Amino Acids (2013) 45:975–981
DOI 10.1007/s00726-013-1554-5

ORIGINAL ARTICLE

Synthesis of novel peptides through Ugi-ligation and their anti-cancer activities

Fatemeh Tahoori · Reza Sheikhnejad · Saeed Balalaie · Mahnaz Sadjadi

Received: 19 April 2013 / Accepted: 28 June 2013 / Published online: 18 July 2013

Abstract Proline-rich heptapeptide was synthesized and its structure was modified through Ugi-ligation. The desired pseudopeptides were separated as diastereomers and their anti-cancer activities were investigated. Their in vitro anti-cancer activities were investigated by treating HL60 (leukemia cancer cells), MCF7 (breast cancer cells) and A549 (lung cancer cells) cells with appropriate amounts of synthesized peptides. Our in vitro studies suggest that compounds **11a–b**, **11i–j**, and **11e** had little or no effect on cancer cells viabilities.

Keywords Ugi-ligation · Ligation of peptides · Anti-cancer activity · Pseudopeptides

Introduction

The interaction of some regulatory proteins with transcriptional regulatory elements within genome might regulate gene expression. Assays were deployed to systematically identify DNA-binding transcriptional regulators in nuclear extracts (Mirzaei et al. 2013). They identified 15 regulators that bound specifically to distinct regions along ~600 bp of the regulatory sequence. More than 100 active regulatory DNAs have been identified in F9 cells correspond to promoter elements, which display several features of endogenous transcriptional regulators, including CpG islands (Yaragatti et al. 2008; Akopov et al. 2007). The availability of complete genome sequence has made it possible to develop computational methods for the detection of transcriptional regulatory elements. The physiological roles of DNA-binding proteins depend upon the precise interactions between amino acids in the DNA-binding protein and nucleotides in the DNA-binding site.

How can we identify features of a DNA-binding site or DNA-binding protein? Many DNA-binding proteins have common structural motifs involved in DNA–protein interactions. But it is not possible to identify the amino acids involved in DNA-binding by simple sequence gazing. Therefore, the critical nucleotides in a DNA-binding site and the interacting amino acids in DNA-binding protein must be determined empirically (Maloy et al. 1996).

Based on DNA–Protein interactions, we have taken specific regions of ras oncogene promoter within the CpG islands into consideration to design some hypothetical heptapeptides (Scheme 1). The proposed mechanism of action, although has not been determined yet, may be due to the direct/or indirect repression of oncogenic activities at transcriptional level. At the extreme, they can also function as "switches", which can turn a gene on and off.

The selected heptapeptide was then chemically modified to enhance its potency as a potential anti-cancer drug. The selected chemical modification was combination of biologically active peptide segments. There are several methods for the coupling of peptide segments; among them, native chemical ligation is the most useful method employed. The native chemical ligation (NCL) reaction is a powerful method to join two unprotected peptides in

Dedicated to Dr. Mostafa Ghanei for his brilliant role in pharmaceutical research in Iran.

F. Tahoori · S. Balalaie (✉)
Peptide Chemistry Research Center, K. N. Toosi University of Technology, P.O. Box 15875-4416, Tehran, Iran
e-mail: balalaie@yahoo.com; balalaie@kntu.ac.ir

R. Sheikhnejad · M. Sadjadi
Tofigh Daru Research and Engineering Company, 61st. Km 18 Karaj Highway, P.O. Box 37515-375, Tehran, Iran

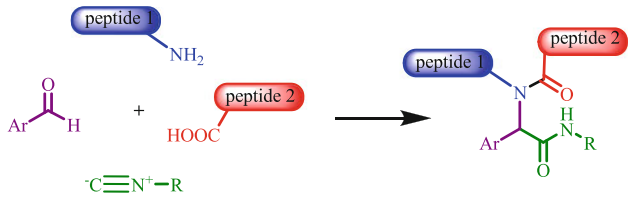
H_2N-Ser- Ala-Pro-Pro-Pro-Arg-Lys -OH **(1)**

H_2N-Gly- Ala- Pro- Pro- Gly- Arg- Asp- OH (**2**)

H_2N-Arg- Pro- Pro- Gly- Ser- Pro- Ala- OH (**3**)

H_2N-Phe- Ala- Gly- Arg- Ser- Arg- Gly- OH **(4)**

Scheme 1 The compositions of the designed heptapeptides **1–4**



Scheme 2 Ugi-ligation for the conjunction of two peptide segments

aqueous solution (Haase et al. 2008; Dose and Seitz 2005; Mende and Seitz 2007; Hackenberger and Schwarzer 2008; Kumar and Brik 2010). The rare cysteine requirement limits the applicability of the naturally occurring protein synthesis. Although NCL can provide a facile route for the synthesis of proteins, this amide-bond forming chemistry has been limited to the sequential ligation of peptides in the *C*-to-*N* direction, and most synthetic proteins have been prepared by sequential NCL of 2–4 peptide segments (Siman et al. 2012; Brik and Wong 2007). Another approach for the ligation is Staudinger reaction (Nepomniaschiy et al. 2008; Tian and Wang 2004; Lin et al. 2005). Ugi-ligation was used for the conjunction of peptides with additional amide bonds and also lipophilic moieties (Arabanian et al. 2009; Saleh-Abady et al. 2010).

Designing of novel MCRs for the synthesis of biologically active compounds is an interesting task in organic synthesis and has been reviewed (Dömling et al. 2012). In continuation of our research work to use of Ugi-four-component reaction (Ugi-4CR) for the synthesis of novel compounds (Bararjanian et al. 2010, 2011; Balalaie et al. 2011, 2012; Haghighatnia et al. 2012; Ramezanpour et al. 2013), herein we wish to report an approach for the synthesis of designed peptides **1–4** and also the novel peptides through Ugi-4CR from *C*-terminus pentapeptides, *N*-terminus dipeptide, aromatic aldehydes and isocyanides. The synthesized peptides contain lipophilic moieties and several amide bonds (Scheme 2).

Results and discussion

At first, the heptapeptides **1–4** were synthesized based on the known solid phase peptide synthesis. The purification of peptides was done using preparative HPLC. The

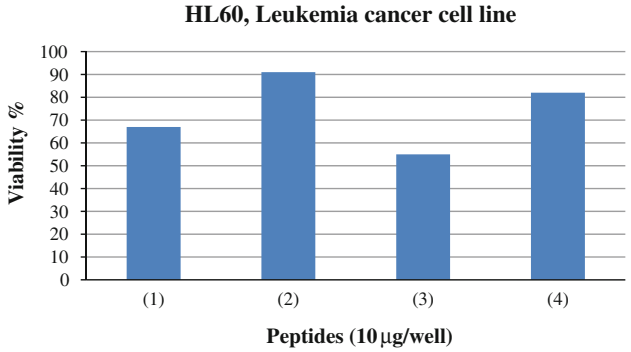


Fig. 1 The anti-cancer activity of a specific heptapeptides **1–4** on HL 60 cell viabilities

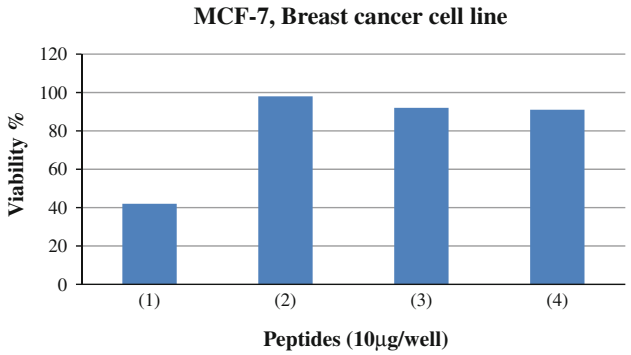


Fig. 2 The anti-cancer activity of a specific heptapeptides **1–4** on MCF7 cell viabilities

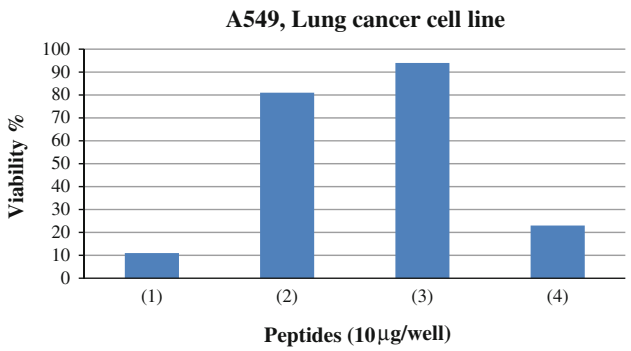
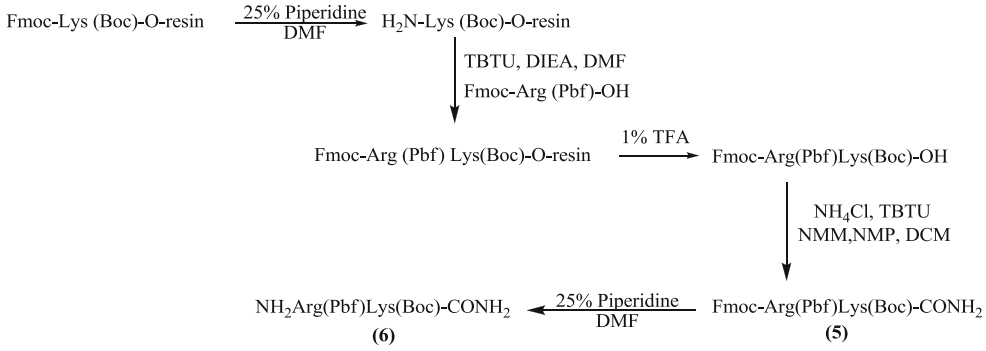


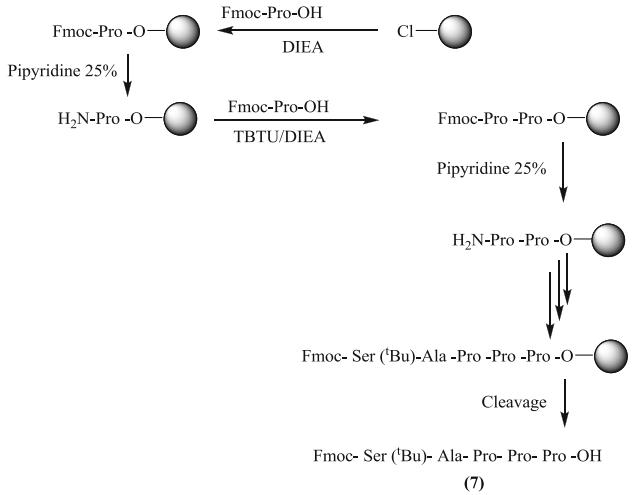
Fig. 3 The anti-cancer activity of a heptapeptides **1–4** on A549 cell viabilities

structure of the synthesized peptides was confirmed by their HR-mass (ESI) spectral data. Their in vitro anti-cancer activities were investigated by treating HL60 (leukemia cancer cells), MCF7 (breast cancer cells) and A549 (lung cancer cells) cells with appropriate amounts of our peptides (Figs. 1, 2, 3).

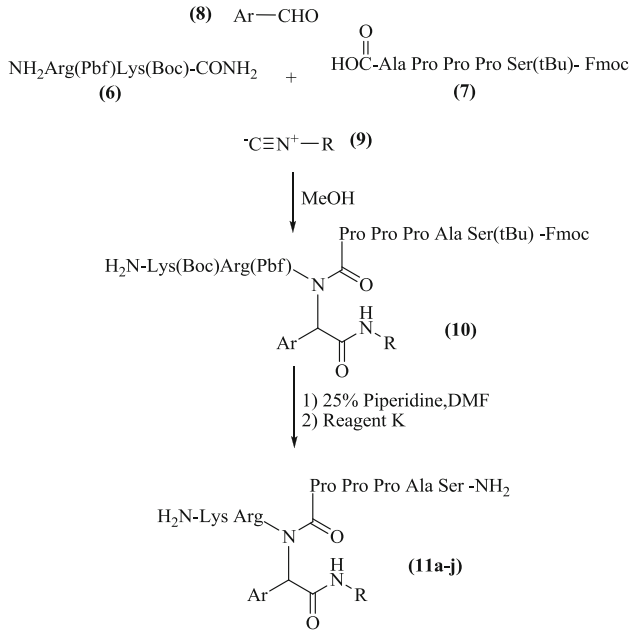
Based on our in vitro anti-cancer activity results, heptapeptide **1** was selected as a model to be modified based on the ligation of peptide segments. This study was focused

Scheme 3 General method for the synthesis of amidated dipeptide **6**





Scheme 4 General method for the synthesis of pentapeptide **7**



Scheme 5 Ugi-ligation approach for the synthesis of pseudopeptides **11a–j**

on the design and synthesis of *C*-terminus pentapeptide and *N*-terminus dipeptide as our starting materials. The *C*-terminus pentapeptide **2** was synthesized using known Fmoc solid phase peptide synthesis strategy. Meanwhile, TBTU was used as the coupling reagent for the synthesis of *C*-terminus pentapeptide and *N*-terminus dipeptide. The dipeptide **3** was synthesized in solution phase with Boc/*Z* strategy as well as Fmoc solid phase strategy. The details for the synthesis of *N*-terminus dipeptide are shown in Scheme 3.

The synthesis of dipeptide **6** started with the coupling reaction of loaded Fmoc-Lys (Boc)-OH (2-chlorotrityl chloride was used as resin) with Fmoc-Arg (Boc)-OH in presence of TBTU as coupling reagent and base to afford the protected dipeptide **6**. Cleavage of the peptide from the surface of resin was done using TFA (1 %). Amidation of *C*-terminal was done using ammonium chloride in the presence of coupling reagent. Final Fmoc deprotection was performed using 25 % piperidine to obtain the deprotected dipeptide.

The *C*-terminus pentapeptide (**7**) was synthesized on the surface of 2-chlorotrityl chloride resin and Fmoc-protected amino acids, and they were coupled according to known methods. The synthetic details were clarified in the experimental section. The details were shown in Scheme 4.

After synthesis of *C*-terminal pentapeptide and *N*-terminal dipeptide, four-component reaction of these synthesized peptides with isocyanide and aromatic aldehyde was done. This approach has two-point of diversity. Different aromatic aldehydes and two isocyanides were used in this reaction (Scheme 5). The results were summarized in Table 1.

The products were purified using preparative HPLC and their structures were confirmed by ESI mass spectrometry method. The products were mixture of two diastereomers and their ratios were obtained based on HPLC data and were shown in Table 1. The diastereomers were separated using preparative HPLC, and their anti-cancer activities were investigated separately. The diastereomers were shown as *d*1, and *d*2 for every compound. The results are summarized in Figs. 4 and 5.

One of advantage of synthesized peptide was the number of amide bond in its structure. In this way, we synthesized amidated *C*-terminal heptapeptide as a standard and its anti-cancer activity was compared to the synthesized peptides **11a–j**. Amidated *C*-terminal peptide was synthesized using reaction of *C*-terminal peptide with ammonium chloride in the presence of *N*-methyl morpholine (Scheme 6) (Arabanian et al. 2010).

Table 1 Synthesis of functionalized peptides through Ugi-ligation

Entry	Ar	*R*	Product	Yield (%)	Diastereomeric ratio
1	4-NC-C_6H_4	Cy-Hexyl	**11a**	79	60:40
2	4-NC-C_6H_4	*t*-Bu	**11b**	59	62:38
3	4-F-C_6H_4	Cy-Hexyl	**11c**	44	59:41
4	4-F-C_6H_4	*t*-Bu	**11d**	36	63:37
5	4-Cl-C_6H_4	Cy-Hexyl	**11e**	83	55:45
6	4-Cl-C_6H_4	*t*-Bu	**11f**	45	63:37
7	4-Br-C_6H_4	Cy-Hexyl	**11g**	72	50:50
8	4-Br-C_6H_4	*t*-Bu	**11h**	45	57:43
9	2-Thienyl	Cy-Hexyl	**11i**	53	59:41
10	2-Thienyl	*t*-Bu	**11j**	84	77:33

The unique resemblance of this particular heptapeptide to ABC transporter might facilitate its entrance into the cells. The specific anti-cancer activity of this peptide may prove the concept but it needs further investigation to determine its specific target(s) and the mechanism of action. The prepared peptide has more amide bonds, and it seems that it could be more stable against proteases, thus a better candidate for the therapeutic purposes. The presence of aryl, cyclohexyl and alkyl groups in the structure of the products mimics the peptide backbone and increases hydrophobic properties of the molecule that could affect its biological activity. The anti-cancer activities of our specific synthesized amidated heptapeptide **12** and its chemically modified variants (**11a–j**) were determined against breast and lung cancer cell viabilities. The results are shown in Figs. 4 and 5.

Anti-cancer activity results

Based on in vitro cancer cells viabilities, compounds **11d, f** and **g** seem to be more active compared to peptide A. Meanwhile the two diastereomers showed different activities. The selected heptapeptide was originally active against A549, lung cancer cells in its *C*-terminal-OH form.

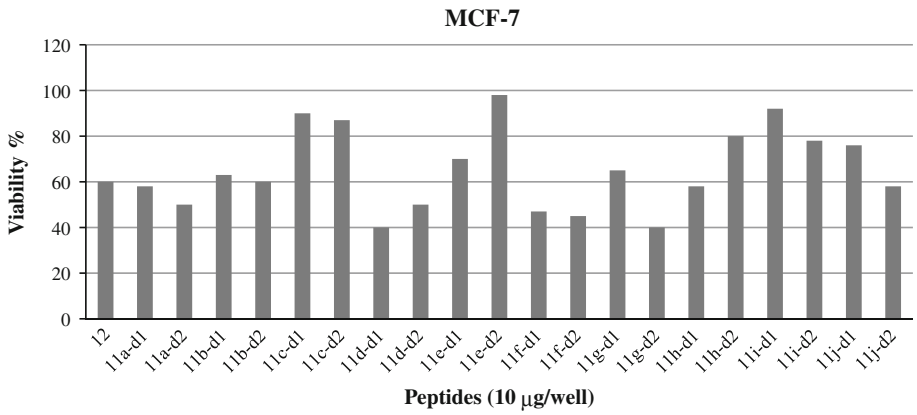


Fig. 4 The anti-cancer activity of a specific heptapeptide and its chemically modified variants on MCF7, breast cancer cell viabilities

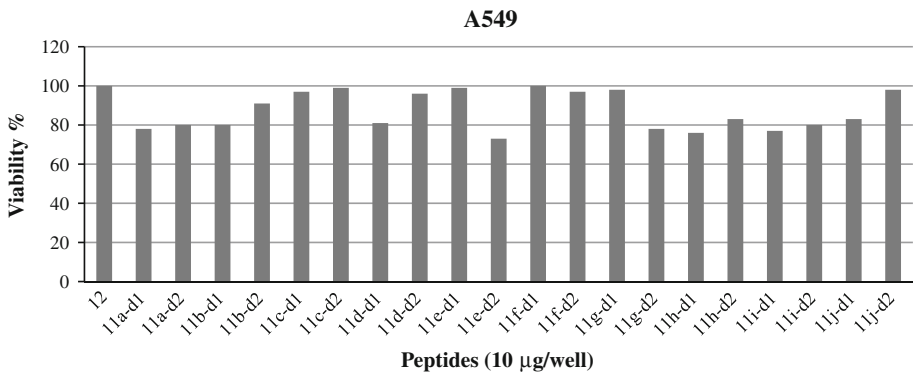
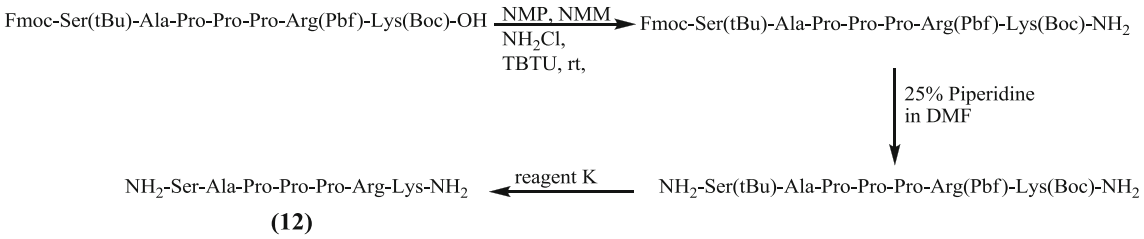
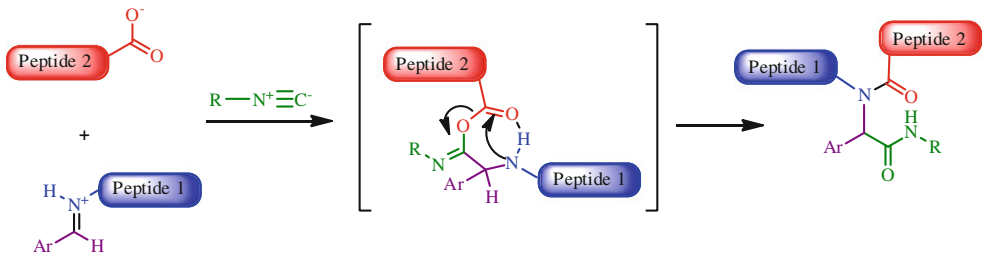


Fig. 5 The anti-cancer activities of a specific heptapeptides and its chemically modified variants on A549, lung cancer cell viabilities



Scheme 6 Synthesis of amidated peptide **12**



Scheme 7 Proposed mechanism for the synthesis of peptides **11a–j**

In this study, we synthesized *C*-terminal-NH_2 version of this peptide and further modified it using Ugi-4CR. Surprisingly, the amidated form was not active against A549 but it had some effects (40 %) on MCF7, breast cancer cells. The Ugi-4CR modification, however, significantly affects the anti-cancer activity of this heptapeptide. In the structure of peptides **11a–b** the 4-cyano benzaldehyde was used as aldehyde and in the cases **11i–j** where 2-thienyl derivatives were used they had little or no effect on either cell lines. However, compound **11e** had some effects on A549 cells (25 %) but no effect on breast cancer cells (MCF7). This may be due to different ras oncogenic activities in these two cell lines (c-ha-ras in MCF7, ki-ras in A549). The compound **11d** has 60 % activity on MCF7 but when *t*-butyl isocyanide was replaced with cyclohexyl isocyanide **(11c)** the activity was reduced to only 10 %. Contrary to fluoro aryl derivative, when 4-bromo benzaldehyde was used as aldehyde and cyclohexyl isocyanide to form **11h** was 60 % active while **11g** which contained *t*-butyl moiety had only 10 % effects on cell viability.

The generally accepted mechanism of the Ugi-4CR involves four elementary steps, with the last step being irreversible. In the first step, the aldehyde condenses with the amino group of *N*-terminus dipeptide to form an imine that could be converted to produce iminium salt in the presence of *C*-terminus pentapeptides. Second, the isocyanide is added to the iminium salt to produce a nitrilium ion. Third, a reactive *O*-acyl iminolate is formed via the addition of carboxylate anion to the nitrilium ion. The final step involves the *O*- to *N*-acyl transfer (Mumm rearrangement) to afford the desired peptide (Scheme 7).

Conclusion

In summary, we are reporting the synthesis of novel functionalized peptides through Ugi-ligation. The desired products **11a–j** were formed in good yields and good diastereo-selectivity. The anti-cancer activities of isolated diastereomers were investigated. The broad scope, operational simplicity, practicability, high yields and mild reaction conditions render it an attractive approach for the generation of different functionalized peptides. In some case, the Ugi-ligation products showed better anti-cancer activity compared to linear heptapeptide. The synthesized peptides are examples of modified proline-rich peptides. Our in vitro studies suggest that compounds **11a–b**, **11i–j**, and **11e** had little or no effect on cancer cells viabilities. However, **11d** and **11g** both had about 60 % activity on MCF7 cells. Interestingly, when *t*-butyl isocyanide and cyclohexyl isocyanide were replaced by one another, the activity was sharply reduced to only 10 %. It may related to the size and spacing of substituents. The research to find the more active compounds is in progress in our laboratory.

Experimental section

Commercially available materials were used without further purification. High resolution mass spectra were recorded on Mass-ESI-POS (Apex Qe-FT-ICR instrument) spectrometer.

AAPTEC Focus-Xi instrument was used as peptide synthesizer for the synthesis of *C*- and *N*-terminal peptides.

General procedure for the synthesis of pentapeptides-COOH (**7**)

Synthesis was carried out using 2-chlorotrityl chloride resin (1.0 mmol/g) following standard Fmoc strategy. Fmoc-Pro-OH (3.37 g, 10 mmol) was attached to the 2-CTC resin (5.0 g) with DIPEA (6.85 ml, 40 mmol) in anhydrous DCM:DMF (50 ml, 1:1) at room temperature for 2 h. After filtration, the remaining trityl chloride groups were capped by a solution of DCM/MeOH/DIPEA (17:2:1, 120 ml) for 30 min. It was then filtered and washed thoroughly with DCM (1 × 20 ml), DMF (4 × 20 ml) and MeOH (5 × 20 ml). The loading capacity was determined by weight after drying the resin under vacuum and was 1.0. The resin-bound Fmoc-amino acid was washed with DMF (3 × 20 ml) and treated with 25 % piperidine in DMF (65 ml) for 30 min and the resin was washed with DMF (3 × 20 ml). Then a solution of Fmoc-Pro-OH (2.53 g, 7.5 mmol), TBTU (2.40 g, 7.5 mmol), DIPEA (3.0 ml, 17.5 mmol) in 30 ml DMF was added to the resin-bound free amine and shaken for 1 h at room temperature. After completion of coupling, resin was washed with DMF (4 × 20 ml) and DCM (1 × 20 ml). The coupling was repeated as the same methods for other amino acids of their sequences. In all cases for the presence or absence of free primary amino groups, Kaiser test was used. Fmoc determination was done using UV spectroscopy method. After completion of couplings, resin was washed with DMF (4 × 20 ml), DCM (1 × 20 ml). The produced pentapeptide was cleaved from resin by treatment of TFA (1 %) in DCM (275 ml) and neutralization with pyridine (4 %) in MeOH (85 ml). The solvent was removed under reduced pressure and precipitated in water. The yield was 82 % (3.0 g of pentapeptide **7**).

The syntheses of heptapeptides **1–4** were done based on the same procedure with appropriate protected amino acids.

HR-mass (ESI) heptapeptides

Peptide (**1**): $C_{33}H_{58}N_{11}O_9$ $[M+1]^+$ found 752.44183, calc. 752.44189. $C_{33}H_{57}N_{11}NaO_9$ $[M + Na]^+$ found 774.42400, calc. 774.42409.

Peptide **(2)**: $C_{27}H_{45}N_{10}O_{10}$ $[M+1]^+$ found 669.33266, calc. 669.33284.

Peptide **(3)**: $C_{29}H_{49}N_{10}O_9$ $[M+1]^+$ found 681.36879, calc. 681.36893.

Peptide **(4)**: $C_{31}H_{52}N_{13}O_9$ $[M+1]^+$ found 750.40269, calc. 750.40298.

Amidation of C-terminal of dipeptide

After cleavage of the dipeptide **(5)** from the resin, purification was performed by washing with distilled water. Then dipeptide (1.0 mmol) and 0.55 ml NMM (5.0 mmol) were added to a solution of 0.483 g TBTU (1.5 mmol) and 0.106 g NH_4Cl (2.0 mmol) in 2 ml DCM:NMP (50:50). The mixture was stirred overnight. The progress of reaction was monitored using TLC. The dipeptide was precipitated in water and the *C*-terminal amidated dipeptide **(6)** was dried.

Amidated peptide **(12)**: $C_{33}H_{59}N_{12}O_8$ $[M+1]^+$ found 751.44190, calc. 751.44221.

General procedure for the synthesis of peptide **11a–j** via Ugi-4CR

The Ugi-4CR between an aldehyde, an amine, a carboxylic acid and an isocyanide allows the rapid preparation of α-aminoacyl amide derivatives. Here, after Fmoc deprotection from the dipeptide, a solution of 0.653 g H_2N-Arg (Pbf)-Lys (Boc)-$CONH_2$ (1 mmol), benzaldehyde derivatives or thiophene-2-carbaldehyde (1 mmol) derivatives, in 5 ml MeOH was added for the formation of imine. After 5 h, 0.745 g Fmoc-heptapeptide-OH (0.745 g, 1 mmol) was added, this reaction followed with the addition of isocyanide (1 mmol) derivatives. The mixture was stirred for 48 h. Reaction followed with TLC (10:2:1 EtOAc:-MeOH:H_2O). After completion of the reaction, products precipitated in water. Final Fmoc deprotection was done with 25 % piperidine in DMF, and final deprotection was done with reagent *K*. All of the products were purified using prep-HPLC. The structures of products were confirmed according to their spectral data and ESI (HR)-mass spectra.

*HR-mass (ESI) of **11a–j***

11a: $C_{48}H_{75}N_{14}O_9$ $[M+H]^+$ found 992.04463, calc. 992.04458.

11b: $C_{45}H_{70}N_{14}O_8$ $[M-(CH_2OH)+H]^+$ found 935.48385, calc. 935.48362, $C_{45}H_{69}N_{14}NaO_8$ $[M-(CH_2OH)+Na]^+$ found 957.46574, calc. 957.46551.

11c: $C_{47}H_{75}FN_{13}O_9$ $[M+H]^+$ found 985.74825, calc. 985.74786.

11d: $C_{45}H_{73}FN_{13}O_9$ $[M+H]^+$ found 957.46642, calc. 957.46679.

11e: $C_{47}H_{75}ClN_{13}O_9$ $[M+H]^+$ found 1,000.40718, calc. 1,000.40785.

11f: $C_{45}H_{73}ClN_{13}O_9$ $[M+H]^+$ found 974.33309, calc. 974.33396, $C_{45}H_{71}ClN_{13}O_8$ $[M-H_2O+H]^+$ found 957.46632, calc. 957.46648.

11g: $C_{47}H_{75}BrN_{13}O_9$ $[M+H]^+$ found 1,043.55787, calc. 1,043.55794.

11h: $C_{45}H_{73}BrN_{13}O_9$ $[M+H]^+$ found 1,019.77406, calc. 1,019.77443.

11i: $C_{45}H_{74}N_{13}O_9S$ $[M+H]^+$ found 972.24586, calc. 972.24566.

11j: $C_{43}H_{72}N_{13}O_9S$ $[M+H]^+$ found 946.66710, calc. 946.66747.

General in vitro experiments

Cancer cells were all seeded at 5,000 cells/well in a 96-well plate and the culture was maintained in RPMI 1640 supplemented with 10 % fetal bovine serum, 1 % L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin overnight. The media was replaced with fresh media containing up to 50 μg of peptides and incubated for 48 h in a humidified atmosphere of 95 % air and 5 % CO_2 at 37 °C until the control cultures were confluent. The media was then removed and plate was washed two times with phosphate-buffered saline (PBS). Serum free media (100 μl) containing 0.5 mg/ml MTT dye was added into each well and incubated at 37 °C for 2 h. The media with dye was removed, washed with PBS and the reactive dye was solubilized by addition of 100 μl dimethyl sulfoxide. The absorbance was read using an automatic multi-well spectrophotometer. The experiment was always performed in triplicates.

Acknowledgments S. B. gratefully acknowledges Iran National Science Foundation (INSF). We are also thanking Iranian ministry of health and medicinal education and also presidential office deputy of science and technology for financial support.

Conflict of interest The authors declare that they have no conflict of interest.

References

Akopov SB, Chernov IP, Bulanenkova SS, Skvortsova YV, Vetchinova AS, Nikolaev LG (2007) Methods for identification of epigenetic elements in mammalian long multigenic genome sequences. Biochemistry 72:589–594

Arabanian A, Mohammadnejad M, Balalaie S (2009) Synthesis of novel GnRH analogues using Ugi-4CR. Bioorganic Med Chem Lett 19:887–890

Arabanian A, Mohammadnejad M, Balalaie S (2010) A novel and efficient approach for the amidation of *C*-terminal peptides. J Iran Chem Soc 7:840–845

Balalaie S, Bararjanian M, Rominger F, Hosseinzadeh S, Bijanzadeh R, Wolf E (2011) Designing a sequential Ugi/Ullmann type reaction for the synthesis of indolo[1,2-a]quinoxalinones catalyzed by CuI/L-proline. Tetrahedron 67:7294–7300

Balalaie S, Motaghedi H, Tahmassebi D, Bararjanian M, Bijanzadeh HR (2012) A facile and efficient synthesis of 2,2,2-trifluoroethyl 2-[(E)-N-phenylcinnamamido]-2-phenylacetates in trifluoroethanol via sequential Ugi four-component reaction/esterification. Tetrahedron Lett 53:6177–6181

Bararjanian M, Balalaie S, Rominger F, Movassagh B, Bijanzadeh HR (2010) Six-component reactions for the stereoselective synthesis of 3-arylidene-2-oxindoles via sequential one-pot Ugi/Heck carbocyclization/Sonogashira/nucleophilic addition. J Org Chem 75:2806–2812

Bararjanian M, Balalaie S, Movassagh B, Rominger F, Bijanzadeh HR (2011) Novel and efficient one-pot five- and six-component reactions for the stereoselective synthesis of highly functionalized enaminones and dithiocarbamates. Mol Divers 15:583–594

Brik A, Wong CH (2007) Sugar-assisted ligation for the synthesis of glycopeptides. Chem Eur J 13:5670–5675

Dömling A, Wang W, Wang K (2012) Chemistry and biology of multicomponent reactions. Chem Rev 112:3083–3135

Dose C, Seitz O (2005) Convergent synthesis of peptide nucleic acids by native chemical ligation. Org Lett 7:4365–4368

Haase C, Rohde H, Seitz O (2008) Native chemical ligation at valine. Angew Chem Int Ed 47:6807–6810

Hackenberger CPR, Schwarzer D (2008) Chemoselective ligation and modification strategies for peptides and proteins. Angew Chem Int Ed 47:10030–10074

Haghighatnia Y, Balalaie S, Bijanzadeh HR (2012) Designing and synthesis of novel amidated fentanyl analogs. Helv Chim Acta 95:818–824

Kumar KSA, Brik A (2010) Accessing posttranslationally modified proteins through thiol positioning. J Pep Sci 16:524–529

Lin FL, Hoyt HM, Halbeek HV, Bergman RG, Bertozzi CR (2005) Mechanistic investigation of the Staudinger ligation. J Am Chem Soc 127:2686–2695

Maloy S, Stewart V, Taylor R (1996) Genetic analysis of pathogenic bacteria. Cold Spring Harbor Laboratory Press, New York

Mende F, Seitz O (2007) Solid-phase synthesis of peptide thioesters with self-purification. Angew Chem Int Ed 46:4577–4580

Mirzaei H, Knijnenburg TA, Kim B, Robinson M, Picotti P, Carter GW, Li S, Dilworth DJ, Eng JK, Aitchison JD, Shmulevich I, Galitski T, Aebersold R, Ranish J (2013) Systematic measurement of transcription factor-DNA interactions by targeted mass spectrometry identifies candidate gene regulatory proteins. Proc Natl Acad Sci. doi:10.1073/pnas.1216918110

Nepomniaschiy N, Grimminger V, Cohen A, Digiovanni S, Lashuel HA, Brik A (2008) Switch peptide via Staudinger reaction. Org Lett 10:5243–5246

Ramezanpour S, Balalaie S, Rominger F, Bijanzadeh HR (2013) An efficient and diastereoselective synthesis of hydrazino amides via a novel one-pot three-component reaction. Tetrahedron 69:3480–3485

Saleh-Abady MM, Naderi-Manesh H, Alizadeh A, Shamsipour F, Balalaie S, Arabanian A (2010) Anticancer activity of a new gonadotropin releasing hormone analogue. Pept Sci 94:292–297

Siman P, Vedhamarayanan K, Brik A (2012) Native chemical ligation at glutamine. Org Lett 14:1520–1523

Tian WQ, Wang YA (2004) Mechanisms of Staudinger reactions within density functional theory. J Org Chem 69:4299–4308

Yaragatti M, Basilico C, Dailey L (2008) Identification of active transcriptional regulatory modules by the functional assay of DNA from nucleosome-free regions. Genome Res 18:930–938