Bioorganic & Medicinal Chemistry Letters 22 (2012) 6391–6396

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Serum stability of selected decapeptide agonists of KISS1R using pseudopeptides

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ARTICLE INFO

Article history:
Received 5 June 2012
Revised 15 August 2012
Accepted 17 August 2012
Available online 23 August 2012

Keywords:
Kisspeptin
KISS1R agonist
Metastin
Metastin(45–54)
Metastin analog
Serum stability

ABSTRACT

Metastin/kisspeptin, a 54-amino acid peptide, is the ligand of the G-protein-coupled receptor KISS1R which plays a key role in pathways that regulate reproduction and cell migration in many endocrine and gonadal tissues. The N-terminally truncated decapeptide, metastin(45–54), has 3–10 times higher receptor affinity and intracellular calcium ion-mobilizing activity but is rapidly inactivated in serum. In this study we designed and synthesized stable KISS1R agonistic decapeptide analogs with selected substitutions at positions 47, 50, and 51. Replacement of glycine with azaglycine (azaGly) in which the α-carbon is replaced with a nitrogen atom at position 51 improved the stability of amide bonds between Phe^{50}-Gly^{51} and Gly^{51}-Leu^{52} as determined by in vitro mouse serum stability studies. Substitution for tryptophan at position 47 with other amino acids such as serine, threonine, β-(3-pyridyl)alanine, and D-tryptophan (D-Trp), produced analogs that were highly stable in mouse serum. D-Trp^{47} analog **13** showed not only high metabolic stability but also excellent KISS1R agonistic activity. Other labile peptides may have increased serum stability using amino acid substitution.

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Metastin/kisspeptin is the endogenous ligand of an orphan G-protein-coupled receptor GPR54, also known as AXOR12 or OT7T175, and has recently been renamed the KiSS1 receptor (KISS1R).[1–3] Recent studies have suggested that metastin acts as a critical potentiator of gonadotropin-releasing hormone secretion following acute administration in several mammalian species including rats,[4] mice,[5] and human males,[6] but lowers testosterone levels as a consequence of continuous administration.[7] These results strongly suggest that appropriate KISS1R agonists can modulate the hypothalamic-pituitary-gonadal axis and may have the potential to be useful for the prevention or treatment of a number of sex hormone-dependent diseases.

N-terminally truncated human metastin(45–54), Tyr-Asn-Trp-Asn-Ser-Phe-Gly-Leu-Arg-Phe-NH_2, is 3–10 times more active than metastin in vitro.[1] However, both metastin and metastin(45–54) are susceptible to enzyme-catalyzed hydrolysis.[8] In our laboratory, N^{ω}-methylarginine [Arg(Me)] substitution has been shown to be highly effective in preventing the cleavage between amino acids Arg^{53} and Phe^{54}.[9] Consequently, an analog possessing Arg(Me) was synthesized and found to be metabolically resistant to trypsin-like proteases.[9] There remains a great demand for more stable analogs with other amino acid replacements at fragile sites to improve their pharmacological properties by maintaining or improving agonistic activity and serum stability. In this report, we provide a rational strategy for the design of decapaptide analogs of KISS1R agonists to improve their stability in murine serum.

All peptides were synthesized by standard *N*-(9-fluorenylmethoxycarbonyl;Fmoc)-based solid phase synthetic methodology except where specifically described. The $[Ca^{2+}]_i$-mobilizing activities of all peptides are shown as the concentration at 50% of the maximum response (EC_{50} values) calculated using sigmoidal dose response curves in Tables 1 and 2. The receptor binding affinities of the synthesized peptides were determined as IC_{50} values.

The ability of selected peptides to prevent enzymatic hydrolysis was evaluated by incubation in mouse serum at 37 °C. The residual ratio, defined as the percent of compound remaining after a 1 h incubation at initial concentrations of 0.1 mM, is given in Tables 1 and 2.

Metastin(45–54) was rapidly metabolized in mouse plasma (Fig. 1). More than 50% of the peptide was metabolized after a 1-min incubation at 37 °C (data not shown). The metabolites

Abbreviations: Arg(Me), N^{ω}-methylarginine; azaGly, azaglycine; $[Ca^{2+}]_i$, intracellular calcium concentration; Cha, β-cyclohexylalanine; DMF, dimethylformamide; ESI-MS, electrospray ionization mass spectrometry; FLIPR, fluorometric imaging plate reader; Fmoc, 9-fluorenylmethoxycarbonyl; GnRH, gonadotropin-releasing hormone; HPLC, high-performance liquid chromatography; KISSR, kisspeptin receptor; MBHA, 4-methylbenzhydrylamine; MMP-9, metalloproteinase-9; NEP, neutral endopeptidase; Nal(2), β-(2-naphthyl)alanine; Pya(3), β-(3-pyridyl)alanine; Pya(4), β-(4-pyridyl)alanine; T/C, treated/control; TFA, trifluoroacetic acid.

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http://dx.doi.org/10.1016/j.bmcl.2012.08.069

Table 1
Biological activities of metastin analogs substituted between positions 50 and 51

H-AA^{45}-Asn-Trp-Asn-Ser-AA$^{50-51}$-Leu-AA^{53}-Phe-NH$_2$							
Compound-no.	AA^{45}	AA$^{50-51}$	AA^{53}	Agonist activity[a] EC$_{50}$ (nM)	Binding affinity[c] IC$_{50}$ (nM)		Stability in mouse serum
					Human	Rat	Residual ratio (%)[e]
Metastin(45–54)	Tyr	Phe-Gly	Arg	0.049[b]	0.082[d]	0.11[d]	ND[f]
1	D-Tyr	Phe-Gly	Arg(Me)	0.065	0.11	0.26	18.1
2	D-Tyr	Phe-ψ(CSNH)-Gly	Arg(Me)	0.041	2.8	3.3	35.3
3	D-Tyr	Phe-ψ(CH$_2$NH)-Gly	Arg(Me)	0.62	0.61	1.4	12.1
4	D-Tyr	Phe-ψ(NHCO)-Gly	Arg(Me)	0.95	0.51	2.9	46.8
5	D-Tyr	Phe-azaGly	Arg(Me)	0.050	0.16	0.17	39.5

[a] Agonist activities of all peptide analogs were evaluated in a functional assay of human OT7T175, an intracellular calcium mobilization assay using fluorometric imaging plate reader technology. EC$_{50}$ values of all peptide analogs were calculated using sigmoidal dose response curves.
[b] EC$_{50}$ values of metastin(45–54) were calculated as the average value of 13 independent experiments.
[c] Receptor binding affinities of the synthesized peptides were determined as IC$_{50}$ values.
[d] IC$_{50}$ value of metastin(45–54) was calculated as the average value of three independent experiments.
[e] Residual ratio after incubation in mouse serum 1 h at 37 °C.
[f] Not determined.

Table 2
Biological activities of decapeptide metastin analogs substituted at position 47

H-AA^{45}-Asn-AA^{47}-Asn-Ser-Phe-AA^{51}-Leu-AA^{53}-Phe-NH$_2$								
Compound-no.	AA^{45}	AA^{47}	AA^{51}	AA^{53}	Agonist activity[a] EC$_{50}$ (nM)	Binding affinity[c] IC$_{50}$ (nM)		Stability in mouse serum
						Human	Rat	Residual ratio (%)[e]
Metastin(45-54)	Tyr	Trp	Gly	Arg	0.049[b]	0.082[d]	0.11[d]	ND[f]
6	D-Tyr	Ser	azaGly	Arg(Me)	0.73	0.49	0.43	50.0
7	D-Tyr	Thr	azaGly	Arg(Me)	0.24	0.86	0.83	62.5
8	D-Tyr	Ile	azaGly	Arg(Me)	0.13	0.43	0.46	ND[f]
9	D-Tyr	Cha	azaGly	Arg(Me)	0.10	0.19	0.24	32.0
10	D-Tyr	Pya(3)	azaGly	Arg(Me)	0.24	0.70	0.89	50.6
11	D-Tyr	Pya(4)	azaGly	Arg(Me)	0.11	0.30	0.28	30.7
12	D-Tyr	Nal(2)	azaGly	Arg(Me)	0.098	0.13	0.20	43.6
13	D-Tyr	D-Trp	azaGly	Arg(Me)	0.072	0.19	0.22	56.4
14	D-Tyr	D-Pya(4)	azaGly	Arg(Me)	0.59	0.28	0.28	ND[f]

[a] Agonist activities of all peptide analogs were evaluated in a functional assay of human OT7T175, an intracellular calcium mobilization assay using fluorometric imaging plate reader technology. EC$_{50}$ values of all peptide analogs were calculated using sigmoidal dose response curves.
[b] EC$_{50}$ values of metastin(45–54) were calculated as the average value of 13 independent experiments.
[c] Receptor binding affinities of the synthesized peptides were determined as IC$_{50}$ values.
[d] IC$_{50}$ value of metastin(45–54) was calculated as the average value of three independent experiments.
[e] Residual ratio after incubation in mouse serum 1 h at 37 °C.
[f] Not determined.

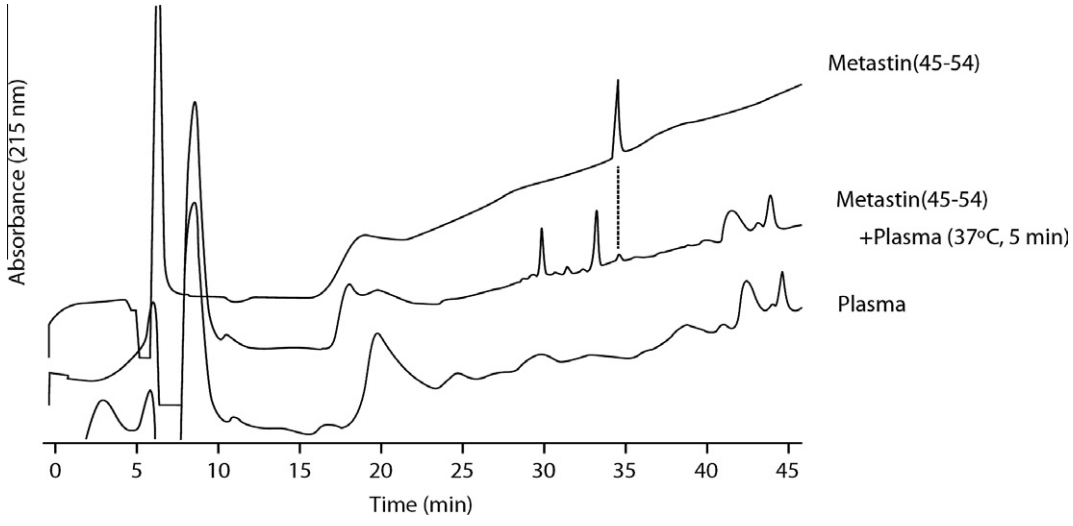

Figure 1. HPLC chromatogram of metastin(45–54) after incubation in mouse plasma.

formed during incubation were identified by HPLC electrospray ionization mass spectrometry (HPLC-ESI-MS) coupling experiments. ESI mass spectra obtained from metastin(45–54) incubated with mouse plasma revealed the presence of more than ten plausible metabolites as shown in Figure 2. Two major peptide fragments, metastin(46–54) and (46–53), were released primarily as

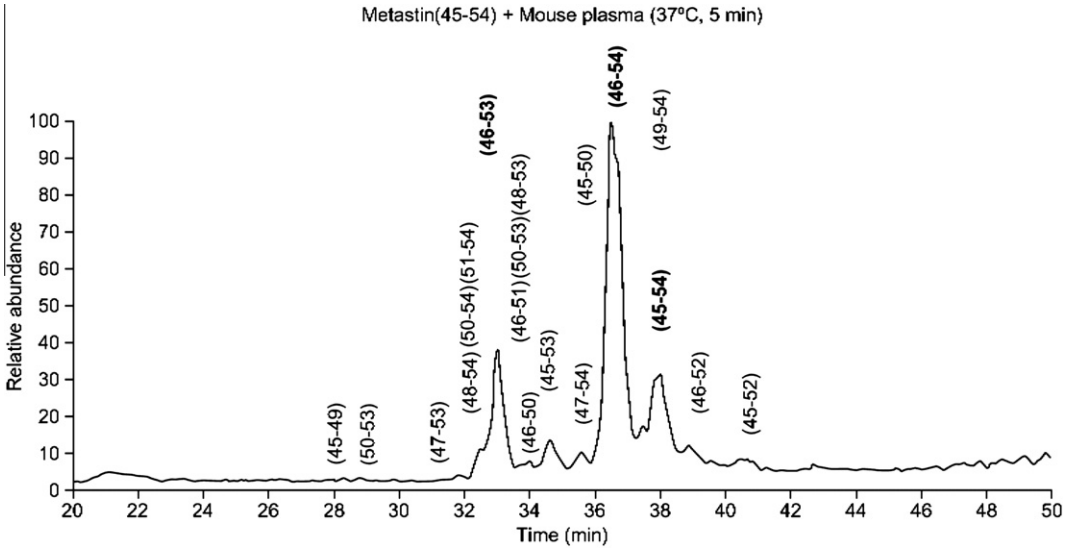

Figure 2. Total ion chromatogram of metastin(45–54) after incubation in mouse plasma.

a result of Tyr^{45}-Asn^{46} and Arg^{53}-Phe^{54} protease cleavage due to aminopeptidase activity and trypsin-like degradation. We determined that Arg(Me) substitution at the 53 position was an optimal approach to improve serum stability by avoiding cleavage between Arg^{53} and Phe^{54}.[9]

Incorporation of D-amino acids has been widely recognized to improve biological potencies of peptides by altering conformational properties[10] and increasing resistance to enzymatic degradation.[11] Peptide **1** with D-Tyr^{45} showed acceptable activity and avoided N-terminal degradation. Mouse plasma stability experiments showed that peptide **1** was apparently resistant to proteolysis after a 1 h incubation in mouse serum and showed a treated/control (T/C) ratio of 18.1%, even though several metabolites were observed in the ESI-MS spectra including metastin(45–47), (45–50), (45–51), and (48–54), possibly due to cleavage by chymotrypsin-like proteases, neutral endopeptidases (NEP), and matrix metalloproteinase-9 (MMP-9), as shown in Figure 3. Peak X in the figure was observed as an unknown substance derived from serum protein.

We hypothesized that more potent and long-acting analogs of compound **1** could be developed if other degradative pathways were successfully inhibited by specific replacement at positions 47, 48, 50, and 51. To examine this hypothesis, we first synthesized a series of analogs with substitution of residues at positions 50 and 51. Preliminary data (not shown) suggested that amino acid substitutions at Phe^{50} and Gly^{51} preserved receptor recognition. D-amino acid replacement improved metabolic stability but reduced in vitro activity. Therefore, we designed and synthesized amide bond isosteres and aza amino acid additions between positions 50 and 51 without altering their side chain moieties. This was expected to improve agonistic activity and avoid enzymatic degradation by changing the overall conformation of compound **1**.

Pseudopeptides have become important for medicinal chemistry.[12] A previous study demonstrated that Gly-Leu dipeptide isosteres, such as (*E*)-alkene- and hydroxyethylene-type isosteres, helped to maintain the agonist activity against MMP-9-mediated cleavage.[12] Metastin analogs with five amino acid residues containing (*E*)-alkene and hydroxyethylene isosteres showed high stability in murine serum.[8]

In this study, we designed and synthesized other simple pseudopeptides including thio peptides, reduced amide peptides, retro-inverso peptides and aza peptides.

Modification of peptides by reduction or thiolation of amide carbonyl oxygen has received attention recently for several reasons. For example, thiolated analogs of biologically active peptides have been reported to show increased enzymatic stability, potency, and selectivity.[13,14]

The reduced amide bond (CH_2NH) in compound **3** was formed by reductive alkylation of a preformed Fmoc-Phe-H with H-Leu-Orn(Mtt)-Phe-Rink MBHA resin in the presence of an excess $NaBH_3CN$ and 1% acetic acid in *N*,*N*-dimethylformamide (DMF).[15–17] Pseudopeptide **4** was synthesized by solid phase synthesis of retro-inverso peptides.[18] The amide functional group of Z-Phe-NH_2 was converted into an amine with bis(trifluoroacetoxy)iodobenzene, followed by condensation with mono-*tert*-butylmalonate and subsequent conversion of the Z group into an Fmoc group. Deprotection of *tert*-butyl ester by TFA treatment for 3 min afforded the desired pseudodipeptide, Fmoc-Phe-ψ(NHCO)-Gly-OH, which was used for the solid phase peptide synthesis.

Aza peptides have an aza amino acid residue in which the α-carbon is replaced with a nitrogen atom. This nitrogen substitution is believed to have little effect on the overall physicochemical profiles of these peptides; however, electronic repulsion between the adjacent nitrogen atoms has been suggested to induce type I and II β-turn conformations.[19–31] This β-turn geometry of aza amino acid-containing peptides may contribute to improve binding affinity of metastin analogs for KISS1R. NMR analysis of the metastin(45–54) analog in dodecylphosphocholine micelles showed that it contained several tight turn structures such as miscellaneous type IV β-turns (residues Asn^{48}–Gly^{51} and Gly^{51}–Phe^{54}).[32] In addition, this manipulation is expected to increase the stability of the aza peptide against proteases that recognize amino acid residues Phe^{50}–Gly^{51} or Gly^{51}–Leu^{52} due to the planar configuration of α-nitrogen between D- and L-amino acids.

We designed an aza peptide and synthesized it using solid phase methodology. All of these analogs including thio peptide **2**, reduced amide peptide **3**, retro-inverso peptide **4**, and aza peptide **5**, retained acceptable $[Ca^{2+}]_i$ mobilizing activities and receptor binding activities (Table 1). Stability against enzymatic hydrolysis was evaluated in mouse serum and the amount of peptide remaining in serum extracts was determined from the area under HPLC peaks. The stability index defined as the percent of a given metastin analog recovered from serum after incubation at 37 °C for 1 h, was calculated relative to the amount of peptide at time 0

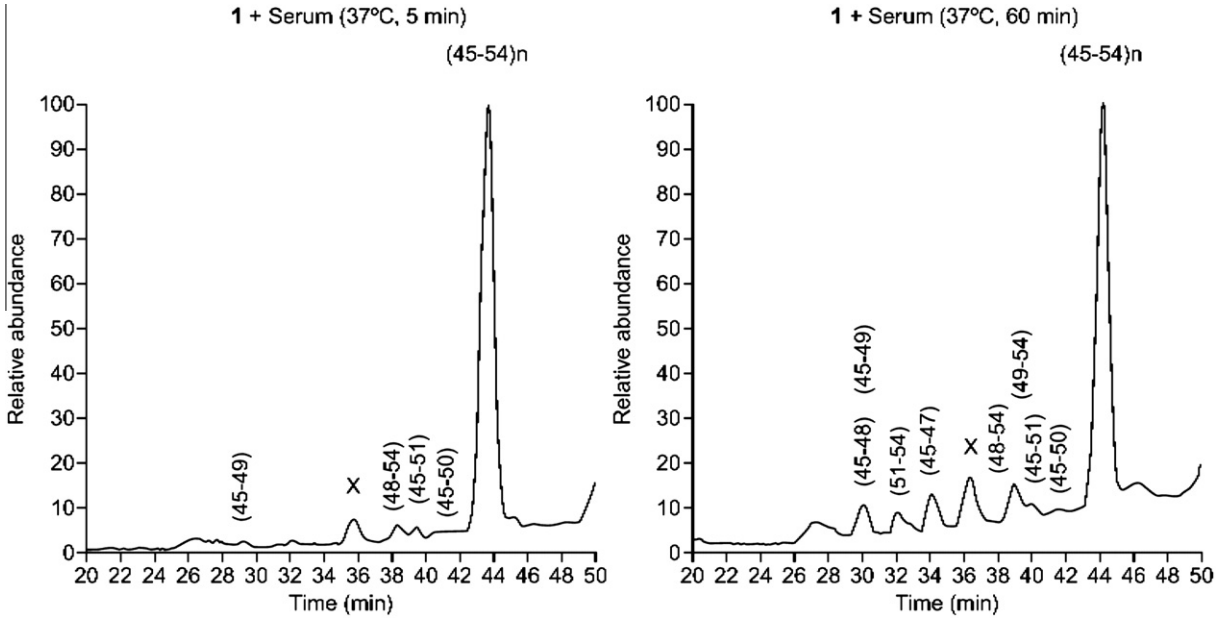

Figure 3. Total ion chromatogram of **1** after incubation in mouse serum.

(Table 1). Thio peptide **2**, retro-inverso peptide **4**, and aza peptide **5** displayed increased stability in mouse serum; however, reduced amide peptide **3** exhibited a lower residual ratio compared with peptide **1**. It was assumed that retro-inverso peptide and aza peptide analogs could avoid cleavage of amide bonds between the residues 50 and 51 and 51 and 52. The azaGly analog was selected for further optimization studies because of its superior water solubility and synthetic characteristics.

The sequence containing tryptophan at position 47 was prone to enzymatic degradation by chymotrypsin-like proteases which are known to exhibit high affinity for aromatic amino acid substrates such as phenylalanine, tyrosine, and tryptophan.[33,34] On the other hand, it was expected that these chymotrypsin-like proteases would exert a relatively low activity against substrates that had unnatural or hydrophilic amino acids. Based on this, the following modifications were carried out to increase the stability of the bond between residues 47 and 48. Replacement of Trp[47] with serine (Ser) yielded analog **6** with relatively low bioactivity but superior metabolic stability compared with compound **5** (Table 2). The residual ratio of compound **6** after a 1 h incubation was 50.0%. Several metabolites were identified by HPLC-ESI-MS coupling experiments following a 1 h incubation of compound **6** which was a prototype model peptide with an azaGly residue (Fig. 4). Peak X in the figure was also observed as an unknown substance derived from serum protein. Compound **6** was not cleaved between positions 50 and 52, and resisted proteolytic degradation in a 15 min human serum incubation in which metastin(45–54) showed rapid decomposition (Table 3).

KISS1R recognition has been reported to be relatively unaffected by the structure of the N-terminal region of metastin(45–54). D-amino acid substitutions at positions 45, 46, and 47 in metastin(45–54) retained potent biological activity comparable

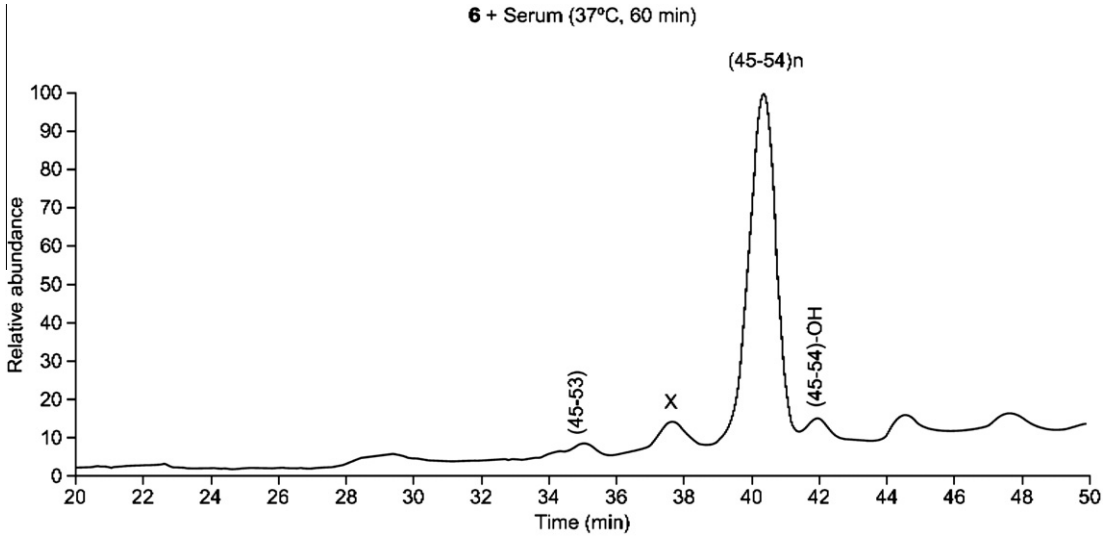

Figure 4. Total ion chromatogram of **6** after incubation in mouse serum.

Table 3
Stabilities of metastin analogs in human serum

Compound	Residual ratio[a]				$t_{1/2}$[b] (min)
	0 min	5 min	10 min	15 min	
Metastin(45–54)	66	0.9	<0.5	<0.5	<1
1	93.4	90.7	85.8	82.7	75
6	86.5	86.5	85.3	86.4	Stable

[a] Residual ratio was determined after incubation in human serum 1 h at 37 °C ($n = 2$).
[b] The half-lives of studied analogs were calculated according to pseudo-first-order kinetic models and optimized using monoexponential regressions.

to metastin(45–54),[35,36] suggesting that optimization at residue 47 with L- or D-amino acids would result in novel analogs with both high $[Ca^{2+}]_i$ mobilizing activities and serum stability.

AzaGly[51] derivatives substituted with unnatural amino acids for Trp[47], β-cyclohexylalanine (Cha) **9**, β-(4-pyridyl)alanine [Pya(4)] **11**, and β-(2-naphthyl)alanine [Nal(2)] **12** analogs demonstrated comparable $[Ca^{2+}]_i$-mobilizing activities compared with Trp analog **5** (Table 2). Of these analogs, Nal(2) analog **12** showed the highest activity and was nearly equivalent to that of metastin(45–54). However, the residual ratios of these compounds was less than 50%. Other L-amino acid substitutions such as threonine (Thr) **7**, isoleucine (Ile) **8**, and β-(3-pyridyl)alanine [Pya(3)] **10**, showed decreased agonistic activities compared to metastin(45–54), although the residual ratios of peptides **7** and **10** were above 50% in serum stability studies.

As for D-amino acid substitutions, D-Trp[47] **13** and D-Pya(4)[47] **14** analogs exhibited good receptor binding affinity, which were comparable to the corresponding L-form amino acid derivatives **5** and **11**. This result suggested that the stereochemistry of the α-carbon at position 47 does not play a critical role in KISS1R recognition, as expected. In addition, D-Trp analog **13** showed excellent serum stability with a residual ratio of 56.4%, compared to 39.5% for Trp analog **5**.

In summary, we designed and synthesized metabolically stable KISS1R decapeptides with selected substitutions at positions 47, 50, and 51. Replacement of Gly with an azaGly residue, which involved a simple nitrogen atom replacement of an α-carbon at Gly, resulted in good agonistic activity for KISS1R. This indicated that some β-turn conformations, which are reported to be observed in aza peptides, may contribute to interactions with KISS1R. Additionally, incorporation of the azaGly[51] residue improved metabolic stability of the Phe[50]-Gly[51] and Gly[51]-Leu[52] bonds, which were resistant to serum proteases such as chymotrypsin, NEP, and MMP-9. Although a variety of aza amino acid analogs of biologically active peptides has been reported with improved biological potency,[37–49] this study is the first report that aza amino acid replacement is a simple way to protect both fragile amide bonds at the N-terminal and C-terminal of the aza amino acid residue.

Substitution of Trp at position 47 with other amino acids, exemplified in this series of analogs with azaGly[51], produced several analogs that were stable to metabolic degradation. Our study demonstrated that the key amino acid replacement with azaGly[51] resulted in metabolically stable analogs that could be easily synthesized using solid-phase methodology without preparing dipeptide isostere units. Further optimization studies on novel azaGly short length metastin agonists with high potency and metabolic stability are ongoing in prostate cancer drug development.

Acknowledgements

The authors acknowledge Stephen Mosley of FireKite for editorial assistance in the development of this manuscript which was supported by Millennium Pharmaceuticals, Inc.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.069.

References and notes

1. Ohtaki, T.; Shintani, Y.; Honda, S.; Matsumoto, H.; Hori, A.; Kanehashi, K.; Terao, Y.; Kumano, S.; Takatsu, Y.; Masuda, Y.; Ishibashi, Y.; Watanabe, T.; Asada, M.; Yamad, T.; Suenaga, M.; Kitada, C.; Usuki, S.; Kurokawa, T.; Onda, H.; Nishimura, O.; Fujino, M. *Nature* **2001**, *411*, 613.
2. Kotani, M.; Detheux, M.; Vandenbogaerde, A.; Communi, D.; Vanderwinden, J. M.; De Poul, E.; Bré zillon, S.; Tyldesley, R.; Suarez-Huerta, N.; Vandeput, F.; Blanpain, C.; Schiffmann, S. N.; Vassart, G.; Parmentier, M. *J. Biol. Chem.* **2001**, *276*, 34631.
3. Muir, A. I.; Chamberlain, L.; Elshourbagy, N. A.; Michalovich, D.; Moore, D. J.; Calamari, A.; Szekeres, P. G.; Sarau, H. M.; Chambers, J. K.; Murdock, P.; Steplewski, K.; Shabon, U.; Miller, J. E.; Middleton, S. E.; Darker, J. G.; Larminie, C. G. C.; Wilson, S.; Bergsma, D. J.; Emson, P.; Faull, R.; Philpott, K. L.; Harrison, D. C. *J. Biol. Chem.* **2001**, *276*, 28969.
4. Matsui, H.; Takatsu, Y.; Kumano, S.; Matsumoto, H.; Ohtaki, T. *Biochem. Biophys. Res. Commun.* **2004**, *320*, 383.
5. Gottsch, M. L.; Cunningham, M. J.; Smith, J. T.; Popa, S. M.; Acohido, B. V.; Crowley, W. F.; Seminara, S.; Clifton, D. K.; Steiner, R. A. *Endocrinology* **2004**, *145*, 4073.
6. Dhillo, W. S.; Chaudhri, O. B.; Patterson, M.; Thompson, E. L.; Murphy, K. G.; Badman, M. K.; McGowan, B. M.; Amber, V.; Patel, S.; Ghatei, M. A.; Bloom, S. R. *J. Clin. Endocrinol. Metab.* **2005**, *90*, 6609.
7. Thompson, E. L.; Murphy, K. G.; Patterson, M.; Bewick, G. A.; Stamp, G. W.; Curtis, A. E.; Cooke, J. H.; Jethwa, P. H.; Todd, J. F.; Ghatei, M. A.; Bloom, S. R. *Am. J. Physiol. Endocrinol. Metab.* **2006**, *291*, 1074.
8. Tomita, K.; Oishi, S.; Ohno, H.; Peiper, S. C.; Fujii, N. *J. Med. Chem.* **2008**, *51*, 7645.
9. Asami, T.; Nishizawa, N.; Ishibashi, Y.; Nishibori, K.; Horikoshi, Y.; Nakayama, M.; Tarui, N.; Matsumoto, H.; Ohtaki, T.; Kitada, C. Unpublished results.
10. Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. F. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5871.
11. Dooley, C. T.; Chung, N. N.; Wilkes, B. C.; Schiller, P. W.; Bidlack, J. M.; Pasternak, G. W.; Houghten, R. N. *Science* **1994**, *266*, 2019.
12. (a) Spatola, A. F. In *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*; Weinstein, B., Ed.; Marcel Dekker: New York, 1983; pp 267–357; (b) Sherman, D. B.; Spatola, A. F. *J. Am. Chem. Soc.* **1990**, *112*, 433; (c) Hirschmann, R. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1278; (d) Gante, J. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699.
13. (a) Michel, A. G.; Ameziane-Hassani, C.; Boulay, G. *Can. J. Chem.* **1989**, *67*, 1312; (b) Mock, W. L.; Chen, J. T.; Tsang, J. W. *Biochem. Biophys. Res. Commun.* **1981**, *102*, 389.
14. Clausen, K.; Spatola, A. F.; Lemieux, C.; Schiller, P. W.; Lawesson, S.-O. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 305.
15. Meyer, J.-P.; Davis, P.; Lee, K. B.; Porreca, F.; Yamamura, H. I.; Hruby, V. J. *J. Med. Chem.* **1995**, *38*, 3462.
16. Fehrentz, J.-A.; Castro, B. *Synthesis* **1983**, 676.
17. Sasaki, Y.; Coy, D. H. *Peptides* **1987**, *8*, 119.
18. Pessi, A.; Pinori, M.; Verdini, A. S.; Viscomi, G. C. *Farmaco* **1983**, *38*, 360.
19. Lee, H.-J.; Ahn, I.-A.; Ro, S.; Choi, K.-H.; Choi, Y.-S.; Lee, K.-B. *J. Pept. Res.* **2000**, *56*, 35.
20. André, F.; Vicherat, A.; Boussard, G.; Aubry, A.; Marraud, M. *J. Pept. Res.* **1997**, *50*, 372.
21. Zouikri, M.; Vicherat, A.; Aubry, A.; Marraud, M.; Boussard, G. *J. Pept. Res.* **1998**, *52*, 19.
22. Lee, H.-J.; Choi, K.-H.; Ahn, I.-A.; Ro, S.; Jang, H. G.; Choi, Y.-S.; Lee, K.-B. *J. Mol. Struct.* **2001**, *569*, 43.
23. Lecoq, A.; Boussard, G.; Marraud, M. *Tetrahedron Lett.* **1992**, *33*, 5209.
24. Didierjean, C.; Del Duca, V.; Benedetti, E.; Aubry, A.; Zouikri, M.; Marraud, M.; Boussard, G. *J. Pept. Res.* **1997**, *50*, 451.
25. André, F.; Boussard, G.; Bayeul, D.; Didierjean, C.; Aubry, A.; Marraud, M. *J. Pept. Res.* **1997**, *49*, 556.
26. Benatalah, Z.; Aubry, A.; Boussard, G.; Marraud, M. *Int. J. Pept. Protein Res.* **1991**, *38*, 603.
27. Lecoq, A.; Boussard, G.; Marraud, M.; Aubry, A. *Biopolymers* **1993**, *33*, 1051.
28. Thormann, M.; Hofmann, H.-J. *J. Mol. Struct.* **1999**, *469*, 63.
29. Lee, H.-J.; Song, J.-W.; Choi, Y.-S.; Ro, S.; Yoon, C. *Phys. Chem. Chem. Phys.* **2001**, *3*, 1693.
30. Zhang, W. J.; Berglund, A.; Kao, J. L.-F.; Couty, J. P.; Gershengorn, M. C.; Marshall, G. R. *J. Am. Chem. Soc.* **2003**, *125*, 1221.
31. Lee, H. J.; Song, J. W.; Choi, Y. S.; Park, H. M.; Lee, K. B. *J. Am. Chem. Soc.* **2002**, *124*, 11881.
32. Lee, J. Y.; Moon, J. S.; Eu, Y. J.; Lee, C. W.; Yang, S. T.; Lee, S. K.; Jung, H. H.; Kim, H. H.; Rhim, H.; Seong, J. Y.; Kim, J. I. *Arch. Biochem. Biophys.* **2009**, *485*, 109.
33. Wilcox, P. E. *Methods Enzymol.* **1970**, *19*, 64.
34. Appel, W. *Clin. Biochem.* **1986**, *19*, 317.
35. Niida, A.; Wang, Z.; Tomita, K.; Oishi, S.; Tamamura, H.; Otaka, A.; Navenot, J. M.; Broach, J. R.; Peiper, S. C.; Fujii, N. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 134.

36. Gutiérrez-Pascual, E.; Leprince, J.; Martínez-Fuentes, A. J.; Ségalas-Milazzo, I.; Pineda, R.; Roa, J.; Duran-Prado, M.; Guilhaudis, L.; Desperrois, E.; Lebreton, A.; Pinilla, L.; Tonon, M. C.; Malagón, M. M.; Vaudry, H.; Tena-Sempere, M.; Castaño, J. P. *Mol. Pharmacol.* **2009**, *76*, 58.
37. Niedrich, H. *J. Prakt. Chem.* **1972**, *314*, 749.
38. Niedrich, H.; Oehme, C. J. *J. Prakt. Chem.* **1972**, *314*, 759.
39. Oehme, P.; Bergmann, J.; Falk, M.; Reich, J. G.; Voigt, W. E.; Niedrich, H.; Pirrwitz, J.; Bersek, C.; Jung, F. *Acta Biol. Med. Ger.* **1972**, *28*, 109.
40. Oehme, P.; Bergmann, J.; Niedrich, H.; Jung, F.; Menzel, G.; Eichstädt, M. *Acta Biol. Med. Ger.* **1970**, *25*, 613.
41. Han, H.; Yoon, J.; Janda, K. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 117.
42. Dutta, A. S.; Gormley, J. J.; Hayward, C. F.; Morley, J. S.; Shaw, J. S.; Stacey, G. J.; Turnbull, M. T. *Life Sci.* **1977**, *21*, 559.
43. Dutta, A. S.; Gormley, J. J.; Hayward, C. F.; Morley, J. S.; Shaw, J. S.; Stacey, G. J.; Turnbull, M. T. *J. Pharmacol.* **1977**, *61*, 481.
44. Ho, T. L.; Nestor, J. J.; McCrae, G. I.; Vickery, B. H. *Int. J. Peptide Protein Res.* **1984**, *24*, 79.
45. Dutta, A. S.; Furr, B. J. A.; Giles, M. B.; Valcaccia, B.; Walpole, A. L. *Biochem. Biophys. Res. Commun.* **1978**, *81*, 382.
46. Furr, B. J. A. *Akt. Onkol.* **1983**, *8*, 243.
47. Furr, B. J. A.; Valcaccia, B. E.; Hutchinson, F. G. *Brit. J. Cancer* **1983**, *48*, 140.
48. Dutta, A. S.; Furr, B. J. A. *Ann. Rep. Med. Chem.* **1985**, *20*, 203.
49. Zhang, W. J.; Berglund, A.; Kao, J. L. F.; Couty, J. P.; Gershengorn, M. C.; Marshall, J. R. *J. Am. Chem. Soc.* **2003**, *125*, 1221.