Prototypic ^{18}F -Labeled Argininamide-Type Neuropeptide Y Y_1R Antagonists as Tracers for PET Imaging of Mammary Carcinoma

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S Supporting Information

ABSTRACT: The neuropeptide Y (NPY) Y_1 receptor (Y_1R) selective radioligand (R)- N^α -(2,2-diphenylacetyl)- N^ω -[4-(2-[^{18}F]-fluoropropanoylamino)butyl]aminocarbonyl- N -(4-hydroxybenzyl)-argininamide ([^{18}F]23), derived from the high-affinity Y_1R antagonist BIBP3226, was developed for imaging studies of Y_1R -positive tumors. Starting from the argininamide core bearing amine-functionalized spacer moieties, a series of fluoropropanoylated and fluorobenzoylated derivatives was synthesized and studied for Y_1R affinity. The fluoropropanoylated derivative 23 displayed high affinity ($K_i = 1.3$ nM) and selectivity toward Y_1R . Radiosynthesis was accomplished via ^{18}F -fluoropropanoylation, yielding [^{18}F]23 with excellent stability in mice; however, the biodistribution study revealed pronounced hepatobiliary clearance with high accumulation in the gall bladder (>100 %ID/g). Despite the unfavorable biodistribution, [^{18}F]23 was successfully used for imaging of Y_1R positive MCF-7 tumors in nude mice. Therefore, we suggest [^{18}F]23 as a lead for the design of PET ligands with optimized physicochemical properties resulting in more favorable biodistribution and higher Y_1R -dependent enrichment in mammary carcinoma.

KEYWORDS: Fluorine-18, antagonist, positron emission tomography, PET, neuropeptide Y, NPY, Y_1R

Neuropeptide Y (NPY), a 36 amino acid peptide, and NPY receptors are widely distributed in the central nervous system and in the periphery. NPY receptor mediated signaling is involved in the regulation and the modulation of numerous physiological processes. In humans, four NPY receptor subtypes, designated Y_1R , Y_2R , Y_4R , and Y_5R , are functionally expressed, all belonging to the superfamily of seven-transmembrane domain (G-protein coupled) receptors. Y_1 and Y_2 receptors were detected in a variety of human cancers and were, therefore, proposed as potential molecular targets for tumor diagnosis and treatment (theranostics).^{1–6} It was reported that 85% of mammary carcinoma are Y_1R positive, whereas Y_2R levels were found to be low.¹ More than two-thirds of breast cancers are classified as estrogen receptor-positive, and in several studies an estrogen-dependent Y_1R expression in breast cancer cells was described.^{7,8}

Positron emission tomography (PET) allows noninvasive in vivo imaging of receptors expressed on tumors with high sensitivity and quantification of receptor densities, making PET

a powerful imaging technique in diagnostic nuclear medicine, provided that appropriate radiotracers are available for in vivo use.⁹ As yet, efforts to develop radiolabeled Y_1R ligands for tumor diagnosis were based on peptidic agonists.^{3,4,10–15} However, diaminopyridine-type ^{18}F -labeled Y_1R antagonists, developed as brain-penetrant tracers for Y_1R imaging in rhesus monkey brain,^{16,17} could represent potential molecular tools for PET imaging of peripheral Y_1R -expressing tumors, too. Recently, in vivo imaging of mammary carcinoma using an ^{18}F -labeled NPY peptide analogue in MCF-7 tumor-bearing mice,¹⁸ and whole body scintimammography using a Y_1R selective $^{99\text{m}}\text{Tc}$ -labeled peptide in humans¹³ were reported. In general, the use of nonpeptidic receptor ligands (antagonists) for tumor imaging has

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been explored in a few cases only, such as imaging of neurotensin receptor-positive tumors by ^{18}F -labeled¹⁹ or ^{111}In -labeled radioligands of the diarylpyrazole type.²⁰

In this study we report on the exploration of ^{18}F -labeled PET tracers for the Y_1R derived from the argininamide BIBP3226 (**1a**, Figure 1, Table 1), a highly potent and selective Y_1R antagonist.²¹

Figure 1. Radiolabeled Y_1R ligands derived from argininamide-type antagonists such as **1c**.

Lately, the bioisosteric substitution of the guanidine group in the argininamide moiety by an acyl- or carbamoylguanidine group (e.g., compounds **1b**, **1c**, Figure 1) was shown to be an effective strategy to prepare highly potent and selective fluorescent ligands,^{22,23} bivalent ligands,^{24–26} and tritiated radioligands for the human Y_1R .^{27–29} Likewise, the concept of guanidine-/acylguanidine nonclassical bioisosterism has been successfully applied to Y_2R selective argininamides^{30,31} and arginine-containing peptidic GPCR ligands.³²

In the present study we demonstrate that this strategy of bioisosterism could be adapted to the design of fluorinated Y_1R selective PET ligands. For this purpose the argininamide **1a** was 2-fluoropropanoylated or 4-fluorobenzoylated either directly at the guanidine entity (compounds **4** and **7**) or at an amine-functionalized spacer attached to the guanidine group (**17–26**, **28**, **32–36**) (Scheme 1). One of the early compounds was N^{ω} -2-fluoropropionylated **1a** (**4**), a congener of the high-affinity Y_1R radioligand [^3H]**1b**.²⁷ In contrast to the latter,^{27,28} compound **4** rapidly decomposed in buffer at pH 7.4 (cf. Supporting Information) and is therefore inappropriate as a radiotracer. Whereas argininamide **4** was synthesized through acylation of compound **2**²⁷ using 4-nitrophenyl 2-fluoropropanoate (**3**), the 4-fluorobenzoylated analogue **7** was obtained via activation of precursor **5** by treatment with trifluoromethanesulfonic acid anhydride and subsequent reaction with the reported amine **6** (Scheme 1).²⁷ The majority of the series of potential PET ligands (compounds **17–26** and **28**) were prepared through convenient acylation of the amine-functionalized precursor molecules **8–15**^{23,26} and **27** with active ester **3** or **16**, affording the products **17–26** and **28** with an average yield of 68% (Scheme 1). Argininamides **33–36** were obtained through guanidinylation of amine **6** with *S*-methylisothiourea derived guanidinylation reagents (**29–32**), which contained the complete N^{ω} -substituent of the resulting argininamide (Scheme 1).

Compounds **38** and **41** represent potential PET ligands, which were inaccessible by 2-fluoropropionylation or 4-fluorobenzoylation. However, **38** was obtained through treatment of **2** with succinimidyl ester **37** and subsequent deprotection (Scheme 1). The triazole derivative **41** was prepared from the alkyne functionalized precursor **39** and 2-deoxy-2-fluoro- β -D-glucosyl azide (**40**)³³ by Cu(I)-promoted “click” reaction.

The Y_1R affinities of the series of argininamides were determined by displacement of [^3H]**1b** on SK-N-MC neuro-

Table 1. Structures and Y_1R Affinities (K_i Values) of Parent Compound **1a**, Amine Precursors **8**, **14**, **15**, Alkyne **39**, and Fluorinated Argininamides **4**, **7**, **17–26**, **28**, **33–36**, **38**, and **41**; Y_1R Antagonism (K_b Values) of **1a** and Fluorinated Argininamides with a K_i below 100 nM; clogP Values of the Potential PET Ligands **4**, **7**, **17–26**, **28**, **33–36**, **38**, and **41** (Calculated with ACD/Labs 12.0 Using the Depicted Guanidine Tautomeric Form)

2FP = 2-fluoropropanoyl
4FB = 4-fluorobenzoyl

No.	R	K_i [nM] ^a	K_b [nM] ^b	clogP
1a	H	1.3 ± 0.2 ^c	1.5 ± 0.1 ^c	n.d.
1b	2FP	1.0 ± 0.2 ^d	0.25 ± 0.07	n.d.
1c	4FB	1.2 ± 0.2 ^e	0.59 ± 0.03	n.d.
4	2FP	n.d.	n.d.	3.6 ± 0.7
7	4FB	3,100 ± 80	n.d.	5.1 ± 0.7
8	A = -CH ₂ -	19 ± 5 ^c	n.d.	n.d.
14	A = -NH-	4.3 ± 0.2 ^c	7.3 ± 0.95 ^c	n.d.
15		7.3 ± 1 ^f	n.d.	n.d.
17		25 ± 4	8.2 ± 1	3.9 ± 0.7
18		15 ± 1	79 ± 8	2.8 ± 0.8
19		37 ± 10	140 ± 50	4.2 ± 0.9
20		51 ± 11	30 ± 1	5.4 ± 0.9
21		24 ± 2	35 ± 4	4.5 ± 0.9
22		64 ± 3	34 ± 2	4.1 ± 0.8
23	X = 2FP	1.3 ± 0.4	0.7 ± 0.02	3.4 ± 0.7
24	X = 4FB	7.2 ± 2	0.5 ± 0.01	4.8 ± 0.7
25	X = 2FP	19 ± 4	19 ± 3	2.6 ± 0.8
26	X = 4FB	16 ± 5	3.5 ± 0.2	4.4 ± 0.9
28		51 ± 5	22 ± 0.04	4.3 ± 0.9
33		59 ± 5	4.1 ± 0.2	5.2 ± 0.7
34		34 ± 3	30 ± 3	5.7 ± 0.7
35		1,200 ± 80	n.d.	4.8 ± 0.7
36		280 ± 30	n.d.	4.9 ± 0.8
38		30 ± 5	7.1 ± 0.4	5.7 ± 0.7
39		1.3 ± 0.6	n.d.	n.d.
41		2,000 ± 180	n.d.	2.5 ± 0.9 ^g

^aDissociation constant determined from the displacement of [^3H]**1b** (K_d = 1.2 nM, c = 1.5 nM) on SK-N-MC cells; mean values ± SEM from at least two independent experiments each performed in triplicate. ^bInhibition of 10 nM pNPY induced [Ca^{2+}]_i mobilization in HEL cells; mean values ± SEM from at least two independent experiments. ^cReported by Keller et al. 2011.²³ ^dReported by Keller et al. 2008.²⁷ ^eReported by Keller et al. 2011.²⁸ ^fReported by Keller et al. 2013.²⁶ ^gclogP value was calculated for the drawn structure.

Scheme 1. Synthesis of the Fluorinated Argininamides 4, 7, 17–26, 28, 33–36, 38, and 41^a

^aReagents: (a) (1) triethylamine, acetonitrile; (2) TFA, acetonitrile, 84%; (b) (1) trifluoromethanesulfonic anhydride, triethylamine, CH₂Cl₂; (2) amine 6, CH₂Cl₂, 40%; (c) triethylamine, acetonitrile or DMF, 48–91%; (d) (1) triethylamine, CH₂Cl₂; (2) TFA, CH₂Cl₂, 50%; (e) (1) HgCl₂, triethylamine, DMF; (2) TFA, CH₂Cl₂, 54–85%; (f) (1) triethylamine, CH₂Cl₂; (2) TFA, CH₂Cl₂, 71%; (h) CuSO₄·5H₂O, sodium ascorbate, EtOH/H₂O 1:1, 37%.

blastoma cells (Table 1). Most of the derivatives of 1a proved to be high-affinity Y₁R ligands with K_i values between 1 and 65 nM. For the potential PET ligands with K_i values <100 nM (compounds 17–26, 28, 33, 34, 38) Y₁R antagonism was proven in a spectrofluorimetric Fura-2 Ca²⁺ assay on human erythroleukemia (HEL) cells³⁴ (Table 1). As described recently, acylation of amine precursor 14, which contains a carbamoylguanidine moiety, with tritiated propionic acid afforded a Y₁R

radioligand ([³H]1c, K_d = 2.0 nM).²⁸ Likewise, acylation of 14 with 2-fluoropropionic acid gave 23, the compound with the highest Y₁R affinity (K_i = 1.3 nM) among the fluorinated ligands.

Acylation of the guanidine group in 1a with 4-fluorobenzoic acid, affording 7, and the introduction of N^ω-alkoxycarbonyl substituents (35, 36) resulted in compounds with considerably decreased Y₁R affinity (Table 1). Even though alkyne derivative 39 showed high Y₁R binding (K_i = 1.3 nM), the glucosylated derivative 41 exhibited significantly decreased Y₁R affinity (K_i = 2.0 μM). Therefore, the synthesis of such fluoroglucosylated Y₁R ligands as potential PET tracers was discontinued.

In addition to Y₁R binding constants determined on SK-N-MC neuroblastoma cells, the Y₁R affinity of compounds 18, 23, and 24 was investigated at MCF-7-Y₁²⁸ breast cancer cells. The K_i values amounted to 31 ± 11, 3.3 ± 0.1, and 6.8 ± 0.5 nM, respectively, and were in good agreement with the K_i values provided in Table 1 (SK-N-MC cells). The Y₁R selectivity was confirmed for argininamides 23 and 24 in flow cytometric binding studies using cyanine labeled peptides (Cy5-pNPY, Dy-635-pNPY, Cy5-[K⁴]hPP) and cells transfected with the human Y₂, Y₄, and Y₅ receptor (Table S1).

According to the in vitro Y₁R binding data, the 2-fluoropropionylated derivative 23 exhibited the most promising Y₁R affinity among the potential PET ligands under study. In addition to receptor binding characteristics, the hydrophilicity of a PET tracer for imaging of peripheral tumors is also a critical issue with regard to the ratio between compound accumulation in the tissue of interest and elimination/excretion. The acyl- or carbamoylguanidine entity of the herein reported argininamide-type Y₁R ligands exhibit a considerably lower basicity (pK_a ≈ 7–8) than alkylguanidines (pK_a ≈ 12–13), though high enough to account for a partial protonation of the compounds at pH 7.4 and thus sufficient water solubility (clogD(pH 7.4) < clogP). Nevertheless, the 4-fluorobenzoylated derivatives showed clogP values as high as 4.1–5.7 (Table 1), predicting unfavorable biodistribution due to high lipophilicity. The most promising derivative 23 demonstrated lower lipophilicity with a clogP value of 3.4 when compared to the series of compounds under study (Table 1).

In a first approach toward a suitable PET ligand for Y₁R, [¹⁸F]17 was prepared via acylation of amine precursor 8 using 4-nitrophenyl-2-[¹⁸F]fluoropropionate ([¹⁸F]NPFP, [¹⁸F]3)³⁵ as shown in Scheme 2, and the biodistribution was studied in nude

Scheme 2. Synthesis of the PET ligands [¹⁸F]17 and [¹⁸F]23^a

for R¹ cf. Scheme 1

^aReagents and conditions: [¹⁸F]17: 1.22 μmol of 8, triethylamine, acetonitrile/DMSO 10:1, 50 °C, 15 min, RCY = 60%. [¹⁸F]23: 1.47 μmol of 14, triethylamine, acetonitrile/DMSO 11:1, 50 °C, 5 min, RCY = 94%.

mice bearing subcutaneous SK-N-MC xenografts (Figure 2A). The radiosynthesis afforded [¹⁸F]17 in radiochemical yield (RCY) of 60% for the final reaction step, when the preliminary biodistribution study showed high uptake of [¹⁸F]17 in the gall bladder and only marginal uptake by the tumor (Figure 2A). Taking into account the highly laborious radiosynthesis of [¹⁸F]3,³⁵ together with difficulties in the detection of the SK-N-

Figure 2. Biodistribution data of [^{18}F]17 (A) and [^{18}F]23 (B) in SK-N-MC or MCF-7 tumor-bearing mice. Each bar represents the mean $\pm$ SEM of 3–4 mice (except for SK-N-MC tumor, $n = 2$).

MC tumors with [^{18}F]17 in PET imaging experiments (results not shown), we envisaged an improved PET ligand and imaging of Y_1R -positive breast cancer. The in vitro data, presented in Tables 1 and S, prompted us to synthesize the ^{18}F -labeled form of 23, which has a 20-fold higher Y_1R affinity compared to compound 17 (cf. Table 1). Moreover, the in vivo studies were performed with nude mice bearing MCF-7 tumors, for which we proved the expression of Y_1R by immunofluorescence analysis (Figure S4, Supporting Information), confirming our previous studies.^{8,27,28}

Apart from higher Y_1R affinity, 23 showed slightly higher hydrophilicity over 17 ($\text{clogP} = 3.4$ vs 3.9), and most importantly, 23 contains a carbamoylguanidine structure, which was recently reported to be highly stable at pH 7.4, contrary to the acylguanidine moiety of 17.^{23,28,32}

[^{18}F]23 was prepared by treatment of an excess of amine precursor 14 with [^{18}F]3 (Scheme 2). [^{18}F]3 was prepared following a previously established protocol,³⁵ with some important modifications to optimize the overall synthesis time and RCY (Supporting Information): The ^{18}F -fluorination of the 9'-anthrylmethyl-2-bromopropionate precursor was performed under mild reaction conditions in the presence of KH_2PO_4 ,³³ resulting in a reduced precursor concentration needed for radiolabeling (30 mM instead of 116 mM³⁵). This allowed us to omit the HPLC separation after the ^{18}F -fluorination step and to perform the subsequent reaction steps of the radiosynthesis of [^{18}F]3 by a simple one-pot procedure (Supporting Information). After isolation of [^{18}F]3 by semipreparative HPLC and conjugation to amine 14, the radioligand [^{18}F]23 was obtained in an overall RCY of 5–8% (uncorrected for decay) within an overall synthesis time of only 70–80 min and in a radiochemical purity of >99% and molar radioactivity of 12–21 GBq/ μmol ($n = 6$).

Because [^{18}F]23 revealed excellent in vitro Y_1R binding affinity and sufficient RCY from the optimized radiosynthesis, we proceeded with the evaluation of the radioligand in vitro and in

vivo using tumor-bearing nude mice with Y_1R -positive MCF-7 tumors. The experimental $\text{logD}_{7.4}$ determination of [^{18}F]23 revealed a value of 2.34 ± 0.03 , which differed from the calculated $\text{clogD}_{7.4}$ value of 3.2 (ACD/Laboratories), possibly due to an underestimation of the protonated fraction of the ligand at physiological pH. Stability experiments in vivo displayed excellent stability of [^{18}F]23, as no degradation products could be observed in the blood of mice at 90 min postinjection (p.i.) (Figure S2, Supporting Information).

Using MCF-7 tumor-bearing nude mice with a tumor mass of 80–240 mg, we studied whether [^{18}F]23 could be suitable for the visualization of Y_1R positive tumors. As shown in Figure 2B, [^{18}F]23 revealed fast blood clearance in nude mice bearing MCF-7 xenografts at 30 and 90 min p.i., low uptake in the liver, but exceptionally high uptake in the gall bladder (>100 %ID/g). The biodistribution of [^{18}F]23 was surprisingly similar to that of [^{18}F]17 (Figure 2A). In this context, it has to be noted that these data cannot be strictly compared due to the use of different animal models.

[^{18}F]23 showed marginally higher uptake in the intestines and lower uptake in the femur. Since decomposition of ^{18}F -fluoropropionylated radiotracers frequently leads to increased accumulation of [^{18}F]fluoride in the bones,^{36,37} the lower uptake of [^{18}F]23 in the femur could be ascribed to the higher stability of the carbamoylguanidine as compared to the acylguanidine structure of [^{18}F]17. The uptake of [^{18}F]23 in MCF-7 tumors was 0.51 ± 0.04 %ID/g at 30 min p.i., and the tracer demonstrated high tumor retention with an uptake of 0.43 ± 0.08 %ID/g at 90 min p.i. (Figure 2B). Consequently, the tumor/blood ratio was about 2, also at the later time point at 90 min p.i., suggesting a suitable signal-to-noise ratio for PET imaging. In comparison with the MCF-7 tumor uptake of the previously described ^{18}F -labeled NPY peptide analogue (0.7 %ID/g at 120 min p.i.),¹⁸ the tumor uptake of [^{18}F]23 (0.4 %ID/g at 90 min p.i.) is lower, as the biodistribution of [^{18}F]23 and its pronounced uptake in the gall bladder clearly hampers the suitability of [^{18}F]23 for PET imaging purposes. However, [^{18}F]23 revealed a significantly reduced kidney uptake by a factor of about 10 when compared with the peptide tracer, which is, as expected, the major advantage of the nonpeptide radioligand compared with a peptide ligand for PET imaging.

[^{18}F]23 was injected into MCF-7 xenografted nude mice for PET imaging studies and demonstrated displaceable and specific Y_1R -mediated tumor uptake (Figure 3A,B), regardless of its biodistribution with predominant accumulation in the gall bladder, intestines, and kidneys (Figure 3C). The in vivo specificity of [^{18}F]23 was proven by coinjection of 1a (40 μg /animal, bolus injection of 100 μL over a few seconds), demonstrating a significantly diminished tracer uptake in the tumors of coinjected animals (0.42 ± 0.13 %ID/g ($n = 5$, Figure 3A) vs 0.21 ± 0.07 %ID/g ($n = 4$, Figure 3B), $P < 0.05$, Mann–Whitney U test, two-tailed). In addition, to exclude that differences in tumor uptake could be a result of overall biodistribution changes when 1a is administered prior to tracer injection, we determined tumor-to-muscle ratios by PET image analysis, confirming significantly diminished tumor uptake of [^{18}F]23 by coinjection of 1a (3.1 ± 0.9 ($n = 5$, control) vs 1.8 ± 0.7 ($n = 4$, coinjection), $P < 0.05$, paired t test). These results confirmed the visualization of Y_1R -positive MCF-7 tumors by the antagonist [^{18}F]23 in vivo by PET.

In conclusion, we developed the antagonist radioligand [^{18}F]23 as the first nonpeptide ^{18}F -labeled tracer that has been evaluated for in vivo small animal PET imaging of Y_1R -positive

Figure 3. Representative coronal PET images of a MCF-7 tumor-bearing mouse injected with [^{18}F]23 alone (A) or with [^{18}F]23 and 1a (40 μg , B). The blue crosshair indicates the tumor. (C) PET image of the same mouse with adjusted scale bar showing the biodistribution of [^{18}F]23 in the plane of the intestines (left) and in the kidneys (right): b, bladder; g, gall bladder; i, intestines; k, kidneys; l, liver.

mammary carcinoma. The biodistribution of [^{18}F]23 is characterized by its hepatobiliary clearance with very slow clearance of the gall bladder. We suggest [^{18}F]23 as a lead for the design of improved PET ligands with more favorable biodistribution and higher Y_1R -dependent tumor uptake to enable PET imaging studies. Further preclinical studies of derivatives of [^{18}F]23 are currently underway.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.6b00467.

Experimental description of the synthesis and analytical data of compounds 3–5, 7, 16–26, 28–39, 41 and further intermediates. RP-HPLC chromatograms of compounds 4, 7, 17–26, 28, 33–36, 38, 39 and 41. Investigations on the chemical stability of 4. Pharmacology-related experimental protocols. Data on Y_1R selectivity of 23 and 24. Experimental protocols for the synthesis of the PET ligands [^{18}F]17 and [^{18}F]23, for animal studies, PET imaging, Western blot, and immunofluorescence analysis (PDF)

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Notes

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■ ABBREVIATIONS

brs, broad singlet; CH_2Cl_2 , dichloromethane; DIPEA, diisopropylethylamine; EDC·HCl, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride; EtOAc, ethyl acetate; HEL cells, human erythroleukemia cells; HOBt, 1-hydroxybenzotriazole; hPP, human pancreatic polypeptide; k, retention (or capacity) factor (HPLC); K_d , dissociation (or binding) constant obtained from a saturation binding experiment; K_i , dissociation (or binding) constant obtained from a competition binding experiment; $\log D_{7.4}$, logarithm of distribution coefficient (pH of the aqueous phase = 7.4); MeCN, acetonitrile; NPY, neuropeptide Y; p.i., postinjection; pNPY, porcine NPY; RCY, radiochemical yield; RP-HPLC, reversed-phase HPLC; SEM, standard error of the mean; TBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; Y_1R , NPY Y_1 receptor

■ REFERENCES

- (1) Reubi, J. C.; Gugger, M.; Waser, B.; Schaer, J. C. Y_1 -mediated effect of neuropeptide Y in cancer: breast carcinomas as targets. *Cancer Res.* **2001**, *61*, 4636–41.
- (2) Körner, M.; Reubi, J. C. NPY receptors in human cancer: a review of current knowledge. *Peptides* **2007**, *28*, 419–25.
- (3) Reubi, J. C.; Maecke, H. R. Peptide-based probes for cancer imaging. *J. Nucl. Med.* **2008**, *49*, 1735–8.
- (4) Zwanziger, D.; Beck-Sicking, A. G. Radiometal targeted tumor diagnosis and therapy with peptide hormones. *Curr. Pharm. Des.* **2008**, *14*, 2385–400.

- (5) Lu, C.; Everhart, L.; Tilan, J.; Kuo, L.; Sun, C. C. J.; Munivenkatappa, R. B.; Joensson-Rylander, A. C.; Sun, J.; Kuan-Celari, A.; Li, L.; Abe, K.; Zukowska, Z.; Toretzky, J. A.; Kitlinska, J. Neuropeptide Y and its Y2 receptor: potential targets in neuroblastoma therapy. *Oncogene* **2010**, *29*, 5630–5642.
- (6) Morgat, C.; Mishra, A. K.; Varshney, R.; Allard, M.; Fernandez, P.; Hindie, E. Targeting neuropeptide receptors for cancer imaging and therapy: perspectives with bombesin, neurotensin, and neuropeptide-Y receptors. *J. Nucl. Med.* **2014**, *55*, 1650–1657.
- (7) Amlal, H.; Farouqi, S.; Balasubramanian, A.; Sheriff, S. Estrogen up-regulates neuropeptide Y₁ receptor expression in a human breast cancer cell line. *Cancer Res.* **2006**, *66*, 3706–3714.
- (8) Memminger, M.; Keller, M.; Lopuch, M.; Pop, N.; Bernhardt, G.; von Angerer, E.; Buschauer, A. The neuropeptide Y Y₁ receptor: a diagnostic marker? Expression in MCF-7 breast cancer cells is down-regulated by antiestrogens in vitro and in xenografts. *PLoS One* **2012**, *7*, e51032.
- (9) Li, Z.; Conti, P. S. Radiopharmaceutical chemistry for positron emission tomography. *Adv. Drug Delivery Rev.* **2010**, *62*, 1031–1051.
- (10) Langer, M.; La Bella, R.; Garcia-Garayoa, E.; Beck-Sickinger, A. G. ^{99m}Tc-Labeled Neuropeptide Y Analogues as Potential Tumor Imaging Agents. *Bioconjugate Chem.* **2001**, *12*, 1028–1034.
- (11) Zwanziger, D.; Khan, I. U.; Neundorff, I.; Sieger, S.; Lehmann, L.; Friebe, M.; Dinkelborg, L.; Beck-Sickinger, A. G. Novel chemically modified analogues of neuropeptide Y for tumor targeting. *Bioconjugate Chem.* **2008**, *19*, 1430–8.
- (12) Khan, I. U.; Beck-Sickinger, A. G. Targeted tumor diagnosis and therapy with peptide hormones as radiopharmaceuticals. *Anti-Cancer Agents Med. Chem.* **2008**, *8*, 186–99.
- (13) Khan, I. U.; Zwanziger, D.; Bohme, I.; Javed, M.; Naseer, H.; Hyder, S. W.; Beck-Sickinger, A. G. Breast-cancer diagnosis by neuropeptide Y analogues: from synthesis to clinical application. *Angew. Chem., Int. Ed.* **2010**, *49*, 1155–8.
- (14) Guerin, B.; Dumulon-Perreault, V.; Tremblay, M.-C.; Ait-Mohand, S.; Fournier, P.; Dubuc, C.; Authier, S.; Benard, F. [Lys(DOTA)₄]BVD15, a novel and potent neuropeptide Y analog designed for Y₁ receptor-targeted breast tumor imaging. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 950–953.
- (15) Chatenet, D.; Cescato, R.; Waser, B.; Erchevgy, J.; Rivier, J. E.; Reubi, J. C. Novel dimeric DOTA-coupled peptidic Y₁-receptor antagonists for targeting of neuropeptide Y receptor-expressing cancers. *EJNMMI Res.* **2011**, *1*, 21.
- (16) Kameda, M.; Ando, M.; Nakama, C.; Kobayashi, K.; Kawamoto, H.; Ito, S.; Suzuki, T.; Tani, T.; Ozaki, S.; Tokita, S.; Sato, N. Synthesis and evaluation of a series of 2,4-diaminopyridine derivatives as potential positron emission tomography tracers for neuropeptide Y Y₁ receptors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5124–5127.
- (17) Hostetler, E. D.; Sanabria-Bohorquez, S.; Fan, H.; Zeng, Z.; Gantert, L.; Williams, M.; Miller, P.; O'Malley, S.; Kameda, M.; Ando, M.; Sato, N.; Ozaki, S.; Tokita, S.; Ohta, H.; Williams, D.; Sur, C.; Cook, J. J.; Burns, H. D.; Hargreaves, R. Synthesis, characterization, and monkey positron emission tomography (PET) studies of [¹⁸F]Y₁-973, a PET tracer for the neuropeptide Y Y₁ receptor. *NeuroImage* **2011**, *54*, 2635–2642.
- (18) Hofmann, S.; Maschauer, S.; Kuwert, T.; Beck-Sickinger, A. G.; Prante, O. Synthesis and in Vitro and in Vivo Evaluation of an ¹⁸F-Labeled Neuropeptide Y Analogue for Imaging of Breast Cancer by PET. *Mol. Pharmaceutics* **2015**, *12*, 1121–1130.
- (19) Lang, C.; Maschauer, S.; Hübner, H.; Gmeiner, P.; Prante, O. Synthesis and evaluation of a ¹⁸F-labeled diarylpyrazole glycoconjugate for the imaging of NTS1-positive tumors. *J. Med. Chem.* **2013**, *56*, 9361–9365.
- (20) Schulz, J.; Rohracker, M.; Stiebler, M.; Grosser, O. S.; Pethe, A.; Goldschmidt, J.; Osterkamp, F.; Reineke, U.; Smerling, C.; Amthauer, H. Comparative Evaluation of the Biodistribution Profiles of a Series of Nonpeptidic Neurotensin Receptor-1 Antagonists Reveals a Promising Candidate for Theranostic Applications. *J. Nucl. Med.* **2016**, *57*, 1120–3.
- (21) Rudolf, K.; Eberlein, W.; Engel, W.; Wieland, H. A.; Willim, K. D.; Entzeroth, M.; Wienen, W.; Beck-Sickinger, A. G.; Doods, H. N. The first highly potent and selective non-peptide neuropeptide Y Y₁ receptor antagonist: BIBP3226. *Eur. J. Pharmacol.* **1994**, *271*, R11–3.
- (22) Schneider, E.; Keller, M.; Brennauer, A.; Hoefelschweiger, B. K.; Gross, D.; Wolfbeis, O. S.; Bernhardt, G.; Buschauer, A. Synthesis and characterization of the first fluorescent nonpeptide NPY Y₁ receptor antagonist. *ChemBioChem* **2007**, *8*, 1981–8.
- (23) Keller, M.; Erdmann, D.; Pop, N.; Pluym, N.; Teng, S.; Bernhardt, G.; Buschauer, A. Red-fluorescent argininamide-type NPY Y₁ receptor antagonists as pharmacological tools. *Bioorg. Med. Chem.* **2011**, *19*, 2859–2878.
- (24) Weiss, S.; Keller, M.; Bernhardt, G.; Buschauer, A.; Koenig, B. Modular synthesis of non-peptidic bivalent NPY Y₁ receptor antagonists. *Bioorg. Med. Chem.* **2008**, *16*, 9858–9866.
- (25) Keller, M.; Teng, S.; Bernhardt, G.; Buschauer, A. Bivalent Argininamide-Type Neuropeptide Y Y₁ Antagonists Do Not Support the Hypothesis of Receptor Dimerization. *ChemMedChem* **2009**, *4*, 1733–1745.
- (26) Keller, M.; Kaske, M.; Holzammer, T.; Bernhardt, G.; Buschauer, A. Dimeric argininamide-type neuropeptide Y receptor antagonists: chiral discrimination between Y₁ and Y₄ receptors. *Bioorg. Med. Chem.* **2013**, *21*, 6303–6322.
- (27) Keller, M.; Pop, N.; Hutzler, C.; Beck-Sickinger, A. G.; Bernhardt, G.; Buschauer, A. Guanidine-Acylguanidine Bioisosteric Approach in the Design of Radioligands: Synthesis of a Tritium-Labeled N^G-Propionylargininamide ([³H]-UR-MK114) as a Highly Potent and Selective Neuropeptide Y Y₁ Receptor Antagonist. *J. Med. Chem.* **2008**, *51*, 8168–8172.
- (28) Keller, M.; Bernhardt, G.; Buschauer, A. [³H]UR-MK136: A Highly Potent and Selective Radioligand for Neuropeptide Y Y₁ Receptors. *ChemMedChem* **2011**, *6*, 1566–1571.
- (29) Keller, M.; Weiss, S.; Hutzler, C.; Kuhn, K. K.; Mollereau, C.; Dukorn, S.; Schindler, L.; Bernhardt, G.; Koenig, B.; Buschauer, A. N^ω-Carbamoylation of the argininamide moiety: an avenue to insurmountable NPY Y₁ receptor antagonists and a radiolabeled selective high-affinity molecular tool ([³H]UR-MK299) with extended residence time. *J. Med. Chem.* **2015**, *58*, 8834–8849.
- (30) Pluym, N.; Brennauer, A.; Keller, M.; Ziemek, R.; Pop, N.; Bernhardt, G.; Buschauer, A. Application of the Guanidine-Acylguanidine Bioisosteric Approach to Argininamide-Type NPY Y₂ Receptor Antagonists. *ChemMedChem* **2011**, *6*, 1727–1738.
- (31) Pluym, N.; Baumeister, P.; Keller, M.; Bernhardt, G.; Buschauer, A. [³H]UR-PLN196: a selective nonpeptide radioligand and insurmountable antagonist for the neuropeptide Y Y₂ receptor. *ChemMedChem* **2013**, *8*, 587–593.
- (32) Keller, M.; Kuhn, K. K.; Einsiedel, J.; Huebner, H.; Biselli, S.; Mollereau, C.; Wifling, D.; Svobodova, J.; Bernhardt, G.; Cabrele, C.; Vanderheyden, P. M. L.; Gmeiner, P.; Buschauer, A. Mimicking of Arginine by Functionalized N^ω-Carbamoylated Arginine As a New Broadly Applicable Approach to Labeled Bioactive Peptides: High Affinity Angiotensin, Neuropeptide Y, Neuropeptide FF, and Neurotensin Receptor Ligands As Examples. *J. Med. Chem.* **2016**, *59*, 1925–1945.
- (33) Maschauer, S.; Prante, O. A series of 2-O-trifluoromethylsulfonyl-D-mannopyranosides as precursors for concomitant ¹⁸F-labeling and glycosylation by click chemistry. *Carbohydr. Res.* **2009**, *344*, 753–761.
- (34) Müller, M.; Knieps, S.; Gesselle, K.; Dove, S.; Bernhardt, G.; Buschauer, A. Synthesis and neuropeptide Y Y₁ receptor antagonistic activity of N,N-disubstituted omega-guanidino- and omega-aminoalkanoic acid amides. *Arch. Pharm.* **1997**, *330*, 333–42.
- (35) Wester, H.-J.; Hamacher, K.; Stöcklin, G. A comparative study of n.c.a. fluorine-18 labeling of proteins via acylation and photochemical conjugation. *Nucl. Med. Biol.* **1996**, *23*, 365–372.
- (36) Okamoto, M.; Naka, K.; Kitagawa, Y.; Ishiwata, K.; Yoshimoto, M.; Shimizu, I.; Toyohara, J. Synthesis and evaluation of 7α-(3-[¹⁸F]fluoropropyl) estradiol. *Nucl. Med. Biol.* **2015**, *42*, 590–7.
- (37) Kuchar, M.; Mamat, C. Methods to Increase the Metabolic Stability of ¹⁸F-Radiotracers. *Molecules* **2015**, *20*, 16186–220.