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Evaluation of Fluorine-labeled Gastrin-Releasing Peptide Receptor (GRPR) Agonists and Antagonists by LC/MS

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Abstract

An LC/MS method was used to evaluate 2-fluoropropionyl (FP) and 4-fluorobenzoyl (FB) modified bombesin peptides: GRPR agonist [Aca-QWAVGHLM-NH₂] and antagonist [fQWAVGHL-NH₂], and their hydrophilic linker modified counterparts with the attachment of GGGRDN sequence. This study developed strategies to evaluate the *in vitro* receptor mediated cell uptake and metabolic profile of the various GRPR agonists and antagonists. We identified the metabolites produced by rat hepatocytes, and quantitatively analyzed the uptake and internalization of the ligands in PC-3 human prostate cancer cells. The major metabolites of both GRPR agonists and antagonists were the result of peptide bond hydrolysis between WA and AV. The agonists also formed a unique metabolite resulting from hydrolysis of the C-terminal amide. The antagonists showed significantly higher stability against metabolism compared to the agonists in rat hepatocytes. The directly modified agonists (FP-BBN and FB-BBN) had higher internalization with similar cell binding compared to the unmodified agonist (BBN), whereas the hydrophilic linker modified agonists (G-BBN and FG-BBN) had much lower total cell uptake. The labeled antagonist (FP-NBBN, FB-NBBN, G-NBBN and FP-G-NBBN) displayed lower internalization. The optimal imaging agent will depend on the interplay of ligand metabolism, cellular uptake, and internalization *in vivo*.

Keywords

LC/MS; Gastrin releasing peptide receptor (GRPR); Bombesin (BBN); Agonist; antagonist; PET

Introduction

The gastrin-releasing peptide receptor (GRPR) is an important target for cancer imaging and therapy since over-expression of GRPR is related to proliferation and growth of human cancers from a variety of origins, including breast, lung, pancreatic, and prostate (Fleischmann et al., 2005; Fleischmann et al., 2007; Markwalder and Reubi, 1999; Reubi et al., 2004; Sun et al., 2000). Both GRPR agonists and antagonists have been developed as therapeutic agents (Abd-Elgaliel et al., 2008; Abiraj et al., 2010; Ananias et al., 2011; Cescato et al., 2008; Mansi et al., 2009; Reubi et al., 2004; Schroeder et al., 2009; Smith et

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al., 2005). Molecular imaging of GRPR has also been explored to determine the presence, concentration, and therapeutic efficacy in various tumor types (Cornelio et al., 2007a; Cornelio et al., 2007b; Gugger and Reubi, 1999; Markwalder and Reubi, 1999; Smith et al., 2005). An optimal radiotracer for GRPR should meet several criteria. Firstly, the ligand must display high affinity for GRPR and demonstrate specific tumor uptake. Secondly, it must exhibit ideal pharmacokinetic properties and high enough metabolic stability so that images provide high target to background ratios and contain quantitative information. (Yan et al., 2010; Yang et al., 2011). Using LC/MS, the pharmacokinetics and metabolic properties of potential imaging probes can be evaluated without the need to conduct radiolabeling (Gu et al., 2011; Ma et al., 2009). In addition, LC/MS could provide the structural information of metabolism as well.

We and others have evaluated a number of radiolabeled BBN peptide derivatives for positron emission tomography (PET) imaging of GRPR expressing tumors (Chen et al., 2004b; Linder et al., 2009; Liu et al., 2009a; Liu et al., 2009c; Nock et al., 2003; Shi et al., 2008; Yan et al., 2010; Yang et al., 2011; Yang et al., 2006; Zhang et al., 2006). However, imaging with BBN analogs always suffered with low signal to noise ratios, which may result from the poor metabolic stability of the peptides. Indeed, it has been found that BBN derivatives, especially agonists, were stable over time in human plasma, but degraded rapidly in the kidneys and liver homogenates (Linder et al., 2009; Shipp et al., 1991; Zhang et al., 2004).

BBN analogs displayed either agonist properties or antagonist properties and efficient internalization was predominantly observed for the agonists (Wagner, 1979). However, high-affinity receptor antagonists, which showed poor internalization into tumor cells, performed equally or even better in terms of *in vivo* uptake in animal tumor models than the corresponding agonists (Abd-Elgaliel et al., 2008; Abiraj et al., 2010; Cescato et al., 2008; Mansi et al., 2009; Schroeder et al., 2009). Thus, a comprehensive evaluation of BBN analogs requires determination of several aspects including cellular uptake, internalization, and metabolism.

We had applied LC/MS to evaluate the *in vitro* receptor mediated cell uptake and metabolic profile of a potent BBN agonist (Aca-QWAVGHLM-NH₂, denoted as BBN), a comparably potent BBN antagonist (fQWAVGHL-NHEt, denoted as NBBN) (Gu et al., 2011). The goal of this study was to apply the LC/MS method to evaluate variously modified BBN agonists and antagonists (Fig. 1), including those that have been modified with fluorine-containing prosthetic groups, in rat hepatocytes and PC-3 human prostate cancer cells. These results could provide guidance to develop GRPR imaging agents with improved tumor targeting and metabolic stability and to screen candidate radiotracers without the need for radiolabeled compounds

Methods

Chemicals, reagents, and solutions

Acetonitrile (CH₃CN, HPLC grade) was purchased from Fisher Scientific (Pittsburgh, PA). All other reagents for synthesis and analysis were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise indicated. Aca-QWAVGHLM-NH₂ (BBN) and fQWAVGHL-NHEt (NBBN) were prepared according to the published procedure (Yang et al., 2011). GGGRDN-QWAVGHLM-NH₂ and GGGRDN-fQWAVGHL-NHEt were synthesized in our laboratory using solid-phase Fmoc chemistry and purified by semipreparative reversed-phase HPLC. Identity and purity were established by LC/MS: Aca-QWAVGHLM-NH₂ (m/z 1053.6 [M+H]⁺, 95% purity), fQWAVGHL-NHEt (m/z 984.6 [M+H]⁺, 97% purity), GGGRDN-QWAVGHLM-NH₂ (m/z 1497.1 [M+H]⁺, 93% purity), and GGGRDN-

fQWAVGHL-NHEthyl (m/z 1541.1 $[M+H]^+$, 90% purity). 2-Fluoropropionate (FP) and 4-fluorobenzoate (FB) analogs of the four peptides were prepared using the standard methods (Chen et al., 2004b; Liu et al., 2009a) (Yan et al., 2010) and purified by semipreparative reversed-phase HPLC. Identity and purity were established by LC/MS: FP-Aca-QWAVGHLM-NH₂ (m/z 1127.7 $[M+H]^+$, 97% purity), FP-fQWAVGHL-NHEthyl (m/z 1058.7 $[M+H]^+$, 95% purity), FB-aca-QWAVGHLM-NH₂ (m/z 1175.6 $[M+H]^+$, 93% purity), FB-fQWAVGHL-NHEthyl (m/z 1106.6 $[M+H]^+$, 95% purity), FP-GGGRDN-QWAVGHLM-NH₂ (m/z 1571.1 $[M+H]^+$, 94% purity), and FP-GGGRDN-fQWAVGHL-NHEt (m/z 1615.2 $[M+H]^+$, 94% purity). Stock solutions of the peptides were prepared in water at a concentration of 1 mg/mL.

Qualitative LC/MS

Waters LC-MS system (Waters, Milford, MA) was employed with an Acquity UPLC system coupled to the Waters Q-ToF Premier high resolution mass spectrometer. An Acquity BEH Shield RP18 column (150 × 2.1 mm) was used for chromatography. Elution was achieved with a mixture of two components: solution A was composed of 2 mM ammonium formate, 0.1% formic acid, and 5% CH₃CN; and solution B was composed of 2 mM ammonium formate and 0.1% formic acid in CH₃CN. The elution profile, at 0.2 mL/min, is: 100% (v:v) A and 0% B at initial; gradient 0–40% B over 15 min; isocratic elution at 40% B for an additional 3 min; 40–80% B over 2 min; and re-equilibrated with A for an additional 4 min. The retention time for each compounds were listed in Table 1. The injection volume was 10 μ L. The entire column elute was introduced into the Q-ToF mass spectrometer. Ion detection was achieved in ESI mode using a source capillary voltage of 3.5 kV, source temperature of 100°C, desolvation temperature of 200°C, cone gas flow of 50 L/h (N₂), and desolvation gas flow of 700 L/h (N₂).

Quantitative LC/MS

For quantitative analysis of peptides for cell internalization studies, the LC/MS system consisted of an Agilent 1200 autosampler, Agilent 1200 LC pump, and an AB/MDS Sciex 4000 Q TRAP (Life Technologies Corporation, Carlsbad, California). Separation was achieved on an Agilent RP18 column (1.8 μ m, 100 × 4.6 mm) with gradient system at flow rate of 1.0 mL/min with the same solvent system described in the qualitative section. A gradient of 0% B for three minutes ramped to 50% B was utilized and isocratic elution at 50% B for an additional 4 min; 80% B for 2 min; and re-equilibrated with A for additional 1 min. Different combinations of multiple-reaction monitoring (MRM) and full scan MS/MS experiments were performed. Standards were prepared for each peptide covering the concentration range from 0.001 to 10 μ M in 1:1 CH₃CN-water. Three replicate injections (10 μ L) were made for each concentration level. The specific comparisons made for quantitation used a single MRM transition per analyte. [Aca-QWAVGHLM-NH₂ (527.3/110.1), fQWAVGHL-NHEt (551.3/120.1), GGGRDN-QWAVGHLM-NH₂ (749.1/110.1), GGGRDN-fQWAVGHL-NHEt (771.1/110.1), FP-Aca-QWAVGHLM-NH₂ (1128.6/110.1), FP-fQWAVGHL-NHEt (1059.5/110.1), FB-Aca-QWAVGHLM-NH₂ (1175.6/110.1), FB-fQWAVGHL-NHEt (1106.6/110.1), FP-GGGRDN-QWAVGHLM-NH₂ (786.0/110.1), and FP-GGGRDN-fQWAVGHL-NHEt (808.1/110.1)]

Incubation with Hepatocytes to determine metabolic profile

Cryopreserved hepatocytes from male Sprague-Dawley rats (Celsis In Vitro Technologies, Inc., Baltimore, MD) were used for the *in vitro* metabolism studies. The cells, which were stored in liquid nitrogen (6–12 months), were thawed rapidly at 37°C in a water bath and gradually diluted with cell culture medium (Celsis In Vitro Technologies, Inc.). After washing the cells with the medium and adjusting the viable cell concentration to 1.0×10^6 cells per mL, the resulting cell suspension was incubated at 37°C for 15 min prior to the

introduction of the test compound. The peptides were added to a 1.0 mL suspension of cells so as to achieve a final concentration of test compound of 10 μ M. The suspension was maintained at 37°C. At various time points (10, 30, 60, 120, and 240 min), an aliquot (100 μ L) of cell suspension was removed and added to 100 μ L acetonitrile. This treatment resulted in cell lysis so that the metabolites analyzed could be either bound onto cell surface or internalized into the cells. Each aliquot was centrifuged at 5000 rpm for 5 min. The supernatants (10 μ L) were analyzed by LC-MS.

Internalization Studies in PC-3 Cell Line

The PC-3 human prostate carcinoma cell line was purchased from American Type Culture Collection (ATCC, Manassas, VA). PC-3 cells were grown in DMEM (Mediatech Inc., Manassas, VA) supplemented with 10% (v/v) fetal bovine serum (FBS) (Mediatech), 100 IU/mL penicillin, and 100 μ g/mL streptomycin (Invitrogen, Carlsbad, CA), at 37°C in a humidified atmosphere containing 5% CO₂.

For the uptake assay, PC-3 cells were seeded into 6-well plates at a density of 1×10^6 cells per well and incubated for 24 hr. Cells were rinsed 3 times with phosphate-buffered saline (PBS), followed by the addition of tested peptides to the cultured wells in triplicate (1 nmol/well). After incubation at 37°C for 15, 30, 60, and 120 min, the samples were processed in two different manners at each time point. For the first set, the media was collected for quantitative analysis of the unbound ligand. After washing twice with 1 mL saline, the cells were lysed by addition of 500 μ L 0.1 N NaOH, and then neutralized with 500 μ L 0.1N HCl. The final solution was collected for analysis of total cellular binding. In another set of plates, after the medium was separated, the cells were washed twice (0.5 mL) with 50 mM glycine and 0.1 M NaCl (pH 2.8). The acid washing buffer was collected since it contained the cell surface binding component. The cells were then lysed by adding 0.1 N NaOH and 0.1 N HCl and the resulting mixture was analyzed for internalized ligand. In total, four fractions were collected and analyzed by LC/MS, including unbound ligand, total bound ligand, cell surface binding ligand, and internalized ligand.

Results

Metabolism in Rat Hepatocytes

All agonist and antagonist peptides were incubated with the rat hepatocytes for metabolite analyses. All of the metabolites, whether intracellular or extracellular, are sampled by treating the aliquot of the cell suspension with CH₃CN to lyse the cells. The metabolites were analyzed by LC/MS and their structures were proposed based on the sequence of the individual peptide (Table 1). Both GRPR agonist [R-QWAVGHLM-NH₂] and antagonist [R-fQWAVGHL-NHEt] contain a homologous center of the sequence (WAVGHL). Consequently, the most common metabolites derived from all peptides resulted from peptide bond hydrolysis between W and A, which produced N-terminal sequences (R-QW-OH) for BBN (Fig. 2) and (R-fQW-OH) for NBBN. The peptides were also hydrolyzed between A and V, forming the amino terminal fragment R-QWA-OH and the carboxy terminal VGHLM-NH₂ (m/z 555) for the BBN peptides and R-fQWA-OH and VGHL-NHEt (m/z 452) for NBBN. The BBN peptides also derived a unique metabolite from hydrolysis of the C-terminal amide. The parent amide (R-QWAVGHLM-NH₂) exhibited a molecular ion [M + 1] that was 1 amu lower than that of its acid metabolite (R-QWAVGHLM-OH). The ability to conduct high resolution mass measurements was important to allow the characterization of this metabolite.

The metabolic profile changed after addition of the sequence GGGRDN to the amino terminus. The metabolites of R-GGGRDN-fQWAVGHL-NHEt result from peptide bond

hydrolysis between either D and N to generate a carboxyl terminal sequence N-fQWAVGHL-NH₂ (m/z 1098.8) or between R and D to generate a carboxyl terminal sequence [DN-fQWAVGHL-NH₂] (m/z 1213.9). No hydrolysis of the ethylamide was observed.

The metabolic stability of the peptides was evaluated in the same preparation of rat hepatocytes, based on the rate of disappearance of the parent peptides. Within 2 hours, the BBN peptides were almost undetectable from the assay solution while the half-lives of parent peptides with rat hepatocytes for BBN, FP-BBN, FB-BBN, G-BBN and FP-G-BBN were estimated to be 4, 25, 3.8, 27 and 68 min respectively (Fig. 3a). The NBBN peptides were more resistant to the hepatocytes. The half-life values of parent for NBBN, FP-NBBN, FB-NBBN, G-NBBN and FPG-NBBN were estimated to be 32, 130, 37, 44, 154 min respectively (Fig. 3b).

Cell Uptake and Internalization

The highly sensitive 4000 Qtrap MS system was used to quantitate ligand binding and internalization with PC-3 cells. Standard curves of the instrument were established with various concentrations of the peptides in CH₃CN:water (1:1). A linear response was observed from 0.002–10.0 μ M (0.02–100 pmol on column). The detection limit (s/n >3) for most ligands was approximately 1 nM or less.

The direct binding study was performed by incubating the peptides with GRPR expressing PC-3 prostate cancer cells (Markwalder and Reubi, 1999). At specified time points, aliquots were taken, processed as described in the methods section, and the various extracts quantitatively analyzed for parent peptide using LC/MS (Fig. 4 & 5). We found that all ligands were more stable during PC-3 cell incubation compared with hepatocyte incubation. The proportion of parent compound exceeded 55% in all compartments (data not shown). Four components from each incubation were analyzed including unbound ligand from the media, surface bound ligand from the acidic wash of the cells, internalized ligand from the lysed cells after acid wash and total surface bound and internalized ligand from lysed cells without acid wash. The cell uptake was expressed as the percent of total ligand added. Agonists (BBN, FPBBN and FB-BBN) showed higher internalization with concomitantly lower surface binding while antagonists (NBBN, FP-NBBN, FB-NBBN, G-NBBN and FP-G-NBBN) showed very low internalization compared with level of cell surface binding. The modified peptide G-BBN and its FP-derivative, FP-G-BBN, showed much lower total cell uptake compared with FP-BBN and FB-BBN.

Discussion

LC/MS is an efficient and sensitive method for screening radiotracer candidates for metabolic profile and tissue uptake without the need for radiolabeling (Ma et al., 2003). In this study, the LC/MS technique was used to evaluate metabolism and internalization of several BBN derived ligands for GRPR. The method provides not only information for structural identification of metabolites but also highly selective, accurate quantitation of ligands in biological extracts.

BBN derived radiotracers are limited in their application to in vivo tumor imaging due to the rapid metabolism and poor stability. After incubation with rat hepatocytes, we found that analogs of the agonist (BBN) are metabolized more quickly than similar analogs of the antagonist (NBBN). Interestingly, the metabolic stability was enhanced by capping the amino terminus with FP but not with FB. Both fluorine labeled BBN and NBBN were metabolized in a similar pathway to what had previously been elucidated (Linder et al., 2009). Metabolic proteolysis between the WA and AV were expected for the BBN derived

peptides and this cleavage was observed for both agonist and antagonist peptides. In addition, all of the labeled BBN peptides showed hydrolysis of the C-terminal amide. The shorter half-life of the labeled BBN peptides could be attributed to the relatively rapid formation of the amide hydrolysis product and the difference in peptide sequence compared to the antagonist. The differences in sequence between the two classes of peptides, a C-terminal methionine in the agonist and an N-terminal R-phenylalanine in the antagonist, could also influence the enzymatic hydrolysis. FP-G-BBN was metabolized slower than other fluorine-labeled BBN, which may result in better tumor imaging properties even though it exhibited lower *in vitro* PC-3 cell uptake.

We also used LC/MS to directly determine binding and internalization of various GRPR ligands to human prostate derived PC-3 cells. The agonist ligands exhibited 2- to 3-fold higher total cell uptake than the antagonists. The increased cell uptake was due to higher internalization of agonists as the cell surface binding was very similar between the two groups of peptides. The antagonists displayed almost no internalization. As GRPR agonists, directly modified BBN (FP-BBN and FB-BBN) had similar cell uptake as compared to the unmodified agonist (BBN). However, FP-BBN was previously found to have better *in vivo* tumor imaging than FB-BBN presumably because it was more polar and more slowly metabolized. It has been well established that introduction of PEG, sugar moiety and oligo-glycine linkers could improve the *in vivo* kinetics of various peptides (Lee et al., 2010). Previously, we have also successfully used PEG and oligo-Glycine linkers to improve the pharmacokinetics of RGD peptides (Chen et al., 2004a; Liu et al., 2009b). The modified BBN with hydrophilic linker (GGGRDN-BBN and FP-GGGRDN-BBN) was found to have much lower PC-3 cell uptake in both surface and internalization. Meanwhile, the NBBN analogs (FP-NBBN, FB-NBBN, G-NBBN, FP-G-NBBN) were very similar with the unmodified antagonist NBBN which showed more efficient cell surface binding than internalization (Fig. 5).

Conclusion

The combination of LC and MS/MS can provide both structural information for identification of metabolites and accurate quantitation of ligands in cell binding experiments. The metabolism profiles of BBN derived GRPR agonist and antagonist peptides were identified by LC/MS. The major metabolites resulted from peptide bond hydrolysis between W and A in rat hepatocyte incubation. The antagonist peptides were more metabolically stable than the agonist peptides. The agonist ligands (BBN, FP-BBN and FB-BBN) had higher cell uptake in PC-3 cells due to high internalization. The addition of the hydrophilic linker reduced the cell uptake of the ligands G-BBN and FP-G-BBN by interrupting internalization. The optimal imaging agent will depend on the interplay of ligand metabolism, cellular uptake, and internalization *in vivo*.

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a

BBN R = Aca-
 FP-BBN R = FP-Aca-
 FB-BBN R = FB-Aca-
 G-BBN R = GGGRDN-
 FP-G-BBN R = FP-GGGRDN-

b

NBBN R = H-
 FP-NBBN R = FP-
 FB-NBBN R = FB-
 G-NBBN R = GGGRDN-
 FP-G-NBBN R = FP-GGGRDN-

Fig. 1.
 Structures of GRPR agonists [R-QWAVGHLM-NH₂] (**a**) and antagonists [R-fQWAVGHL-NHEt] (**b**) peptides. FP = 2-fluoropropionate, FB = 4-fluorobenzoate, and Aca = 5-amino caproic acid

Fig. 2.

The Total Ion Chromatography (TIC) of agonist [FP-BBN] metabolites after 30 min of incubation in rat hepatocytes. Other peaks (X) except for labeled are from incubation solution which was confirmed by compared with blank.

a **b** **Fig. 3.**

a The metabolic rate of BBN, FP-BBN, FB-BBN, G-BBN and FP-G-BBN presented as the percent of parent ligand at various time points during 120 min of incubation with rat hepatocytes. **b** The metabolic rate of NBBN, FP-NBBN, FB-NBBN, G-NBBN and FP-G-NBBN presented as the percent of parent ligand at various time points during 120 min of incubation with rat hepatocytes.

Fig. 4.

The percent of parent ligand of FP-BBN, FB-BBN, G-BBN and FP-G-BBN observed in total cell binding, surface binding and internalization during 120 min of incubation in PC-3 cells.

Fig. 5. The percent of parent ligand of labeled FP-NBBN, FB-NBBN, G-NBBN and FP-GNBBN observed in total cell binding, surface binding and internalization during 120 min of incubation in PC-3 cells.

Table 1

Proposed structures of the metabolites formed by rat hepatocytes and the molecular weight peaks [m/z] observed in positive ESI mass spectrometry

Name/retention time (mins)	Structure	Metabolite 1	Metabolite 2	Metabolite 3
BBN/ 10.47	Aca-QWAVGHLM-NH ₂ [M+H] 1053.6	Aca-QWAVGHLM-OH [M+H] 1054.1	Aca-QW-OH [M+H] 446.5 AVGHLM-NH ₂ [M+H] 626.5	Aca-QW-A-OH [M+H] 517.3 VGHLM-NH ₂ [M+H] 555.4
NBBN/11.47	fQWAVGHL-NHEt [M+H] 984.4		fQW-OH [M+H] 480.3	fQW-A-OH [M+H] 551.4 VGHL-NHEt [M+H] 452.4
FP-BBN /14.81	FP-aca-QWAVGHLM-NH ₂ [M+H] 1127.8	FP-aca-QWAVGHLM-OH [M+H] 1128.8	FP-aca-QW-OH [M+H] 520.3 AVGHLM-NH ₂ [M+H] 626.5	FP-aca-QW-A-OH [M+H] 591.4 VGHLM-NH ₂ [M+H] 555.4
FP-NBBN/17.56	FP-fQWAVGHL-NHEt [M+H] 1058.7		FP-fQW-OH [M+H] 554.4	FP-fQW-A-OH [M+H] 625.4
FB-BBN/ 17.52	FB-aca-QWAVGHLM-NH ₂ [M-H] 1173.9	FB-aca-QWAVGHLM-OH [M-H] 1174.9	FB-aca-QW-OH [M-H] 566.5	FB-aca-QW-A-OH [M-H] 637.5
FB-NBBN/18.71	FB-fQWAVGHL-NHEt		FB-fQW-OH [M+H] 600.5	FB-fQW-A-OH [M+H] 671.5
G-BBN/ 10.45	GGGRDN-QWAVGHLM-NH ₂ [M+H] 1497.1	GGGRDN-QWAVGHLM-OH [M+H] 1498.1	AVGHLM-NH ₂ [M+H] 626.5	VGHLM-NH ₂ [M+H] 555.4
G-NBBN/11.85	GGGRDN-fQWAVGHL-NHEt [M+H] 1541.1		WAVGHL-NHEt [M+H] 523.5	VGHL-NHEt [M+H] 452.4
FP-G-BBN /11.66	FP-GGGRDN-QWAVGHLM-NH ₂ [M+H] 1571.1	FP-GGGRDN-QWAVGHLM-OH [M+H] 1572.1	FP-GGGRDN-QW-OH [M+H] 963.6 AVGHLM-NH ₂ [M+H] 626.5	FP-GGGRDN-QW-A-OH [M+H] 1034.7
FP-G-NBBN/13.11	FP-GGGRDN-fQWAVGHL-NHEt [M+H] 1615.2		FP-GGGRDN-fQW-OH [M+H] 1110.8	FP-GGGRDN-fQW-A-OH [M+H] 1181.8