



**HAL**  
open science

# Design, Synthesis, and Biological Evaluation of the First Radio-Metalated Neurotensin Analogue Targeting Neurotensin Receptor 2

Sacha Bodin, Santo Previti, Emmanuelle Jestin, Delphine Vimont, Imade Ait-Arsa, Frédéric Lamare, Emmanuelle Rémond, Elif Hindié, Florine Cavelier, Clément Morgat

## ► To cite this version:

Sacha Bodin, Santo Previti, Emmanuelle Jestin, Delphine Vimont, Imade Ait-Arsa, et al.. Design, Synthesis, and Biological Evaluation of the First Radio-Metalated Neurotensin Analogue Targeting Neurotensin Receptor 2. ACS Omega, 2023, 8 (7), pp.6994 - 7004. 10.1021/acsomega.2c07814 . hal-04023539

**HAL Id: hal-04023539**

**<https://hal.science/hal-04023539>**

Submitted on 10 Mar 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Design, Synthesis, and Biological Evaluation of the First Radiometalated Neurotensin Analogue Targeting Neurotensin Receptor 2

Sacha Bodin, Santo Previti, Emmanuelle Jestin, Delphine Vimont, Imade Ait-Arsa, Frédéric Lamare, Emmanuelle Rémond, Elif Hindié, Florine Cavelier,<sup>\*,#</sup> and Clément Morgat<sup>\*,#</sup>



Cite This: *ACS Omega* 2023, 8, 6994–7004



Read Online

ACCESS |



Metrics & More



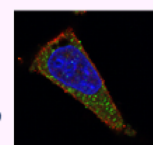
Article Recommendations



Supporting Information

**ABSTRACT:** Neurotensin receptor 2 (NTS<sub>2</sub>) is a well-known mediator of central opioid-independent analgesia. Seminal studies have highlighted NTS<sub>2</sub> overexpression in a variety of tumors including prostate cancer, pancreas adenocarcinoma, and breast cancer. Herein, we describe the first radiometalated neurotensin analogue targeting NTS<sub>2</sub>. JMV 7488 (DOTA-(βAla)<sub>2</sub>-Lys-Lys-Pro-(D)Trp-Ile-TMSAla-OH) was prepared using solid-phase peptide synthesis, then purified, radiolabeled with <sup>68</sup>Ga and <sup>111</sup>In, and investigated *in vitro* on HT-29 cells and MCF-7 cells, respectively, and *in vivo* on HT-29 xenografts. [<sup>68</sup>Ga]Ga-JMV 7488 and [<sup>111</sup>In]In-JMV 7488 were quite hydrophilic (logD<sub>7.4</sub> = -3.1 ± 0.2 and -2.7 ± 0.2, respectively, *p* < 0.0001). Saturation binding studies showed good affinity toward NTS<sub>2</sub> (K<sub>D</sub> = 38 ± 17 nM for [<sup>68</sup>Ga]Ga-JMV 7488 on HT-29 and 36 ± 10 nM on MCF-7 cells; K<sub>D</sub> = 36 ± 4 nM for [<sup>111</sup>In]In-JMV 7488 on HT-29 and 46 ± 1 nM on MCF-7 cells) and good selectivity (no NTS<sub>1</sub> binding up to 500 nM). On cell-based evaluation, [<sup>68</sup>Ga]Ga-JMV 7488 and [<sup>111</sup>In]In-JMV 7488 showed high and fast NTS<sub>2</sub>-mediated internalization of 24 ± 5 and 25 ± 11% at 1 h for [<sup>111</sup>In]In-JMV 7488, respectively, along with low NTS<sub>2</sub>-membrane binding (<8%). Efflux was as high as 66 ± 9% at 45 min for [<sup>68</sup>Ga]Ga-JMV 7488 on HT-29 and increased for [<sup>111</sup>In]In-JMV 7488 up to 73 ± 16% on HT-29 and 78 ± 9% on MCF-7 cells at 2 h. Maximum intracellular calcium mobilization of JMV 7488 was 91 ± 11% to that of levocabastine, a known NTS<sub>2</sub> agonist on HT-29 cells demonstrating the agonist behavior of JMV 7488. In nude mice bearing HT-29 xenograft, [<sup>68</sup>Ga]Ga-JMV 7488 showed a moderate but promising significant tumor uptake in biodistribution studies that competes well with other nonmetalated radiotracers targeting NTS<sub>2</sub>. Significant uptake was also depicted in lungs. Interestingly, mice prostate also demonstrated [<sup>68</sup>Ga]Ga-JMV 7488 uptake although the mechanism was not NTS<sub>2</sub>-mediated.

[M<sup>+</sup>]M-DOTA-(βAla)<sub>2</sub>-Lys-Lys-Pro-(D)Trp-Ile-TMSAla-OH



Targeting NTS<sub>2</sub>-expressing tumors for PET imaging and therapy

## INTRODUCTION

G-protein coupled receptors (GPCRs) represent numerous targets for drug development,<sup>1</sup> particularly in nuclear medicine. Recent advances in nuclear oncology include the use of [<sup>68</sup>Ga]Ga-DOTATOC and [<sup>177</sup>Lu]Lu-DOTATATE for diagnosis and peptide receptor radionuclide therapy (PRRT) of digestive neuroendocrine tumors overexpressing the somatostatin receptor subtype 2 (SST<sub>2</sub>), which is part of the class A GPCR family. Among this same family, neurotensin (NT) receptors represent also promising targets for imaging and therapy. Neurotensin is a 13 amino acid neuropeptide,<sup>2</sup> which binds to three receptors, two GPCRs, namely, NTS<sub>1</sub> and NTS<sub>2</sub>, and the single transmembrane domain NTS<sub>3</sub>/sortilin receptor. NTS<sub>1</sub> and NTS<sub>2</sub> are widely expressed in the central nervous system (CNS) where they are involved in opioid independent analgesia, hypothermia, and psychiatric effects,<sup>3</sup> but they also exhibit peripheral actions.<sup>4,5</sup>

Numerous NTS<sub>1</sub> targeting ligands carrying the pharmacophore NT(8-13) hexapeptide sequence (H-Arg-Arg-Pro-Tyr-Ile-Leu-OH)<sup>6,7</sup> have been developed and studied in different types of peripheral tumors, such as colorectal cancer, prostate adenocarcinoma, breast cancer, or pancreatic cancer.<sup>8–12</sup> Contrarily, NTS<sub>2</sub> targeting peptides have been studied for

their central opioid-independent analgesic potency.<sup>13–17</sup> Whereas very few analogues have been developed for imaging of peripheral tumors,<sup>18</sup> NTS<sub>2</sub>'s role in tumor development remains unclear and requires more investigations. Indeed, in prostate cancer, NTS<sub>2</sub> mRNA expression was found only in malignant well-differentiated (androgen receptors +) cell-lines.<sup>19</sup> A recent study also demonstrated its involvement in apoptosis resistance in B-cell chronic lymphocytic leukemia.<sup>20</sup>

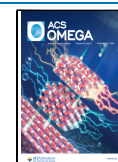
Only few studies have investigated the expression of NTS<sub>2</sub> in normal organs. In healthy tissues, NTS<sub>2</sub> has been found to be expressed in lungs.<sup>21</sup>

Herein, we report the pharmacological and biological properties of JMV 7488, a new silylated NTS<sub>2</sub>-targeting hexapeptide radiolabeled with gallium-68 and indium-111. This ligand is derived from the N-terminal elongation of JMV

Received: December 7, 2022

Accepted: January 30, 2023

Published: February 10, 2023



5504, a modified NT(8-13) analogue, which has high affinity and selectivity toward the NTS<sub>2</sub> receptor.<sup>22</sup>

## MATERIALS AND METHODS

Chemistry, radiolabeling procedure, determination of the octanol/water partition coefficient, cell culture, western blot, confocal imaging, and *blood plasma* stability methods are fully presented in the [Supporting Information](#).

**In Vitro Studies.** HT-29 and MCF-7 cells were seeded into 24-wells plates at the density of  $2 \times 10^5$  cells per well, the day before the experiment. The plates were incubated at 37 °C and 5% CO<sub>2</sub>. All the binding and internalization experiments were carried out in quadruplicate and efflux studies in octuplicate.

**Saturation Binding Studies.** Plates were stored at 4 °C for 30 min in order to reduce cell processing and then incubated for 2 h at 37 °C with 250 μL of complete medium containing the radiolabeled peptide at increasing concentrations (0.1, 1, 10, 100, 250, and 500 nM) with and without saturation ligands (neurotensin, a specific agonist of NTS<sub>1</sub> or levocabastine, a specific agonist of NTS<sub>2</sub>, final concentration of 1 μM). After incubation, cells were rinsed twice with ice cold DPBS (250 μL, 14190-144, Gibco). Then, cells were lysed with NaOH 1 M (750 μL) to collect the bound fraction. Radioactivity of each fraction was determined in a gamma-counter (Wizard2, PerkinElmer, USA). Affinity ( $K_D$ ) was determined by nonlinear regression using Prism 6.01 software (GraphPad Software Inc., USA). Experiments were performed three times in quadruplicates.

**Cell-Associated Radioactivity and Internalization Studies.** A total of 1 MBq of [<sup>68</sup>Ga]Ga-JMV 7488 or 50 kBq of [<sup>111</sup>In]In-JMV 7488 in 250 μL of complete medium with and without 1 μM of saturation ligands was added in each well. Plates were incubated for 10, 30, or 60 min (plus 120 and 240 min for <sup>111</sup>In radiolabeled JMV 7488) at 37 °C. Three minutes before the selected time point, plates were stored at 4 °C to stop the internalization process. Media were removed, and wells were rinsed three times with ice cold DPBS (250 μL). A total of 250 μL of sodium acetate (20 mM, pH 5) was added in each well twice and then collected in tubes after 5 min incubation. Then, cells were lysed with NaOH 1 M (750 μL) to collect the bound fraction. Radioactivity of each fraction was determined in a gamma-counter. Cell-associated radioactivity results are expressed as percentage of specific binding. Experiments were performed four times in quadruplicates.

**Efflux Studies.** A total of 1 MBq of [<sup>68</sup>Ga]Ga-JMV 7488 or 50 kBq [<sup>111</sup>In]In-JMV 7488 in 250 μL of complete medium was added in each well. Plates were incubated 30 min at 37 °C. Media were removed, and cells were rinsed with ice cold DPBS (250 μL). A total of 250 μL of sodium acetate (20 mM, pH 5) was added in each well and removed after 5 min of incubation. Cells were rinsed again with ice cold DPBS (250 μL). Then, 250 μL of medium were added in each well and plates were incubated for 5, 15, 30, or 45 min (or 5, 15, 60, and 120 min for <sup>111</sup>In radiolabeled JMV 7488) at 37 °C. Media were collected in tubes, and each well was rinsed twice with ice cold DPBS (250 μL). Then, cells were lysed with NaOH 1 M (750 μL) to collect the bound fraction. Radioactivity of each fraction was determined in a gamma-counter. Results are expressed as percentage of total binding. Experiments were performed three times in octuplicate.

**Calcium Imaging Assay.** HT-29 cells were cultured as previously described. The day before the experimentation, cells

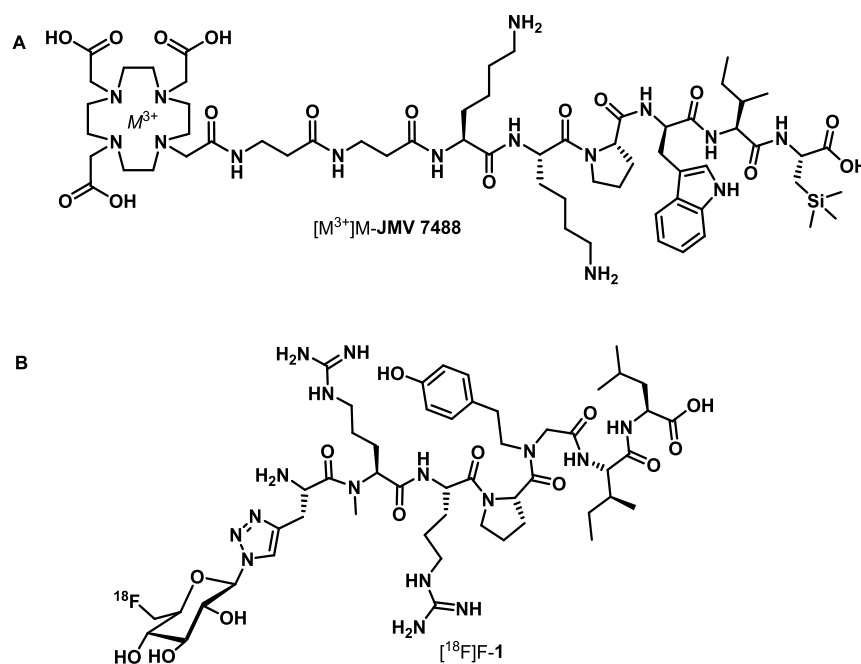
were grown on 15 mm Thermanox glass slides (ThermoFischer, USA) in 12-wells plates at the density of  $5 \times 10^5$  cells in 500 μL of complete culture medium. Night incubation at 37 °C and 5% CO<sub>2</sub> allows cell adherence on the slides. Glass slides were individually loaded for 30 min in the dark with a 500 μL loading solution containing 100 μM fluo-8 AM (ATT Bioquest, USA), 0.4% (w/v) pluronic acid (AAT Bioquest, USA), and 2.5 mM probenecid (Invitrogen, ThermoFischer, USA) into RPMI medium. Slides were then placed in a recording chamber perfused with buffer solution (containing (in mM): 130 NaCl, 3 KCl, 2.5 CaCl<sub>2</sub>, 1.3 MgSO<sub>4</sub>, 0.58 NaH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>, and 10 glucose) equilibrated with 95% O<sub>2</sub>/5% CO<sub>2</sub>, adjusted to pH 7.4 at room temperature. A 10 min delay was respected to wash out extracellular fluo-8 AM and enable fluo-8AM intracellular hydrolysis. Increasing concentrations ( $10^{-11}$  to  $10^{-5}$  M) of JMV 7488 were added *via* a rapid perfusion system. Positive control experiments were performed by adding similar concentrations of levocabastine. Imaging of intracellular Ca<sup>2+</sup> changes was performed following excitation of cells at 490 nm with analysis of fluorescence emission at 514 nm using an Eclipse E600FN microscope (Nikon, Japan) coupled with an Infinity 3 detector (Lumenera, Canada). Changes in fluorescence ( $F_T$ ) were calculated for each cell relative to the averaged baseline fluorescence before stimulation ( $F_{REST}$ ) and expressed as  $\Delta F/F$  (%) =  $[(F_T - F_{REST})/F_{REST}] \times 100$ . The calcium mobilization assay was performed twice in duplicate, and 20 cells were manually analyzed.

**In Vivo Studies.** Animal experiments have been approved and permitted by the Ethics committee n°114 (reference APAFIS#26960-2020061008304944 v4) and were conducted according to European and national guidelines. All the animals were maintained at a constant temperature ( $24 \pm 1$  °C) and a hygrometry between 55 and 65% under a 12 h light/dark cycle and permitted free access to food and water.

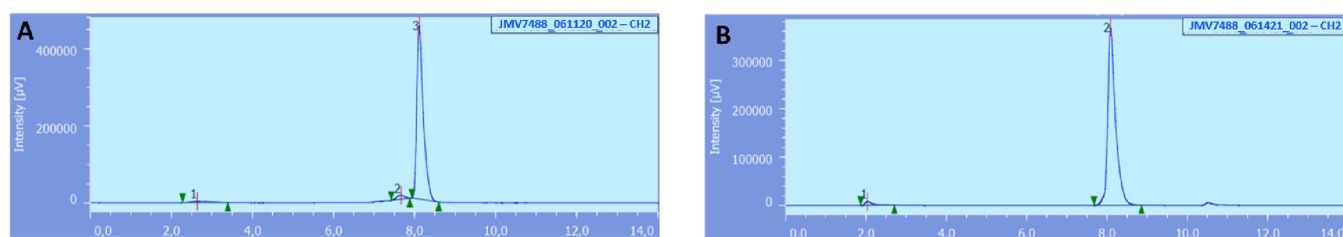
**HT-29 Xenograft Model.** Eight weeks old female Balb-c nude mice have been purchased from JANVIER LABS, and quarantined for 2 weeks. Animals are fed *ad libitum* and maintained in a HEPA-filtered environment with cages, food, and bedding sterilized by autoclaving.

Under anesthesia with isoflurane (2% in air mixture), Balb-c nude mice were injected subcutaneously in the right flank with  $1 \times 10^6$  HT-29 cells suspended in 100 μL of 50:50 Matrigel/collagen I. Tumor growth was monitored externally using calipers. Biodistribution and PET/CT imaging studies were performed in mice when tumor volume reached approximately 150 mm<sup>3</sup>.

**Micro-PET/CT Imaging.** The preclinical imaging studies were performed on a multimodality imaging system (Triumph II, PET/SPECT/CT, Trifoil Imaging, Chatsworth, USA). Mice were anesthetized with isoflurane (3%) and placed under the PET modality on a temperature-controlled bed. Injections of the radiotracers were proceeded in the caudal lateral vein using an insulin syringe 29-gauge under the camera to get the bolus. Four mice were injected with [<sup>68</sup>Ga]Ga-JMV 7488 ( $6.58 \pm 1.54$  MBq,  $4.3 \pm 3.6$  nmol, ~50 μL diluted in 150 μL of saline before administration), and four mice were injected with 0.5 μmol levocabastine 15 min prior to radiotracer injection ( $6.16 \pm 1.21$  MBq). PET acquisitions were done under anesthesia (isoflurane 3% induction and 1.5% maintenance in air) during 75 min. CT acquisitions were carried out at the end of the experiment. Animals were then sacrificed 90 min post-injection, and the organs were collected as described below.



**Figure 1.** (A) Structure of  $[M^{3+}]M$ -JMV 7488 developed in this study.  $M^{3+}$  stands for  $^{68}Ga$  or  $^{111}In$  in this study. Coordination bonds are not figured out. (B) Structure of the  $^{18}F$ -labeled neurotensin peptide  $[^{18}F]F$ -1 previously developed in the literature to target the neurotensine receptor 2.



**Figure 2.** Radio-HPLC chromatogram of  $[^{68}Ga]Ga$ -JMV 7488 (A) and  $[^{111}In]In$ -JMV 7488 (B).

PET data were reconstructed using the OSEM 3D method. CT files were reconstructed using a Feldkamp algorithm after obtaining an isotropic voxel size of 0.170 mm.

**Biodistribution Studies.** Four mice were injected with  $[^{68}Ga]Ga$ -JMV 7488 ( $6.58 \pm 1.54$  MBq,  $4.3 \pm 3.6$  nmol,  $\sim 50$   $\mu$ L diluted in 150  $\mu$ L of saline before administration), and four mice were injected with 0.5  $\mu$ mol levocabastine 15 min prior to radiotracer injection ( $6.16 \pm 1.21$  MBq). Solutions were injected into the tail vein. Animals were sacrificed 90 min post-injection by an intracardiac puncture. Blood, heart, lungs, liver, kidneys, spleen, stomach, intestines, muscle, bone, prostate, brain, and HT-29 tumor were collected. Organs were weighed and  $\gamma$ -counted (Wizard 2470, Perkin Elmer). Uptake levels were expressed as percentage of injected dose per gram of tissue (%ID/g).

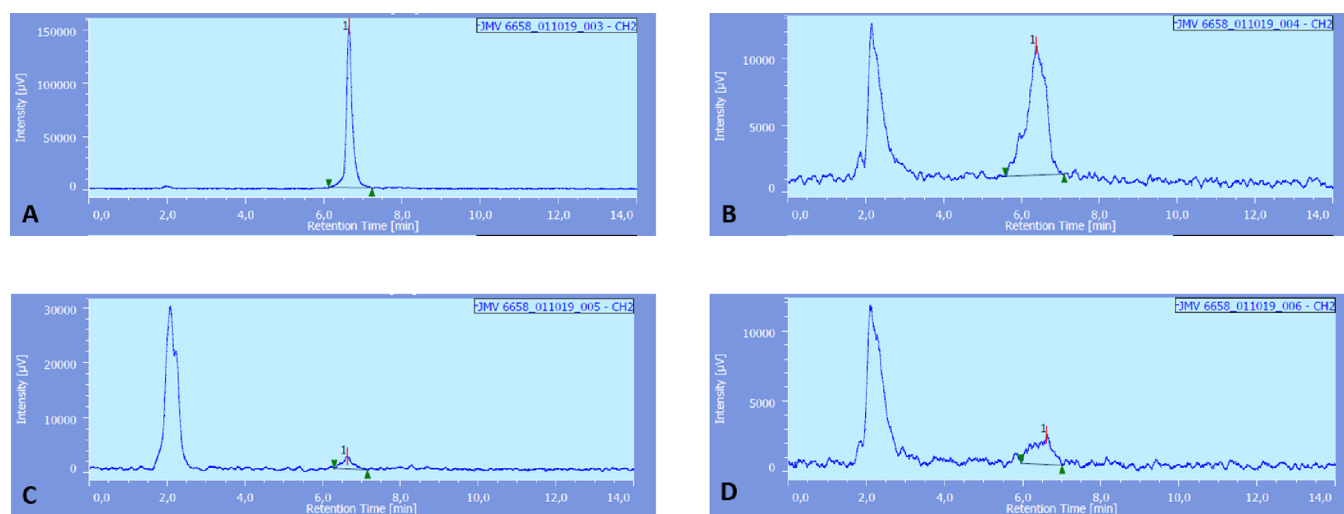
**Statistical Analyses.** Statistical analyses were performed using Prism v6.01 software (GraphPad Software Inc., USA). Unpaired data were used. Quantitative values were expressed as means  $\pm$  SD. Statistical analysis with at least three groups were performed with the multiple *t*-tests corrected for multiple comparisons using the Holm Sidak method. Comparisons between two groups were performed using the bilateral nonparametric Mann–Whitney test. A *P*-value of  $<0.05$  was considered significant.

**Purity Statement.** All compounds are  $>95\%$  pure by HPLC and LCMS analysis. Representative traces are provided in the [Supporting Information](#).

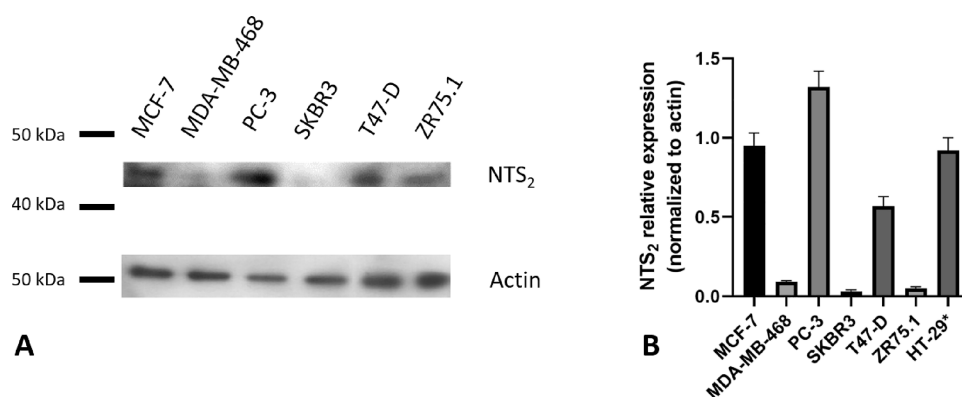
## RESULTS

**Chemistry.** The unnatural amino acid trimethylsilylalanine (TMSAla) carrying the fluorenylmethyloxycarbonyl (Fmoc) as the protecting group was synthesized following the synthetic pathway we already reported.<sup>23</sup> After that, the obtained Fmoc-TMSAla-OH was loaded on 2-chlorotrityl chloride (2-CTC) resin,<sup>24</sup> which was previously treated with  $SOCl_2$  to optimize the reactive sites. A loading of 0.8 mmol/g was detected with UV spectroscopy. On the contrary, the loading of Fmoc-TMSAla-OH resulted to be unproductive if untreated resin was used. The desired NT analogue JMV 7488 (Figure 1) was synthesized using the solid phase peptide synthesis (SPPS) approach with a yield of 31% (0.1 mmol scale). Even though the yield is moderately low, LC–MS analysis of the crude product after final cleavage showed a major peak with minimum amounts of by-products. This good HPLC profile allowed us to purify the desired product very easily. All data concerning the synthesis and characterization of JMV 7488 are detailed in the [Supporting Information](#).

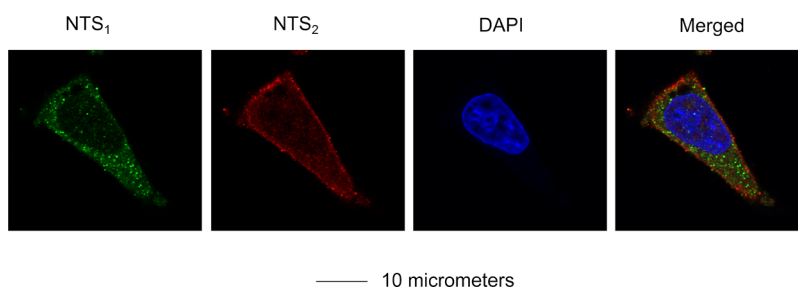
**$^{68}Ga$  and  $^{111}In$  Radiolabeling.** Radiolabeling were achieved with moderate yields of  $53.88 \pm 8.34$  ( $^{68}Ga$ , decay-



**Figure 3.** Radio-HPLC chromatograms of [ $^{68}\text{Ga}$ ]Ga-JMV 7488 in human plasma 0 (A), 15 (B), 30 (C), and 45 min (D) after incubation.



**Figure 4.** (A) Representative western blot of the NTS<sub>2</sub> receptor on cell lysate from MCF-7, MDA-MB-468, PC-3, SKBR3, T47-D, and ZR75.1 cells. (B) Semi-quantification of the NTS<sub>2</sub> signal from the western blots. (\*) Data regarding NTS<sub>2</sub> expression on HT-29 cells are issued from our previous work.<sup>24</sup> Adapted with permission from Fanelli et al. *Bioconjugate Chemistry* 2020, 31, 10, 2339–2349. Copyright 2020 American Chemical Society.



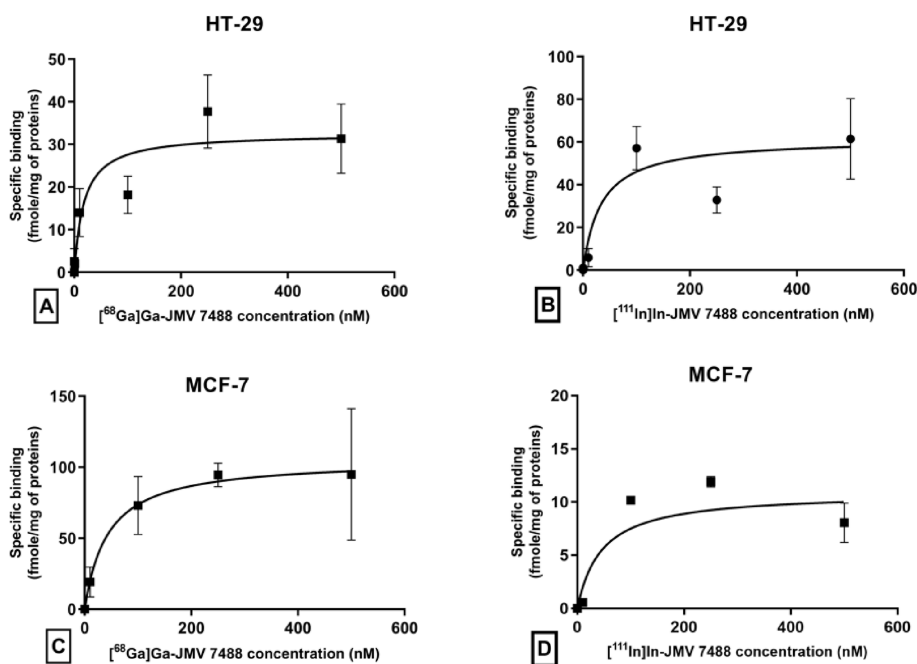
**Figure 5.** Confocal imaging of neurotensin receptors NTS<sub>1</sub> and NTS<sub>2</sub> on MCF-7 cells using indirect immunofluorescence. Images were obtained at 63 $\times$  magnification. (NTS<sub>1</sub>) intracytoplasmic granular expression of the NTS<sub>1</sub> receptor in MCF-7 cells. (NTS<sub>2</sub>) Membrane expression of the NTS<sub>2</sub> receptor in MCF-7 cells. (DAPI) Nucleus staining of alive MCF-7 cells using DAPI. (merged) Combination of NTS<sub>1</sub>, NTS<sub>2</sub>, and DAPI signal in MCF-7 cells.

corrected) and  $63.75 \pm 12.42\%$  ( $^{111}\text{In}$ , decay corrected), high volumetric activities of  $108.86 \pm 15.24$  ( $^{68}\text{Ga}$ ) and  $39.20$  MBq/mL ( $^{111}\text{In}$ ), and apparent molar activities of  $8.77 \pm 1.23$  ( $^{68}\text{Ga}$ ) and  $2.95$  GBq/ $\mu\text{mol}$  ( $^{111}\text{In}$ ). Radiochemical purities were always >95%. No degradation of the radiolabeled product was observed in the vehicle over 8 days (on radio-HPLC analysis using the  $^{111}\text{In}$  radiolabeled compound, Figure 2).

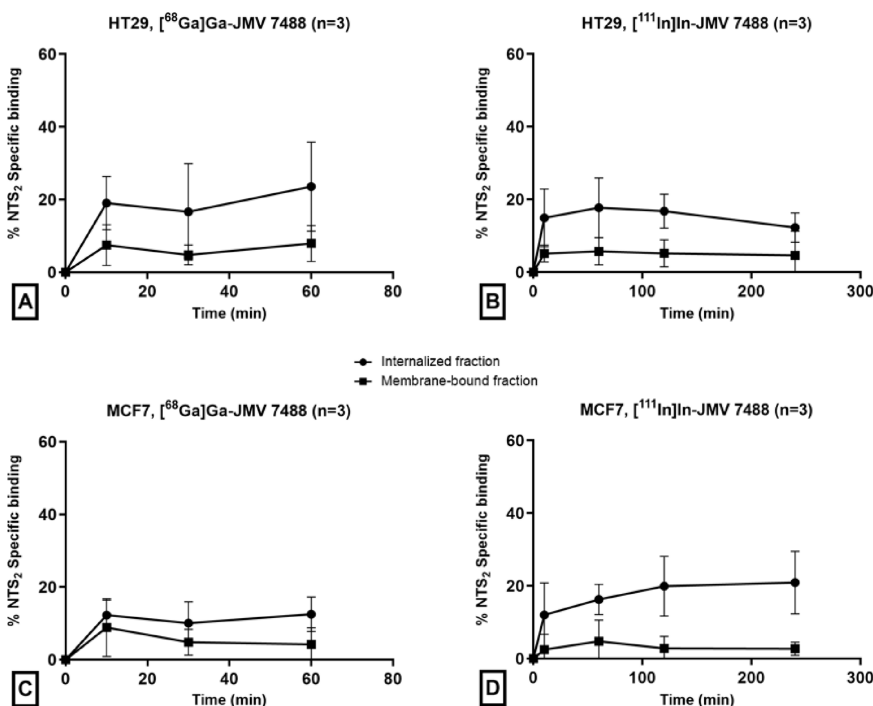
**Human Blood Plasma Stability.** Stability in human blood plasma was performed using [ $^{68}\text{Ga}$ ]Ga-JMV 7488. At 15 min,

23.5% of the peptide remained intact and this value decreased up to 5% after 30 min (Figure 3).

**In Vitro Studies.** NTS<sub>2</sub> expression has already been reported on HT-29 cells in previous studies.<sup>24</sup> To get further insight in the potential of NTS<sub>2</sub> in oncology, we performed western blot experiments on five breast cancer cell lines (MCF-7, MDA-MB-468, SKBR3, T47-D, and ZR75.1), on the prostate cancer cell line PC-3, and on the colorectal adenocarcinoma cell line HT-29. Semi-quantification of the



**Figure 6.** Saturation binding curves of [ $^{68}\text{Ga}$ ]Ga-JMV 7488 on HT-29 cells (A) and MCF-7 cells (C) and [ $^{111}\text{In}$ ]In-JMV 7488 on HT-29 cells (B) and MCF-7 cells (D) toward  $\text{NTS}_2$ . Blocking concentration of levocabastine =  $1 \mu\text{M}$ .



**Figure 7.**  $\text{NTS}_2$  mediated internalizations of [ $^{68}\text{Ga}$ ]Ga-JMV 7488 on HT-29 (A) and MCF-7 (C) cells and [ $^{111}\text{In}$ ]In-JMV 7488 on HT-29 (B) and MCF-7 (D) cells.

western blot showed that among the breast cancer cell line, the MCF-7 cells do express the highest level of  $\text{NTS}_2$ . HT-29 cells express the  $\text{NTS}_2$  receptor. Lastly, PC-3 cells express the highest amount of  $\text{NTS}_2$  among explored cancer cell lines (Figure 4).

Next, we performed confocal imaging on MCF-7 cells to assess the intracellular pattern of expression of  $\text{NTS}_2$ . As a validation step, we confirmed that MCF-7 cells express  $\text{NTS}_1$  and that staining was intracytoplasmic and granular. Interest-

ingly, MCF-7 cells express  $\text{NTS}_2$  and the staining was mostly membranous as depicted in Figure 5. Negative controls did not exhibit staining.

**Lipophilicity.**  $\text{LogD}_{7.4}$  values were  $-3.11 \pm 0.24$  for [ $^{68}\text{Ga}$ ]Ga-JMV 7488 and  $-2.67 \pm 0.18$  for [ $^{111}\text{In}$ ]In-JMV 7488. Values were statistically significant ( $p < 0.0001$ ).

**Affinity Studies.** Saturation binding studies performed on HT-29 and MCF-7 cells revealed that both [ $^{68}\text{Ga}$ ]Ga-JMV 7488 and [ $^{111}\text{In}$ ]In-JMV 7488 displayed a good affinity at

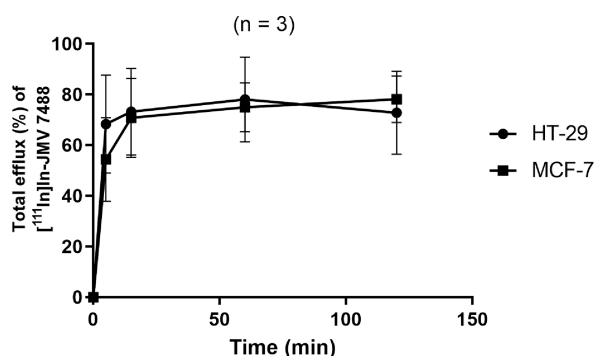
NTS<sub>2</sub> (Figure 6) and no specific binding at NTS<sub>1</sub> up to 0.5  $\mu$ M.  $K_D$  values obtained were  $37.83 \pm 17.20$  nM on HT-29 cells and  $36.37 \pm 9.89$  nM on MCF-7 cells for [<sup>68</sup>Ga]Ga-JMV 7488;  $36.39 \pm 4.02$  nM on HT-29 cells and  $45.94 \pm 0.60$  nM on MCF-7 cells for [<sup>111</sup>In]In-JMV 7488.  $B_{max}$  values at the NTS<sub>2</sub> receptor were  $30.30 \pm 18.46$  fmol/mg of proteins on HT-29 cells and  $50.15 \pm 40.36$  fmol/mg of proteins on MCF-7 cells (mean value obtained with [<sup>68</sup>Ga]Ga-JMV 7488 and [<sup>111</sup>In]In-JMV 7488).

**Cell-Associated Radioactivity and Internalization Studies.** After 1 h incubation at 37 °C, NTS<sub>2</sub>-mediated uptakes (internalized and membranous) were  $23.98 \pm 4.79\%$  of total binding for [<sup>68</sup>Ga]Ga-JMV 7488 on HT-29 cells and  $12.88 \pm 8.23\%$  on MCF-7, and for [<sup>111</sup>In]In-JMV 7488,  $19.72 \pm 9.72$  on HT-29 and  $25.18 \pm 11.73\%$  on MCF-7 cells.

Kinetic studies (Figure 7) indicated that the internalized fraction rapidly reached its maximum 10 min after incubation and that this value remained stable over the experiment timeline (60 min for <sup>68</sup>Ga, 240 min for <sup>111</sup>In). The membrane-bound fraction was always below 8% of total binding.

Specific internalization represented  $13.85 \pm 9.75\%$  of applied dose (AD) on HT-29 cells and  $13.79 \pm 4.78\%$  AD on MCF-7 cells at 60 min.

**Efflux Studies.** [<sup>111</sup>In]In-JMV 7488 demonstrated an intense efflux as early as 5 min ( $68.29 \pm 19.30\%$  on HT-29 cells and  $54.34 \pm 16.54\%$  on MCF-7 cells). This value increased up to  $78.06 \pm 16.64\%$  on HT-29 and  $74.97 \pm 9.56\%$  on MCF-7 cells at 60 min and remained stable until 120 min (Figure 8). In prevision of *in vivo* experiments, efflux studies

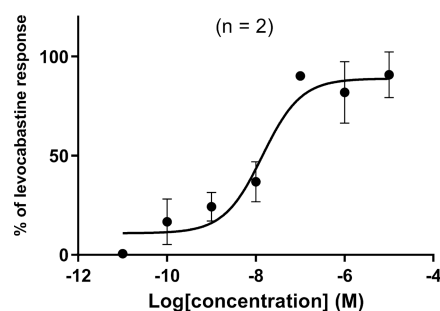


**Figure 8.** Total efflux of [<sup>111</sup>In]In-JMV 7488 on HT-29 and MCF-7 cells.

were also performed with the gallium-68 radiolabeled compound on HT-29 cells. A similar behavior was seen with a high efflux rate of  $45.28 \pm 6.85\%$  at 5 min, reaching  $66.06 \pm 8.05\%$  at 45 min (data not shown).

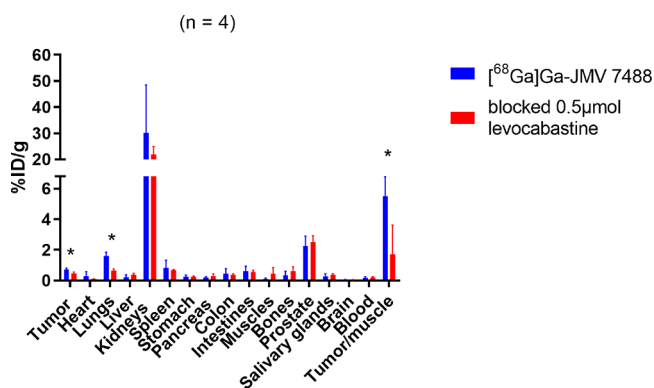
**Calcium Imaging Assay.** Concentration-dependent Ca<sup>2+</sup> mobilization was investigated with JMV 7488 and the NTS<sub>2</sub> agonist levocabastine as the positive control.  $EC_{50}$  values were 431 and 118 nM for JMV 7488 and levocabastine, respectively (Figure 9). JMV 7488 was able to promote the levocabastine-induced maximum dose–response ( $90.88 \pm 11.49\%$ ), meaning that JMV 7488 is a full agonist at the NTS<sub>2</sub>.

**In Vivo Studies. Biodistribution Study.** Four mice were injected with  $6.58 \pm 1.54$  MBq of [<sup>68</sup>Ga]Ga-JMV 7488 and sacrificed 90 min after injection. Four other mice were co-injected with levocabastine (LV) along with  $6.16 \pm 1.21$  MBq of [<sup>68</sup>Ga]Ga-JMV 7488 to demonstrate the specificity of the binding. [<sup>68</sup>Ga]Ga-JMV 7488 exhibited nonspecific uptake in



**Figure 9.** Intracellular mobilization of Ca<sup>2+</sup> induced by JMV 7488. Results are normalized from stimulation induced by levocabastine.

kidneys ( $30.1 \pm 18.4\%$  ID/g) and prostate ( $2.25 \pm 0.65\%$  ID/g). Interestingly, HT-29 tumor and lungs showed specific uptake of [<sup>68</sup>Ga]Ga-JMV 7488. The tumor/muscle ratio was significantly decreased when levocabastine was injected ( $5.5 \pm 1.3$  vs  $1.7 \pm 1.9$  %ID/g,  $p = 0.02$ ). Other organs did not show significant uptake (Figure 10 and Supporting Information Table S1).

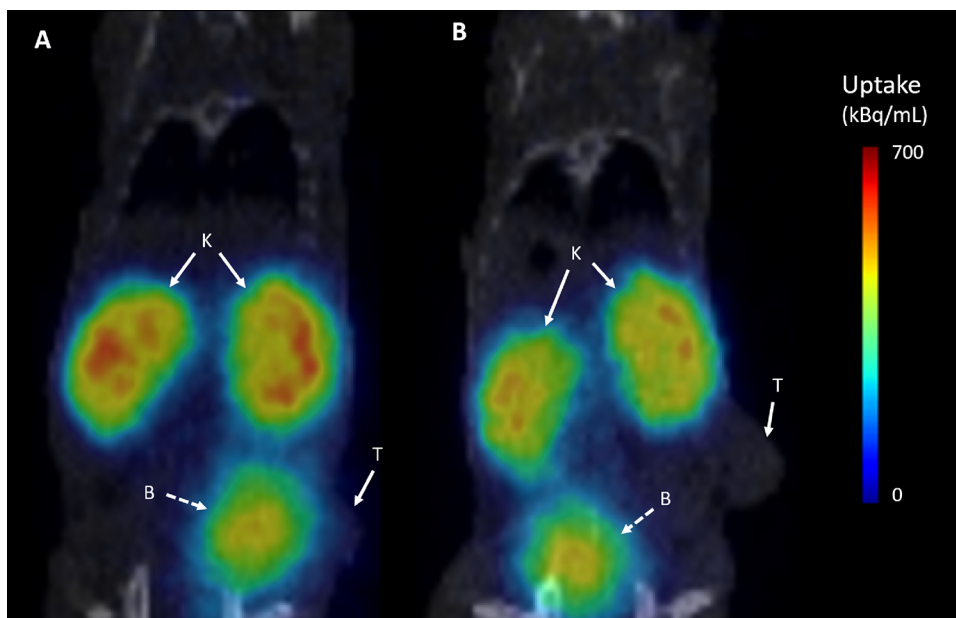


**Figure 10.** Biodistribution of [<sup>68</sup>Ga]Ga-JMV 7488 injected in HT-29 xenografted mice. \* indicates statistical differences ( $p < 0.05$ ).

**PET/CT Imaging.** Whole-body  $\mu$ PET/CT imaging was performed on nude mice bearing HT-29 xenograft injected with [<sup>68</sup>Ga]Ga-JMV 7488 without and with excess of levocabastine to assess NTS<sub>2</sub>-specificity *in vivo*. Elimination of the radiotracer was urinary-exclusive. Unfortunately, no evident uptake on HT-29 was depicted on the PET/CT images at any time point (Figure 11).

## DISCUSSION

Targeting neurotensin and its receptors holds promises in nuclear oncology.<sup>25</sup> The NTS<sub>1</sub> receptor has been shown to be over-expressed in several poor prognosis cancers such as pancreatic adenocarcinoma, triple negative breast cancer and glioblastoma among others.<sup>26–28</sup> However, little is known regarding the expression of the NTS<sub>2</sub> subtype in tumors and very few targeting biomolecules have been designed to target the NTS<sub>2</sub> subtype.<sup>18</sup> Thus, the aim of this work was to synthesize and characterize a new NTS<sub>2</sub>-targeted radiolabeled peptide. As preliminary steps, NTS<sub>2</sub> expression was assessed in a panel of breast cancer cell lines and on a prostate cancer cell line using western blot. Results showed that expression of NTS<sub>2</sub> is variable across breast cancer cell lines, its over-expression being noticed in less aggressive phenotypes represented by MCF-7 and T47D cells (luminal A phenotype).



**Figure 11.**  $\mu$ PET/CT imaging of  $[^{68}\text{Ga}]\text{Ga-JMV 7488}$  in HT-29 tumor-bearing nude mice. (A) Fused coronal PET/CT image at 75 min post-injection of a nude mice injected with 6 MBq of  $[^{68}\text{Ga}]\text{Ga-JMV 7488}$  alone. (B) Fused coronal PET/CT image at 90 min post-injection of a nude mice co-injected with 6 MBq of  $[^{68}\text{Ga}]\text{Ga-JMV 7488}$  and excess of levocabastine (0.5  $\mu\text{mole}$ ). K stands for kidneys, B is the bladder, and T is the HT-29 xenografted tumor.

Confocal imaging of NTS<sub>1</sub>/NTS<sub>2</sub> expression was then assessed on the MCF-7 cell line, and the staining was membranous (Figure 4), which confirmed the rapid recycling properties of NTS<sub>2</sub> to the cell membrane upon stimulation.<sup>29</sup> In prostate cancer, the PC-3 cell line express an even higher amount of NTS<sub>2</sub>. At last, we previously demonstrated that the colon cancer cell line HT-29 does express the NTS<sub>2</sub> subtype suggesting the potential of NTS<sub>2</sub> for imaging cancer types needing novel imaging strategies.<sup>24</sup> In this study, we were interested in developing a specific NTS<sub>2</sub>-radiolabeled peptide (i.e., JMV 7488). HT-29 and MCF-7 cells were chose as models for preclinical characterization because (1) HT-29 is the standard cell line to characterize NTS<sub>1</sub>-analogues, therefore allowing comparison, and (2) the MCF-7 cells, representing the luminal A breast cancer phenotype, need novel imaging agents to overpass intrinsic limitations of  $[^{18}\text{F}]\text{-FDG}$ .

Structurally, JMV 7488 is composed by a chelating agent (DOTA), two  $\beta$ -alanine ( $\beta\text{Ala}$ ) residues, and a modified NT sequence. While several chelating agents have been investigated,<sup>30</sup> DOTA is largely used for the development of radiopharmaceuticals.<sup>31</sup> The role of the linker between the chelating agents and the sequences responsible for the binding was widely discussed.<sup>32,33</sup> In JMV 7488, we inserted two  $\beta\text{Ala}$  residues, with the aim to distance DOTA and the NT sequence. Regarding the latter, we and others identified the residue at the position 11 as being crucial for selectivity.<sup>14,34–39</sup> Considering the double interest of silicon-containing amino acids that confer both resistance to enzyme degradation and lipophilicity,<sup>40</sup> we also demonstrated that the replacement of Leu at position 13 by a TMSAla enhanced the binding affinity of NT ligands for both NTS<sub>1</sub> and NTS<sub>2</sub> by improving the interactions with the hydrophobic ligand binding pocket of both receptors.<sup>41</sup> More recently, we developed a series of modified NT analogues, in which some of them showed a NTS<sub>1</sub>-mediated hypothermic effect.<sup>22</sup> Among all the analogues, the hexapeptide JMV 5504 (H-Lys-Lys-Pro-(D)Trp-

Ile-TMSAla-OH) showed the best selectivity toward hNTS<sub>2</sub>, with Ki values of 8.5 and 3600 nM toward hNTS<sub>2</sub> and hNTS<sub>1</sub>, respectively (selectivity index hNTS<sub>1</sub>/hNTS<sub>2</sub> = 423). Considering the poor NTS<sub>1</sub> binding affinity, no hypothermic effect for JMV 5504 was observed. In the light of our findings, the modified NT sequence of JMV 5504 was selected for the development of the first radiolabeled peptide targeting NTS<sub>2</sub> in peripheral tumors. The SPPS approach used for the synthesis of the desired product resulted to be really efficient, fast, and highly reproducible. After Fmoc-TMSAla-OH loading on 2-CTC resin, Fmoc-deprotections and couplings were carried out as we reported in detail in the Supporting Information. In this context, it has to be noted that the Kaiser test showed an uncomplete coupling reaction when Fmoc-Pro-OH, Fmoc-Lys-OH (for position 10), and DOTA(OtBu)<sub>3</sub>-OH were used. For these reasons, the three above-mentioned coupling reactions were performed in duplicate. The final cleavage resulted to be very efficient after 7 h in vigorous shaking; meanwhile, shorter times have proved to be insufficient for a complete deprotection. In particular, by-products bearing -OtBu protecting groups were observed. After the cleavage, the desired NT analogue JMV 7488 was purified and characterized.

We first looked at the physicochemical parameters of  $[^{68}\text{Ga}]\text{Ga-JMV 7488}$  and  $[^{111}\text{In}]\text{In-JMV 7488}$  using the octanol/PBS partition coefficient method. We found that both radiotracers exhibit a marked hydrophilicity at pH 7.4, consistent with previous neurotensin analogues. Blood plasma stability of  $[^{68}\text{Ga}]\text{Ga-JMV 7488}$  was tested on human plasma, and rapid cleavage was demonstrated (Figure 3). Strategies to improve the stability need to be carried out. They might include the use of enzyme inhibitors,<sup>42</sup> structural modification of the C-terminal hexapeptide binding sequence,<sup>43</sup> modification of the linker/chelate,<sup>30</sup> etc.

**Radiolabeled JMV 7488 Were Then Investigated *In Vitro* on HT-29 Cells and MCF-7 Cells.** Results from



saturation binding experiments showed that both radiolabeled bioconjugates exhibit a quite lower NTS<sub>2</sub> selectivity compared to the unconjugated counterpart JMV 5504.<sup>22</sup> Addition of the M<sup>3+</sup>-DOTA-(βAla)<sub>2</sub> moiety to the binding sequence results in the moderate loss of NTS<sub>2</sub> affinity (8.5 ± 2 nM [Ki] for JMV 5504 vs 38 ± 17 nM on HT-29 cells and 36 ± 10 nM on MCF-7 cells for [<sup>68</sup>Ga]Ga-JMV 7488; 36 ± 4 nM on HT-29 cells and 46 ± 1 nM on MCF-7 cells for [<sup>111</sup>In]In-JMV 7488). Interestingly, no NTS<sub>1</sub>-binding was seen for both radiotracers indicating good selectivity (concentrations higher than 0.5 μM were not tested). The total number of NTS<sub>2</sub> receptor on HT-29 cells obtained by saturation binding experiment was consistent with previously published data.<sup>44</sup> We acknowledge that, compared to the <sup>18</sup>F-labeled NTS<sub>2</sub> glycopeptide <sup>18</sup>F-1,<sup>18</sup> the NTS<sub>2</sub> affinities of our compounds were somewhat lower, but our radiolabeled peptides pave the way for the first time to NTS<sub>2</sub>-directed imaging and treatment thanks to the DOTA macrocycle suitable for radiolabeling with radiometals for PET and/or TRT. This is not achievable with fluorine chemistry. Two other lessons can be deduced from these experiments. First, NTS<sub>2</sub> binding is not sensitive to hydrophilicity as [<sup>111</sup>In]In-JMV 7488 was significantly less hydrophilic than [<sup>68</sup>Ga]Ga-JMV 7488 without impact on NTS<sub>2</sub>-binding. Second, HT-29 and MCF-7 cells provide same biological responses, indicating that breast cancer represents also an interesting model to study NTS<sub>2</sub>-directed radiotracers. We encourage further studies in this setting.

Next, we investigated the intracellular behavior of [<sup>68</sup>Ga]Ga-JMV 7488 in HT-29 cells and [<sup>111</sup>In]In-JMV 7488 on MCF-7 cells. The high internalization rate of 20.53 ± 6.50% for [<sup>68</sup>Ga]Ga-JMV 7488 is in line with its NTS<sub>2</sub> affinity. This value might appear low compared to the internalization capacity of NTS<sub>1</sub>, which is about 70%, but one should keep in mind that the internalization process is the combination of several mechanisms including the β-arrestin coupling of the receptor and the recycling properties of the receptor. In the specific case of the NTS<sub>2</sub> receptor, β-arrestin coupling reached 85% of the specific binding similarly to NTS<sub>1</sub> but NTS<sub>2</sub> is rapidly recycled to the membrane contrarily to NTS<sub>1</sub>, which is intracellularly retained.<sup>29</sup> Collectively, these data seem to indicate that our compounds promote high NTS<sub>2</sub>-internalization, meaning that they behave like agonists regarding the NTS<sub>2</sub>. The low membrane-bound fractions illustrate also well the supposed agonist behavior of [<sup>68</sup>Ga]Ga-JMV 7488 and [<sup>111</sup>In]In-JMV 7488 since agonists are internalized while antagonists usually do not. However, as the possibility for antagonists to undergo internalization has been highlighted,<sup>45</sup> we went deeper in its biological characterization by performing intracellular calcium mobilization experiments (Figure 9). As preliminary steps, we looked at the intracellular calcium mobilization of levocabastine as this point is still matter of debate<sup>46</sup> and was not explored in HT-29 cells. For the first time, we demonstrate that levocabastine is able to induce mobilization of intracellular calcium. Moreover, efficacy of JMV 7488 reaches the maximum intracellular calcium mobilization promoted by levocabastine, indicating that it is a full agonist at the NTS<sub>2</sub>. The lower EC<sub>50</sub> for JMV 7488 (411 vs 118 nM) reflects the lower NTS<sub>2</sub> affinity compared to levocabastine (this experiment was performed using the nonlabeled bioconjugate JMV 7488).

Efflux experiments were finally conducted, given the internalization of [<sup>68</sup>Ga]Ga-JMV 7488 and [<sup>111</sup>In]In-JMV 7488. Our results showed a high efflux, for both radiotracers

(Figure 8). Overall, this is the first detailed *in vitro* characterization of a NTS<sub>2</sub>-directed radiolabeled peptide for peripheral tumor imaging.

Because HT-29 tumors grow fast and do not require hormone supplementation and because of the possibility of quantification using PET, we choose to investigate [<sup>68</sup>Ga]Ga-JMV 7488 *in vivo* in nude mice bearing HT-29 xenograft with the <sup>68</sup>Ga-labeled compound. Specificity of the binding was confirmed by displacement of 44% of [<sup>68</sup>Ga]Ga-JMV 7488 signal by the NTS<sub>2</sub>-preferring agonist levocabastine (full displacement was not reached). In biodistribution experiments performed 90 min after injection, uptake of [<sup>68</sup>Ga]Ga-JMV 7488 in HT-29 tumor was still 0.72 ± 0.10%ID/g. Co-injection of levocabastine significantly decreased this value to 0.45 ± 0.10%ID/g, *p* = 0.03. In comparison with the <sup>18</sup>F-1 agent developed by Prante's lab,<sup>16</sup> our compound provided a higher uptake in tumor at a later time point (90 min vs 60 min), which is a significant improvement. As expected, given the hydrophilicity of the radiotracer, [<sup>68</sup>Ga]Ga-JMV 7488 was eliminated by the kidneys (Figure 11). Unfortunately, the uptake on HT-29 tumor was too low to build an image on the PET/CT presumably due to its low stability. The development of NTS<sub>2</sub>-specific radiotracers allows a better knowledge of the distribution of NTS<sub>2</sub>, and our study went deeper in this characterization compared to the literature. Our results provide for the first time evidence of an uptake [<sup>68</sup>Ga]Ga-JMV 7488 in the mice prostate (translation to humans must be done carefully in the same way as the prostate specific membrane antigen (PSMA), which is expressed in mouse kidneys but absent in human kidneys). Although this nonspecific mechanism remains to be explored in details, this finding represents a caveat for the use of orthotopic models of primary prostate cancer to investigate NTS<sub>2</sub>-targeting agents. Nevertheless, considering that in metastatic prostate cancer, tumor cells are disseminated outside the prostate bed, and that NTS<sub>2</sub> is expressed in PSMA-negative metastases (*i.e.*, PC-3 cells<sup>18</sup> and our data in this work), NTS<sub>2</sub>-directed theranostic is appealing in case of [<sup>177</sup>Lu]Lu-PSMA remains ineffective.<sup>47</sup> Moreover, NTS<sub>2</sub> is absent from salivary glands, which physiologically express the PSMA, therefore explaining xerostomia (partially) responsible for treatment discontinuation when using radiolabeled PSMA-inhibitors.<sup>48</sup> Moreover, the lungs showed moderate uptake of [<sup>68</sup>Ga]Ga-JMV 7488 in line with the known expression of NTS<sub>2</sub>.<sup>21</sup> Other organs do not retain significant uptake and therefore NTS<sub>2</sub> expression.

## CONCLUSIONS

Collectively, targeting the NTS<sub>2</sub> subtype appears highly promising in phenotypes lacking imaging/therapeutic procedures such as low-FDG avid breast cancer cells, PSMA-negative prostate cancer cells, and colorectal cancer cells. Subsequent studies will further explore the expression profile of NTS<sub>2</sub> in human tumors and healthy tissues.

[<sup>68</sup>Ga]Ga-JMV 7488 and [<sup>111</sup>In]In-JMV 7488 showed good affinity and high internalization rate at the NTS<sub>2</sub>. *In vivo*, [<sup>68</sup>Ga]Ga-JMV 7488 showed moderate uptake on HT-29 tumors. Radiolabeled JMV 7488 behaves as a promising tool to assess NTS<sub>2</sub> expression in periphery. Further improvements in tumoral uptake and stability remain needed.

## ■ ASSOCIATED CONTENT

### SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.2c07814>.

Synthesis, analytical characterization of the desired NT analogue JMV 7488, radiolabeling procedures, determination of the octanol/water partition coefficient, cell culture, confocal imaging, *ex vivo* stability methods, and *ex vivo* biodistribution (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Authors

**Florine Cavalier** – Institut des Biomolécules Max Mousseron, IBMM, UMR 5247, CNRS, Université de Montpellier, ENSCM, 34293 Montpellier, France; [orcid.org/0000-0001-5308-6416](https://orcid.org/0000-0001-5308-6416); Email: [florine.cavalier@umontpellier.fr](mailto:florine.cavalier@umontpellier.fr)

**Clément Morgat** – Department of Nuclear Medicine, University Hospital of Bordeaux, 33076 Bordeaux, France; University of Bordeaux, CNRS, EPHE, INCIA, UMR 5287, Bordeaux F-33000, France; [orcid.org/0000-0002-9432-9223](https://orcid.org/0000-0002-9432-9223); Email: [clement.morgat@u-bordeaux.fr](mailto:clement.morgat@u-bordeaux.fr), [clement.morgat@chu-bordeaux.fr](mailto:clement.morgat@chu-bordeaux.fr)

### Authors

**Sacha Bodin** – Department of Nuclear Medicine, University Hospital of Bordeaux, 33076 Bordeaux, France; University of Bordeaux, CNRS, EPHE, INCIA, UMR 5287, Bordeaux F-33000, France; [orcid.org/0000-0001-7331-8536](https://orcid.org/0000-0001-7331-8536)

**Santo Previt** – Institut des Biomolécules Max Mousseron, IBMM, UMR 5247, CNRS, Université de Montpellier, ENSCM, 34293 Montpellier, France

**Emmanuelle Jestin** – Cyclotron Réunion Océan Indien CYROI, 97490 Sainte Clotilde, France

**Delphine Vimont** – University of Bordeaux, CNRS, EPHE, INCIA, UMR 5287, Bordeaux F-33000, France

**Imade Ait-Arsa** – Cyclotron Réunion Océan Indien CYROI, 97490 Sainte Clotilde, France

**Frédéric Lamare** – Department of Nuclear Medicine, University Hospital of Bordeaux, 33076 Bordeaux, France; University of Bordeaux, CNRS, EPHE, INCIA, UMR 5287, Bordeaux F-33000, France

**Emmanuelle Rémond** – Institut des Biomolécules Max Mousseron, IBMM, UMR 5247, CNRS, Université de Montpellier, ENSCM, 34293 Montpellier, France; [orcid.org/0000-0002-3201-4365](https://orcid.org/0000-0002-3201-4365)

**Elif Hindié** – Department of Nuclear Medicine, University Hospital of Bordeaux, 33076 Bordeaux, France; University of Bordeaux, CNRS, EPHE, INCIA, UMR 5287, Bordeaux F-33000, France; Institut Universitaire de France, 75231 Paris, France

Complete contact information is available at:

<https://pubs.acs.org/doi/10.1021/acsomega.2c07814>

### Author Contributions

\*F.C. and C.M. contributed equally to directing this study. S.P. and E.R. synthesized and purified the JMV 7488 compound. S.B., D.V., and C.M. performed radiolabeling and *in vitro* experiments and analyzed data. I.A.A. and E.J. performed *in vivo* experiments. F.L. analyzed microPET data. E.H. critically reviewed the manuscript. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

### Funding

This work was partly funded by the Institut National du Cancer (INCa PLBIO 2017, THERACAN project) and by France Life Imaging (FLI). This study was conducted in the framework of the University of Bordeaux IdEx “Investments for the Future” program RRI “NewMOON” that received financial support from the French Government.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

INCa is acknowledged for postdoctoral fellowship to S.P. The calcium imaging experiments were done at the Phycell facility, Institut de Neurosciences Cognitives et Integratives d'Aquitaine.

## ■ ABBREVIATIONS

<sup>68</sup>Ga, gallium 68; <sup>111</sup>In, indium-111; FBS, fetal bovine serum; GPCR, G protein-coupled receptors; HPLC, high-performance liquid chromatography; PBS, phosphate buffered saline; NT, neurotensin; NTS<sub>1</sub>, NT receptor 1; NTS<sub>2</sub>, NT receptor 2; TFA, trifluoroacetic acid; TLC, thin layer chromatography; TMSAla, (L)-(trimethylsilyl)alanine.

## ■ REFERENCES

- (1) Davenport, A. P.; Scully, C. C. G.; de Graaf, C.; Brown, A. J. H.; Maguire, J. J. Advances in Therapeutic Peptides Targeting G Protein-Coupled Receptors. *Nat Rev Drug Discov* **2020**, *19*, 389–413.
- (2) Carraway, R.; Leeman, S. E. The Isolation of a New Hypotensive Peptide, Neurotensin, from Bovine Hypothalamus. *J. Biol. Chem.* **1973**, *248*, 6854–6861.
- (3) St-Gelais, F.; Jomphe, C.; Trudeau, L.-E. The Role of Neurotensin in Central Nervous System Pathophysiology: What Is the Evidence? *J. Psychiatry Neurosci.* **2006**, *31*, 229–245.
- (4) Vita, N.; Laurent, P.; Lefort, S.; Chalon, P.; Dumont, X.; Kaghad, M.; Gully, D.; Le Fur, G.; Ferrara, P.; Caput, D. Cloning and Expression of a Complementary DNA Encoding a High Affinity Human Neurotensin Receptor. *FEBS Lett.* **1993**, *317*, 139.
- (5) Schulz, S.; Röcken, C.; Ebert, M. P. A.; Schulz, S. Immunocytochemical Identification of Low-Affinity NTS<sub>2</sub> Neurotensin Receptors in Parietal Cells of Human Gastric Mucosa. *J. Endocrinol.* **2006**, *191*, 121–128.
- (6) Barroso, S.; Richard, F.; Nicolas-Ethève, D.; Reversat, J.-L.; Bernassau, J.-M.; Kitabgi, P.; Labbé-Jullié, C. Identification of Residues Involved in Neurotensin Binding and Modeling of the Agonist Binding Site in Neurotensin Receptor 1. *J. Biol. Chem.* **2000**, *275*, 328–336.
- (7) Gonzalez, S.; Dumitrascuta, M.; Eiselt, E.; Louis, S.; Kunze, L.; Blasiol, A.; Vivancos, M.; Previt, S.; Dewolf, E.; Martin, C.; Tourwé, D.; Cavalier, F.; Gendron, L.; Sarret, P.; Spetea, M.; Ballet, S. Optimized Opioid-Neurotensin Multitarget Peptides: From Design to Structure–Activity Relationship Studies. *J. Med. Chem.* **2020**, *63*, 12929–12941.
- (8) Buchegger, F.; Bonvin, F.; Kosinski, M.; Schaffland, A. O.; Prior, J.; Reubi, J. C.; Blauenstein, P.; Tourwé, D.; Garayoa, E. G.; Delaloye, A. B. Radiolabeled Neurotensin Analog, <sup>99m</sup>Tc-NT-XI, Evaluated in Ductal Pancreatic Adenocarcinoma Patients. *J. Nucl. Med.* **2003**, *44*, 1649–1654.
- (9) Maschauer, S.; Einsiedel, J.; Hübner, H.; Gmeiner, P.; Prante, O. <sup>18</sup>F- and <sup>68</sup>Ga-Labeled Neurotensin Peptides for PET Imaging of Neurotensin Receptor 1. *J. Med. Chem.* **2016**, *59*, 6480–6492.
- (10) Prignon, A.; Provost, C.; Alshoukr, F.; Wendum, D.; Couvelard, A.; Barbet, J.; Forgez, P.; Talbot, J.-N.; Gruaz-Guyon, A. Preclinical Evaluation of <sup>68</sup>Ga-DOTA-NT-20.3: A Promising PET Imaging Probe To Discriminate Human Pancreatic Ductal Adenocarcinoma from Pancreatitis. *Mol. Pharmaceutics* **2019**, *16*, 2776–2784.

- (11) Alshoukr, F.; Prignon, A.; Brans, L.; Jallane, A.; Mendes, S.; Talbot, J.-N.; Tourwé, D.; Barbet, J.; Gruaz-Guyon, A. Novel DOTA-Neurotensin Analogues for  $^{111}\text{In}$  Scintigraphy and  $^{68}\text{Ga}$  PET Imaging of Neurotensin Receptor-Positive Tumors. *Bioconjugate Chem.* **2011**, *22*, 1374–1385.
- (12) Deng, H.; Wang, H.; Zhang, H.; Wang, M.; Giglio, B.; Ma, X.; Jiang, G.; Yuan, H.; Wu, Z.; Li, Z. Imaging Neurotensin Receptor in Prostate Cancer With  $^{64}\text{Cu}$ -Labeled Neurotensin Analogs. *Mol Imaging* **2017**, *16*, 1536012117711369.
- (13) Held, C.; Plomer, M.; Hübner, H.; Meltretter, J.; Pischetsrieder, M.; Gmeiner, P. Development of a Metabolically Stable Neurotensin Receptor 2 (NTS<sub>2</sub>) Ligand. *ChemMedChem* **2013**, *8*, 75–81.
- (14) Eiselt, E.; Gonzalez, S.; Martin, C.; Chartier, M.; Betti, C.; Longpré, J.-M.; Cavelier, F.; Tourwé, D.; Gendron, L.; Ballet, S.; Sarret, P. Neurotensin Analogues Containing Cyclic Surrogates of Tyrosine at Position 11 Improve NTS<sub>2</sub> Selectivity Leading to Analgesia without Hypotension and Hypothermia. *ACS Chem. Neurosci.* **2019**, *10*, 4535–4544.
- (15) Besserer-Offroy, É.; Brouillette, R. L.; Lavenus, S.; Froehlich, U.; Brumwell, A.; Murza, A.; Longpré, J.-M.; Marsault, É.; Grandbois, M.; Sarret, P.; Leduc, R. The Signaling Signature of the Neurotensin Type 1 Receptor with Endogenous Ligands. *Eur. J. Pharmacol.* **2017**, *805*, 1–13.
- (16) Tétreault, P.; Besserer-Offroy, É.; Brouillette, R. L.; René, A.; Murza, A.; Fanelli, R.; Kirby, K.; Parent, A. J.; Dubuc, I.; Beaudet, N.; Côté, J.; Longpré, J.-M.; Martinez, J.; Cavelier, F.; Sarret, P. Pain Relief Devoid of Opioid Side Effects Following Central Action of a Silylated Neurotensin Analog. *Eur. J. Pharmacol.* **2020**, *882*, 173174.
- (17) Vivancos, M.; Fanelli, R.; Besserer-Offroy, É.; Beaulieu, S.; Chartier, M.; Resua-Rojas, M.; Mona, C. E.; Previti, S.; Rémond, E.; Longpré, J.-M.; Cavelier, F.; Sarret, P. Metabolically Stable Neurotensin Analogs Exert Potent and Long-Acting Analgesia without Hypothermia. *Behav Brain Res* **2021**, *405*, 113189.
- (18) Maschauer, S.; Greff, C.; Einsiedel, J.; Ott, J.; Tripal, P.; Hübner, H.; Gmeiner, P.; Prante, O. Improved Radiosynthesis and Preliminary in Vivo Evaluation of a  $^{18}\text{F}$ -Labeled Glycopeptide–Peptoid Hybrid for PET Imaging of Neurotensin Receptor 2. *Bioorg. Med. Chem.* **2015**, *23*, 4026–4033.
- (19) Swift, S. L.; Burns, J. E.; Maitland, N. J. Altered Expression of Neurotensin Receptors Is Associated with the Differentiation State of Prostate Cancer. *Cancer Res.* **2010**, *70*, 347–356.
- (20) Abbaci, A.; Talbot, H.; Saada, S.; Gachard, N.; Abraham, J.; Jaccard, A.; Bordessoule, D.; Fauchais, A. L.; Naves, T.; Jauberteau, M. O. Neurotensin Receptor Type 2 Protects B-Cell Chronic Lymphocytic Leukemia Cells from Apoptosis. *Oncogene* **2018**, *37*, 756–767.
- (21) Vita, N.; Oury-Donat, F.; Chalou, P.; Guillemot, M.; Kaghad, M.; Bachy, A.; Thurneyssen, O.; Garcia, S.; Poinot-Chazel, C.; Casellas, P.; Keane, P.; Le Fur, G.; Maffrand, J. P.; Soubrie, P.; Caput, D.; Ferrara, P. Neurotensin Is an Antagonist of the Human Neurotensin NT2 Receptor Expressed in Chinese Hamster Ovary Cells. *Eur. J. Pharmacol.* **1998**, *360*, 265–272.
- (22) Previti, S.; Vivancos, M.; Rémond, E.; Beaulieu, S.; Longpré, J.-M.; Ballet, S.; Sarret, P.; Cavelier, F. Insightful Backbone Modifications Preventing Proteolytic Degradation of Neurotensin Analogs Improve NTS<sub>1</sub>-Induced Protective Hypothermia. *Front. Chem.* **2020**, *8*, 406.
- (23) René, A.; Vanthuyne, N.; Martinez, J.; Cavelier, F. (L)-(Trimethylsilyl)Alanine Synthesis Exploiting Hydroxypinanone-Induced Diastereoselective Alkylation. *Amino Acids* **2013**, *45*, 301–307.
- (24) Fanelli, R.; Chastel, A.; Previti, S.; Hindié, E.; Vimont, D.; Zanotti-Fregonara, P.; Fernandez, P.; Garrigue, P.; Lamare, F.; Schollhammer, R.; Balasse, L.; Guillet, B.; Rémond, E.; Morgat, C.; Cavelier, F. Silicon-Containing Neurotensin Analogues as Radiopharmaceuticals for NTS<sub>1</sub>-Positive Tumors Imaging. *Bioconjugate Chem.* **2020**, *31*, 2339–2349.
- (25) Morgat, C.; Mishra, A. K.; Varshney, R.; Allard, M.; Fernandez, P.; Hindié, E. Targeting Neuropeptide Receptors for Cancer Imaging and Therapy: Perspectives with Bombesin, Neurotensin, and Neuropeptide-Y Receptors. *J. Nucl. Med.* **2014**, *55*, 1650–1657.
- (26) Morgat, C.; Brouste, V.; Chastel, A.; Vélasco, V.; Macrogrogan, G.; Hindié, E. Expression of Neurotensin Receptor-1 (NTS<sub>1</sub>) in Primary Breast Tumors, Cellular Distribution, and Association with Clinical and Biological Factors. *Breast Cancer Res. Treat.* **2021**, *190*, 403–413.
- (27) Körner, M.; Waser, B.; Strobel, O.; Büchler, M.; Reubi, J. C. Neurotensin Receptors in Pancreatic Ductal Carcinomas. *EJNMMI Res.* **2015**, *5*, DOI: 10.1186/s13550-015-0094-2.
- (28) Ouyang, Q.; Chen, G.; Zhou, J.; Li, L.; Dong, Z.; Yang, R.; Xu, L.; Cui, H.; Xu, M.; Yi, L. Neurotensin Signaling Stimulates Glioblastoma Cell Proliferation by Upregulating C-Myc and Inhibiting MiR-29b-1 and MiR-129-3p. *Neuro Oncol* **2016**, *18*, 216–226.
- (29) Martin, S.; Vincent, J.-P.; Mazella, J. Recycling Ability of the Mouse and the Human Neurotensin Type 2 Receptors Depends on a Single Tyrosine Residue. *J. Cell Sci.* **2002**, *115*, 165–173.
- (30) Renard, E.; Moreau, M.; Bellaye, P.-S.; Guillemain, M.; Collin, B.; Prignon, A.; Denat, F.; Goncalves, V. Positron Emission Tomography Imaging of Neurotensin Receptor-Positive Tumors with  $^{68}\text{Ga}$ -Labeled Antagonists: The Chelate Makes the Difference Again. *J. Med. Chem.* **2021**, *64*, 8564–8578.
- (31) Baranyai, Z.; Tircsó, G.; Rösch, F. The Use of the Macrocyclic Chelator DOTA in Radiochemical Separations. *Eur. J. Inorg. Chem.* **2020**, *2020*, 36–56.
- (32) Kim, Y.-S.; Yang, C.-T.; Wang, J.; Wang, L.; Li, Z.-B.; Chen, X.; Liu, S. Effects of Targeting Moiety, Linker, Bifunctional Chelator, and Molecular Charge on Biological Properties of  $^{64}\text{Cu}$ -Labeled Triphenylphosphonium Cations. *J. Med. Chem.* **2008**, *51*, 2971–2984.
- (33) Tolmachev, V.; Feldwisch, J.; Lindborg, M.; Baastrup, B.; Sandström, M.; Orlova, A. Influence of an Aliphatic Linker between DOTA and Synthetic Z(HER2:342) Affibody Molecule on Targeting Properties of the  $^{111}\text{In}$ -Labeled Conjugate. *Nucl. Med. Biol.* **2011**, *38*, 697–706.
- (34) Hapău, D.; Rémond, E.; Fanelli, R.; Vivancos, M.; René, A.; Côté, J.; Besserer-Offroy, É.; Longpré, J.-M.; Martinez, J.; Zaharia, V.; Sarret, P.; Cavelier, F. Stereoselective Synthesis of  $\beta$ -(5-Arylthiazolyl)  $\alpha$ -Amino Acids and Use in Neurotensin Analogues. *Eur. J. Org. Chem.* **2016**, *2016*, 1017–1024.
- (35) Einsiedel, J.; Held, C.; Hervet, M.; Plomer, M.; Tschammer, N.; Hübner, H.; Gmeiner, P. Discovery of Highly Potent and Neurotensin Receptor 2 Selective Neurotensin Mimetics. *J. Med. Chem.* **2011**, *54*, 2915–2923.
- (36) Boules, M.; Johnston, H.; Tozy, J.; Smith, K.; Li, Z.; Richelson, E. Analgesic Synergy of Neurotensin Receptor Subtype 2 Agonist NT79 and Morphine. *Behav. Pharmacol.* **2011**, *22*, 573–581.
- (37) Boules, M.; Shaw, A.; Liang, Y.; Barbut, D.; Richelson, E. NT69L, a Novel Analgesic, Shows Synergy with Morphine. *Brain Res.* **2009**, *1294*, 22–28.
- (38) Fanelli, R.; Floquet, N.; Besserer-Offroy, É.; Delort, B.; Vivancos, M.; Longpré, J.-M.; Renault, P.; Martinez, J.; Sarret, P.; Cavelier, F. Use of Molecular Modeling to Design Selective NTS<sub>2</sub> Neurotensin Analogues. *J. Med. Chem.* **2017**, *60*, 3303–3313.
- (39) Previti, S.; Desgagné, M.; Tourwé, D.; Cavelier, F.; Sarret, P.; Ballet, S. Opening the Amino Acid Toolbox for Peptide-Based NTS<sub>2</sub>-Selective Ligands as Promising Lead Compounds for Pain Management. *J. Pept. Sci.*, e3471, DOI: 10.1002/psc.3471.
- (40) Rémond, E.; Martin, C.; Martinez, J.; Cavelier, F. Silicon-Containing Amino Acids: Synthetic Aspects, Conformational Studies, and Applications to Bioactive Peptides. *Chem. Rev.* **2016**, *116*, 11654–11684.
- (41) Fanelli, R.; Besserer-Offroy, É.; René, A.; Côté, J.; Tétreault, P.; Collette-Tremblay, J.; Longpré, J.-M.; Leduc, R.; Martinez, J.; Sarret, P.; Cavelier, F. Synthesis and Characterization in Vitro and in Vivo of (L)-(Trimethylsilyl)Alanine Containing Neurotensin Analogues. *J. Med. Chem.* **2015**, *58*, 7785–7795.
- (42) Kanellopoulos, P.; Nock, B. A.; Krenning, E. P.; Maina, T. Optimizing the Profile of [ $^{99\text{m}}\text{Tc}$ ]Tc-NT(7-13) Tracers in Pancreatic

Cancer Models by Means of Protease Inhibitors. *Int. J. Mol. Sci.* **2020**, *21*, 7926.

(43) Magafa, V.; Matsoukas, M.-T.; Karageorgos, V.; Dermitzaki, E.; Exarchakou, R.; Stylos, E. K.; Pardalos, M.; Margjoris, A. N.; Varvounis, G.; Tzakos, A. G.; Spyroulias, G. A.; Liapakis, G. Novel Stable Analogues of the Neurotensin C-Terminal Hexapeptide Containing Unnatural Amino Acids. *Amino Acids* **2019**, *51*, 1009–1022.

(44) Maoret, J. J.; Pospai, D.; Rouyer-Fessard, C.; Couvineau, A.; Labois, C.; Voisin, T.; Laburthe, M. Neurotensin Receptor and Its MRNA Are Expressed in Many Human Colon Cancer Cell Lines but Not in Normal Colonic Epithelium: Binding Studies and RT-PCR Experiments. *Biochem. Biophys. Res. Commun.* **1994**, *203*, 465–471.

(45) Pheng, L. H.; Dumont, Y.; Fournier, A.; Chabot, J.-G.; Beaudet, A.; Quirion, R. Agonist- and Antagonist-Induced Sequestration/Internalization of Neuropeptide Y Y<sub>1</sub> Receptors in HEK293 Cells. *Br. J. Pharmacol.* **2003**, *139*, 695–704.

(46) Mazella, J.; Vincent, J.-P. Internalization and Recycling Properties of Neurotensin Receptors. *Peptides* **2006**, *27*, 2488–2492.

(47) Hofman, M. S.; Emmett, L.; Sandhu, S.; Iravani, A.; Joshua, A. M.; Goh, J. C.; Pattison, D. A.; Tan, T. H.; Kirkwood, I. D.; Ng, S.; Francis, R. J.; Gedy, C.; Rutherford, N. K.; Weickhardt, A.; Scott, A. M.; Lee, S.-T.; Kwan, E. M.; Azad, A. A.; Ramdave, S.; Redfern, A. D.; Macdonald, W.; Guminski, A.; Hsiao, E.; Chua, W.; Lin, P.; Zhang, A. Y.; McJannet, M. M.; Stockler, M. R.; Violet, J. A.; Williams, S. G.; Martin, A. J.; Davis, I. D.; TheraP Trial Investigators and the Australian and New Zealand Urogenital and Prostate Cancer Trials Group. TheraP Trial Investigators and the Australian and New Zealand Urogenital and Prostate Cancer Trials Group. [<sup>177</sup>Lu]Lu-PSMA-617 versus Cabazitaxel in Patients with Metastatic Castration-Resistant Prostate Cancer (TheraP): A Randomised, Open-Label, Phase 2 Trial. *Lancet* **2021**, *397*, 797–804.

(48) Feuerecker, B.; Tauber, R.; Knorr, K.; Heck, M.; Beheshti, A.; Seidl, C.; Bruchertseifer, F.; Pickhard, A.; Gafita, A.; Kratochwil, C.; Retz, M.; Gschwend, J. E.; Weber, W. A.; D'Alessandria, C.; Morgenstern, A.; Eiber, M. Activity and Adverse Events of Actinium-225-PSMA-617 in Advanced Metastatic Castration-Resistant Prostate Cancer After Failure of Lutetium-177-PSMA. *Eur. Urol.* **2021**, *79*, 343–350.

## Recommended by ACS

### Design and Development of Novel Glitazones for Activation of PGC-1 $\alpha$ Signaling Via PPAR- $\gamma$ Agonism: A Promising Therapeutic Approach against Parkinson's Disease

Prabitha Prabhakaran, Prashantha Kumar BR, *et al.*

FEBRUARY 10, 2023  
ACS OMEGA

READ 

### Screening Multidrug Resistance Reversal Agents in Traditional Chinese Medicines by Efflux Kinetics of D-Luciferin in MCF-7/DOX<sup>Fluc</sup> Cells

Yue Zhao, Yang Xiong, *et al.*

JANUARY 23, 2023  
ACS OMEGA

READ 

### Acylpyrazoline-Based Third-Generation Selective Antichlamydial Compounds with Enhanced Potency

Bin Lu, Huizhou Fan, *et al.*

FEBRUARY 08, 2023  
ACS OMEGA

READ 

### Vanillin-Based Indolin-2-one Derivative Bearing a Pyridyl Moiety as a Promising Anti-Breast Cancer Agent via Anti-Estrogenic Activity

Onur Bender, Sirajudheen Anwar, *et al.*

FEBRUARY 08, 2023  
ACS OMEGA

READ 

Get More Suggestions >