6.40 (d, J = 3.0 Hz, 1 H), 4.72–4.86 (m, 2 H), 4.40–4.62 (m, 1 H), 2.16–2.41 (m, 3 H), 1.52–1.79 (m, 4 H), 0.81–0.98 (m, 6 H). 13C NMR (CD3OD) δ 213.27, 200.27, 136.16, 138.10, 144.60, 170.62, 175.76. MS (ESI): 456.3 (M−H)+;

[a]D20 = −8.84 (c 0.37, CH3OH). Anal. (C25H27NO2S): C, H, N.

tert-Butyl N-[(5-[(4-Methylphenyl)sulfonyl]amino)-3-(trifluoroacetyl)-1H-indol-1-yl(acetyl)]-L-leucinate (14). 12 (128.4 mg, 0.25 mmol) was dissolved in CH2Cl2 (5 mL) and to this was added trifluoroacetic anhydride (57.8 mg, 0.04 mL, 0.28 mmol) and then triethylamine (0.28 mL, 2 mL) and the reaction was stirred at RT for 2 h. The reaction was then concentrated to a solid film and purified on a 4 g ISCO silica gel column using a 0–60% EtOAc/hexanes gradient. The desired fractions were concentrated to provide 14 (85.9 mg, 56%) as a colorless film.

Calcium Mobilization Assay for NTS2 Receptor. CHO-K1
rNTS2 cells were maintained in DMEM/F12 supplemented with 10% FBS, pen/strep, 100 μg/mL nornocin, and 400 μg/mL Geneticin. For calcium mobilization assays, cells were plated at 25000 cells/well in black, clear-bottom 96-well plates 24 h before the assay and incubated at 37 °C, 5% CO2. Then 100 μL of reconstituted Calcium 5 dye (Molecular Devices, diluted 1:20 in assay buffer (1x HBSS, 20 mM HEPES, 2.5 mM Probenicid, pH 7.4)) was added per well and plates were incubated for 45 min at 37 °C, 5% CO2. For agonist assays, cells were pretreated with 1:10 addition of 10% DMSO, and the plates were returned to 37 °C, 5% CO2. Log-log serial dilutions of the test compounds were made at 10X the desired final concentration in 1% DMSO assay buffer and warmed to 37 °C. After the pretreatment incubation, fluorescence intensity was measured on a FLIPR Tetra fluorometric imaging plate reader (Molecular Devices). Relative fluorescence units (RFUs) were measured for each second for 100 s (14 readings before compound addition to establish baseline fluorescence, 85 after), exposure time 0.53 s, gain = 2000, excitation intensity = 30%, gate open = 10%. For antagonist assays, 1/10 volume of 10X concentration of the test compound dilutions in 10% DMSO in assay buffer was added to cells in lieu of the 10% DMSO only in assay buffer for the pretreatment. Kᵢ assays were run against dose–response curves of the control agonist 3b, and IC5₀ assays were run against the EC5₀ of 3b (73 nM final concentration).

Competitive Binding Assays. Relative binding affinity was evaluated using [3H]labeled neurotensin and CHO-K1 cell lines overexpressing either the rNTS1 or rNTS2 receptor essentially as described by Gendron.

Data Analysis. To determine EC5₀ and IC5₀ values, data were fit to a three-parameter logistic equation using GraphPad Prism software. Kᵢ values for radioligand binding assays were determined from IC5₀ values using the equation of Cheng and Prusoff. All data are from at least three independent experiments run in duplicate wells.

ASSOCIATED CONTENT

Supporting Information

Elemental analysis results for test compounds 5, 13, and 14. This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

NT, neurotensin; NTS1, neurotensin receptor 1; NTS2, neurotensin receptor 2; CNS, central nervous system; H1, histamine receptor 1; FLIPR, fluorometric imaging plate reader; CHO, Chinese hamster ovary.

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