Amino Acids
DOI 10.1007/s00726-013-1663-1

ORIGINAL ARTICLE

Design and synthesis of anti-cancer cyclopeptides containing triazole skeleton

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Received: 4 November 2013 / Accepted: 29 December 2013

Abstract We describe the design and synthesis of some hypothetical heptapeptides specifically to overcome the neoplastic activity of ras oncogene and their anti-cancer activities were studied. To improve the anti-cancer activity of the synthesized peptides, their structure modifications were done based on a sequential Ugi/Huisgen 1,3-Dipolar cyclization reaction. The cyclopeptides which contained triazole skeleton showed significant anti-cancer activity against cancer cells with mutated ras oncogene such as A549, PC3 and C26 cells. This study clearly shows the importance of triazole skeleton in biological activity of the peptides. It might be possible to overcome the difficulties involved in making complex peptides by employing this elegant chemistry.

Keywords Ugi ligation · Ligation of peptides · Anti-cancer activity · Cyclopeptides · Click reaction · Huisgen 1,3-Dipolar reaction

Introduction

Several monoclonal antibodies such as Rituximab (anti-CD20 antibody) and Herceptin (anti-HER-2 antibody) have been approved for the treatment of some cancers. The efficacy of this cancer immunotherapy is, however, limited by its large size and its nonspecific binding to the reticuloendothelial system that causes many undesirable side effects (Aina et al. 2007). Furthermore, the drug research and development has become very expensive and the number of approved drugs has been declining in recent years. Therefore, the demands for alternative approaches are very high. This has contributed to the revival of peptides as potential therapeutic drugs. A large number of peptide-based drugs are now being marketed because new synthetic strategies have been developed in recent years (Vlieghe et al. 2010).

One classical strategy used in drug design is based on the structure of receptor-binding pocket, called "rational structure-based design" (Shoichet et al. 1993; Von Itzstein et al. 1993). Most peptide drugs are designed this way. Here we have used a novel strategy based on DNA–protein binding criteria to design anti-cancer drugs. We focused our interest on finding specific DNA–protein binding sites along the promoter elements of ras oncogene. The precise interactions between amino acid motifs of our designed peptides and ras-specific regulatory sites within the CpG islands might interfere with ras activity at transcriptional level. The most active peptide is then selected based on its in vitro anti-cancer activity to optimize its pharmaceutical value by means of different chemical approaches. One such approach would be the reduction of conformational space by cyclization.

Several hypothetical heptapeptides were designed based on DNA–protein binding criteria known for regulation of gene expression at transcriptional level. These peptides designed to perhaps suppress ras oncogenic activities in human cancer cells. The designed peptides **1–4** were tested for their anti-cancer activities against A549, human lung

Electronic supplementary material The online version of this article (doi:10.1007/s00726-013-1663-1) contains supplementary material, which is available to authorized users.

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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 Compositions of the designed heptapeptides **1–4**

cancer cells in vitro. The most active compound **1** is proline-rich peptide (Ball et al. 2005) selected to further improve its pharmaceutical potential with some specific chemical modifications to create a cyclic peptide (Scheme 1).

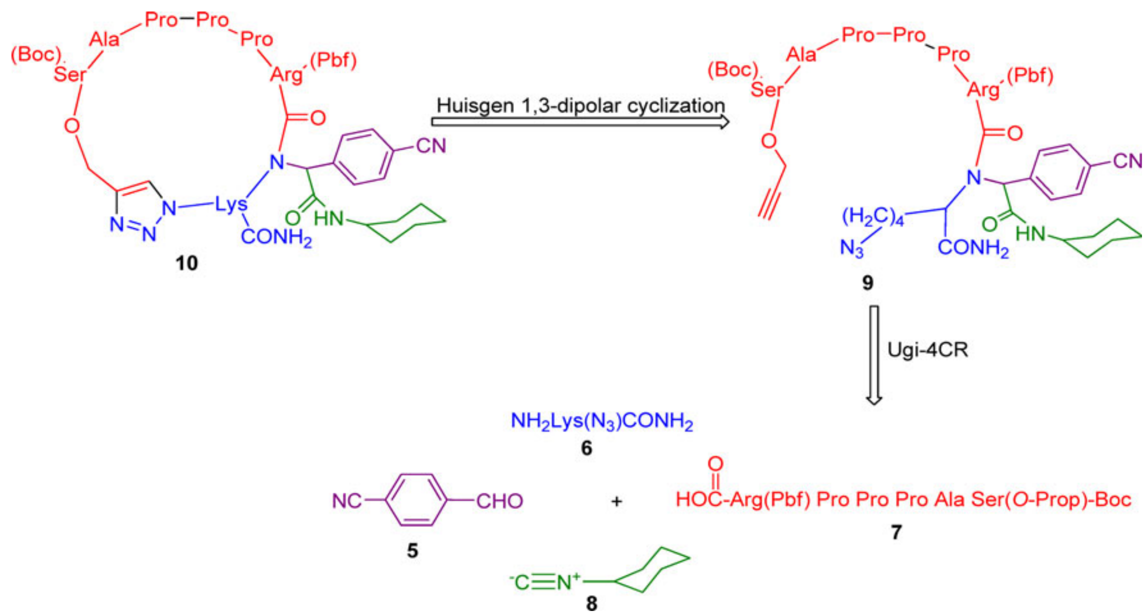
Cyclic peptides are a unique class of compounds that have made great contributions to the treatment of certain diseases such as cancer. Penicillin, vancomycin, cyclosporin, and echinocandins are well-known cyclic peptides. Cilengitide is also a cyclic pentapeptide currently in clinical trial for brain cancer, glioblastomas, and some other cancers (Katsara et al. 2006; Mas-Moruno et al. 2010; Boger 2001; Nicolaou et al. 1999; Rao et al. 1995; Chatterjee et al. 2005). Cyclic peptides, compared to linear peptides, have been considered to have greater potential as therapeutic agents. This may be due to their increased chemical stability, receptor selectivity, as well as improved pharmaco-dynamic properties. Considering these facts, we decided to use one of the known cyclization methods to prepare a unique cyclic heptapeptide. A reaction that would seem ideal for conjugation of peptides and oligonucleotides, due to the compatibility with many other functional groups, is the copper(I) catalyzed 1,3-Dipolar cycloaddition between an azide and an alkyne, commonly referred to as click chemistry (Rostovtsev et al. 2002; Kolb et al. 2001; Moses and Moorhouse 2007; Wu and Fokin 2007; Kolb and Sharpless 2003; Meldal and Tornoe 2008; Tornoe et al. 2002). Moreover, triazole-modified peptidomimetics have been shown as assembling protein-like oligomers and nonpeptidic protein-mimetic foldamers (Angelo and Arora 2005, 2007). Therefore, triazole-modified peptidomimetics have gained considerable attention for designing biological effectors or foldamers (Horne et al. 2004; Kuijpers et al. 2004; Cantel et al. 2008). The synthesis of 1,2,3-triazoles has grown in importance in medicinal (Chabre and Roy 2008; Colombo and Peretto 2008; Hanselmann et al. 2010; Moumne et al. 2010), material (Li et al. 2005; Rozkiewicz et al. 2006; Wyszogrodzka and Haag 2008; Gadzikwa et al. 2009; Golas and Matyjaszewski 2010; Bronisz 2005; Yue et al. 2007; Fazio et al. 2008; Fletcher et al. 2008; Hua and Flood 2010; Rawal et al. 2010), and biological researches (Hahn and Muir 2005; Heal et al. 2008; Ahsanullah et al. 2009; Schneider 2010; Chemama et al. 2009; Nahrwold et al. 2010; Michaels et al. 2010; Mamidyala and Finn 2010). Furthermore, a number of these compounds show a broad spectrum of biological activities, displaying, for example, antibacterial (Genin et al. 2000), herbicidal, fungicidal (Wamhoff 1984), antiallergic (Buckle et al. 1986), or anti-HIV (Alvarez et al. 1994) properties. Recently, 1,2,3-triazoles have also been used as catalysts and ligands in transition metal-based catalyst systems (Chan et al. 2004; Liu et al. 2005; Detz et al. 2006; Colasson et al. 2007; Beyer et al. 2009; Hein et al. 2009; Duan et al. 2009; Mager and Zeiler 2010). Since peptide **1** showed the best anti-cancer activity, we decided to modify its structure using functionalized amino acids which could form triazole scaffold.

Multicomponent reactions (MCR) have become important tools in the preparation of structurally diverse chemical libraries of drug-like polyfunctional compounds. However, to ensure sufficient molecular diversity and complexity of new chemical entities, there is a continuous need for novel reactions with high efficiency and selectivity in novel reaction media (Dömling 2005, 2006; Slobbe et al. 2012; Ruijter et al. 2011; Dömling et al. 2012; Tietze et al. 2006; Tietze and Hauner 2000). We intend to use the Ugi-4CR to construct products with further functional groups which are prone to additional ring closure reactions. This strategy allows us to prepare in a very economic and ecologic way complex systems (Bararjanian et al. 2010, 2011; Balalaie et al. 2011, 2012). Recently, we showed that the Ugi-4CR is an efficient approach for the synthesis of some novel GnRH analogs with better anti-cancer activity (Arabanian et al. 2009; Saleh-Abady et al. 2010). The 2D-NMR spectroscopic data showed that this reaction affects the structure of molecule on folding (Tahoori et al. 2010).

We report the design and synthesis of novel cyclopeptides through the Ugi ligation/click reaction to construct cyclopeptides which have a triazole moiety and also lipophilic moieties (Scheme 2).

Results and discussion

Reduction of conformational flexibility is important to increase the affinity of a peptide for its natural receptor. The first convenient approach to achieve this goal is head-to-tail cyclization. To achieve this goal, chemical modification of starting materials is important for many applications in biology and biotechnology. In order to synthesize cyclopeptides, different strategies were studied. Applying the well-known click chemistry is a known approach to synthesize cyclopeptides with triazole moiety. Functionalizing the scaffold with an alkyne moiety, and also an azide group, to form triazole skeleton is an approach to the synthesis of cyclopeptides.



Scheme 2 Retrosynthetic approach for the synthesis of cyclopeptide **10** through sequential Ugi ligation/Huisgen 1,3-Dipolar reaction

The click reactions have the potential to be further fortified when combined with multicomponent reactions. The idea of using MCRs followed by a Huisgen [3⇓2] copper-catalyzed reaction was investigated and a number of pharmaceutically relevant heterocyclic compounds were synthesized via classical multicomponent reactions combined with click chemistry in separate steps such as sequential Ugi/intermolecular alkyne–azide cycloaddition (IAAC) (Ramachary and Barbas 2004; Akritopoulou-Zanze et al. 2004).

The sequence of Ugi isocyanide multicomponent reaction, followed by post-condensation transformations, constitutes an extremely powerful synthetic tool for the preparation of structurally diverse complex molecules, especially heterocyclic compounds (Orru and Ruijter 2010; Aravind et al. 2011; De Graaff et al. 2012). Ultimately, this one-pot sequential combination of multi-catalysis and multicomponent approach should reduce the cost and waste associated with pharmaceutical synthesis.

The wide variation in starting materials available for IMCRs (isocyanide multicomponent reactions) opens up versatile opportunities for the synthesis of compound libraries. The significant potential of isocyanides for the development of multicomponent reactions is a result of their ability to take part in diverse bond formation processes, their functional group tolerance, and the high levels of chemo-, regio-, and stereoselectivity often observed. According to our design, the copper(I)-catalyzed 1,3-Dipolar cycloaddition between an azide and an alkyne was used. The first moiety to be inserted in the Huisgen reaction is an alkyne group. The alkyne moiety was added to the side chain of serine and the other moiety which was prepared for click chemistry is the amino acid which contains the azide group.

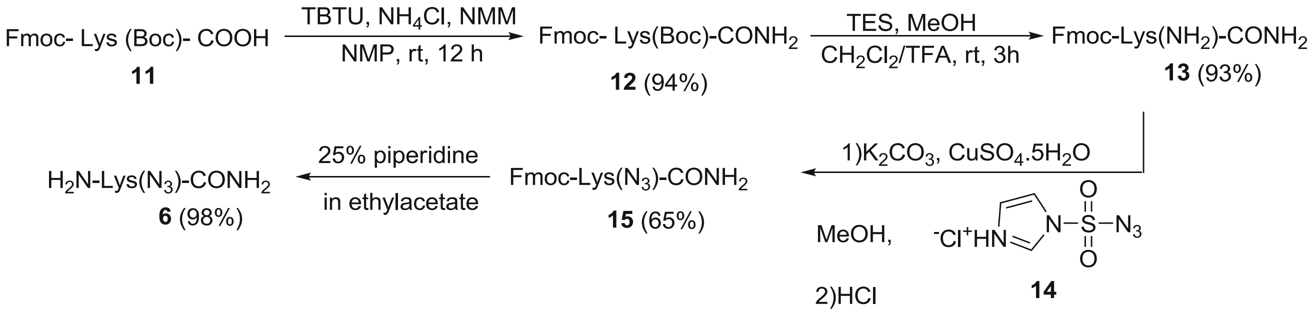
Since peptide **1** showed better anti-cancer activity compared to other heptapeptides, compound **1** was selected as a druggable molecule to be chemically modified. At first, heptapeptide **1** was divided into a hexapeptide **7** which contained an alkyne moiety and an amidated C-terminal Lysine **6** which contains an azide moiety.

The procedure for the synthesis of Fmoc-Lys(N_3)-$CONH_2$ **6** is shown in Scheme 3 in which the amine group in the side chain was converted to azide moiety. To access this molecule, Fmoc-Lys(Boc)-OH was selected as starting material and the synthesis was done in four steps: (a) Amidation of terminal carboxylic acid was done using ammonium chloride in the presence of TBTU as coupling reagent and NMM as base to form compound **12**. (b) The Boc-protecting group was removed with trifluoroacetic acid **13**. (c) The conversion of side-chain amine group to azide could be done using imidazoliumsulfonylazide **14** to obtain compound **15**. (d) Fmoc deprotection using 25 % piperidine afforded H_2N-Lys(N_3)-$CONH_2$ **6**.

The imidazoliumsulfonylazide **14** was synthesized according to the reported method (Johnasson and Pedersen 2012).

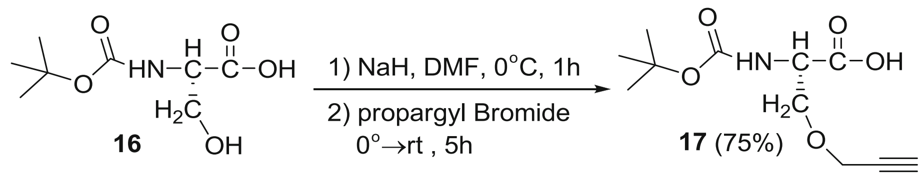
As shown in Scheme 4, Boc-Ser(*O*-Prop)-OH was prepared through the reaction of Boc-Ser-OH and sodium hydride followed by propargyl bromide reaction which led to the desired Boc(*O*-Prop)-OH with 72 % yield.

The linear peptide analogue Boc-Ser(*O*-Prop)-Ala-Pro-Pro–Pro-Arg(pbf)-OH was synthesized by standard solid-phase peptide synthesis (SPPS) strategy on resin. It should be noted that the initial experiments with Fmoc-Arg(Pbf)-

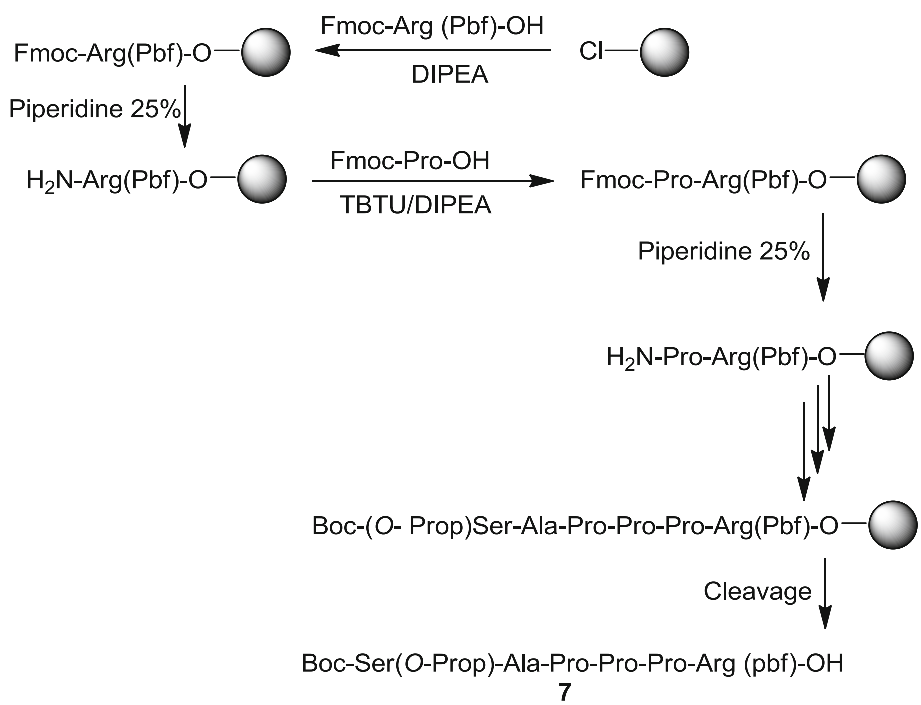


Scheme 3 Synthesis of H_2N-Lys(N_3)-$CONH_2$ **6**

Scheme 4 Synthesis of Boc-Ser(*O*-Prop)-OH **17**



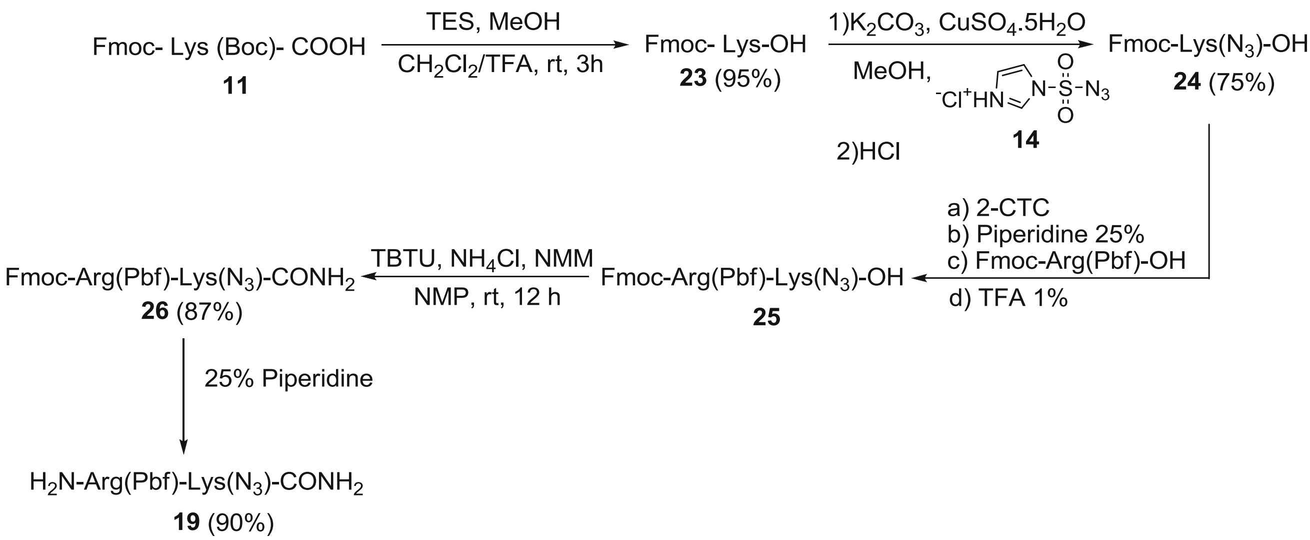
Scheme 5 Solid phase hexapeptide synthesis of **7**



OH coupled to the 2-chlorotrityl chloride resin (2-CTC resin) and the peptides elongated more efficiently with high purity. Coupling reactions were performed using protected amino acids, activated with TBTU in the presence of diisopropyl ethyl amine (DIPEA). Then, three *S*-proline and one L-alanine was used in this sequence of the desired peptide and finally Boc-Ser(*O*-Prop)-OH was added to the peptide sequence to afford the protected hexapeptide **7**. Cleavage of the peptide from the surface of resin was done using TFA (1 %) (Scheme 5).

To twist and bring the two ends closer together for the click reaction, based on our previous experience, we decided to use Ugi ligation. Four-component reaction of hexapeptide with carboxylic acid containing the propargyl group, amidated functionalized lysine containing azide moiety in the side chain as an amine group, 4-cyano-benzaldehyde and cyclohexyl isocyanide in methanol led to Ugi-ligated product **9**.

All structures were confirmed based on spectroscopic data and also high resolution mass spectrometry. Using Ugi-4CR, a new stereocenter was created in the product and the products formed as two diastereomers; the ratio of diastereomers was 80:20. The diastereomers were separated using column chromatography and compound **10** was



Scheme 6 Synthesis of functionalized dipeptide H_2N-Arg(Pbf)-Lys(N_3)-$CONH_2$ **19**

separated as a major and pure stereoisomer. The anti-cancer activity of major and minor diastereomers was investigated and only the major diastereomer showed good biological activity and will be discussed. The minor diastereomer did not show good biological activity.

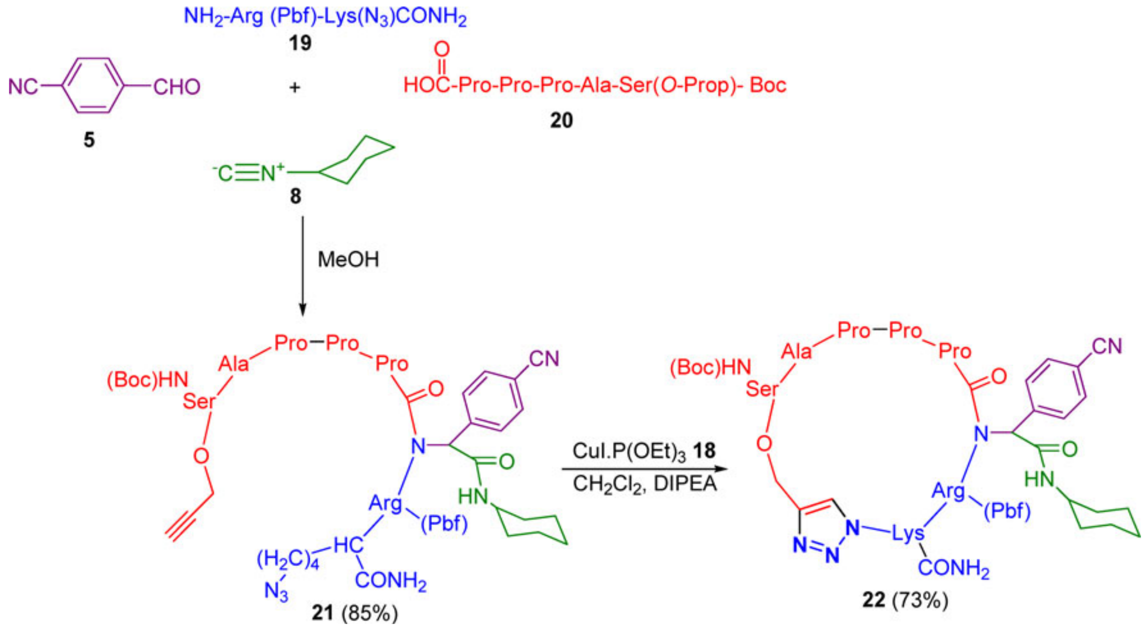
1,3-Dipolar cycloaddition reaction between an azide and alkyne takes place in the presence of a Cu (I) catalyst under mild conditions, resulting in the formation of a triazole link connecting the two molecules. In peptide chemistry, the increasing popularity of the click reaction is largely a result of the unique properties of both azides and the resulting triazoles. Interestingly, the triazole moiety formed by click reaction has a unique similarity to an amide bond. The relative planarity, strong dipole moments, and hydrogen bonding ability of triazole linkage make it as attractive as an amide bond with added advantage that it is less prone to hydrolytic cleavage. Triazole unit may impart rigidity, lipophilicity, enhanced absorption and protease stability, and act as an amide bond. Thus, the incorporation of the triazole unit in the structure of peptide is an added advantage.

There are many reports for the cycloaddition of azides and acetylenes which was done in the presence of CuI or $Cu(OAc)_2$/sodium ascorbate. The reaction was checked according to the reported methods based on CuI and $Cu(OAc)_2$, but the yields were low (Johnasson and Pedersen 2012). Finally, CuI.P$(OEt)_3$ was used as a reagent for the click reaction and cyclopeptide **10** was obtained in 75 % yield.

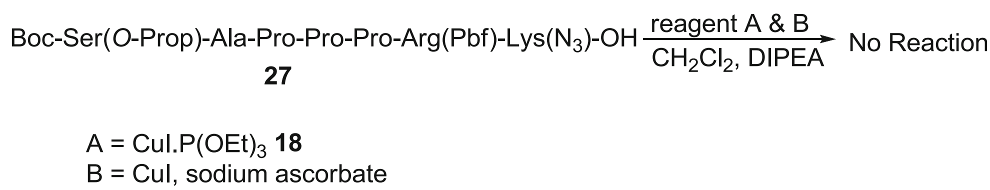
Based on the result of the experiment mentioned above and based on the amino acid sequence in the structure of peptide **1**, another Ugi-4CR was designed. The two pentapeptide **20** and dipeptide **19** segments were selected as the carboxylic acid **20** and amine moiety **19**, respectively. The reaction sequences for the synthesis of dipeptide H_2N-Arg(Pbf)-Lys(N_3)-$CONH_2$ **19** are shown in Scheme 6. The amine group in the side chain of lysine was converted to azide moiety using **14** and Fmoc-Lys(N_3)-OH **23** was loaded on the surface of 2-chlorotrityl chloride resin in the presence of DIPEA. Then Fmoc deprotection was done using piperidine 25 % in DMF and Fmoc-Arg(Pbf)-OH was added to the sequence using TBTU as coupling reagent in the presence of DIPEA. The cleavage of the protected dipeptide was done using 1 % TFA. The amidation of C-terminal was done using ammonium chloride and TBTU in the presence of NMM as base. Finally, Fmoc deprotection was carried out using 25 % piperidine and the desired dipeptide **19** was formed.

The pentapeptide **20** was synthesized based on the standard SPPS method. The Ugi-4CR of pentapeptide **20** as carboxylic acid, dipeptide **19** as amine, 4-cyano-benzaldehyde **5**, cyclohexyl isocyanide **8** led to the formation of compound **21** and finally using CuI.P$(OEt)_3$ **18** (Langille and Jamison 2006), cyclization was carried out and cyclopeptide **22** was obtained (Scheme 7).

To access a cyclopeptide, at first the heptapeptide **27** was synthesized based on SPPS strategy, the sequence of amino acids in this compound is the same as heptapeptide **1**. There are only two changes: instead of Lys and Ser in the sequence, Fmoc-Lys(N_3)-OH and Boc-Ser(*O*-Prop)-OH were used in C- and N-terminal, respectively. The 1,3-Dipolar cycloaddition of peptide **27** was done according to the standard method (CuI) as well as CuI. P$(OEt)_3$ **18**, but in both cases the cyclization was not successful (Scheme 8). It seems that the distance between alkyne and azide moieties is very far. It shows that the Ugi-4CR could affect the folding of molecule and cause efficient cyclization.



Scheme 7 Sequential Ugi ligation/Huisgen 1,3-Dipolar reaction to construct cyclopeptide **22**



Scheme 8 Try for cyclization of peptide **27**

Peptide **27** had no biological activity. The click reaction did not occur; perhaps due to the amino acid sequence of this peptide or because the two ends failed to connect. Compound **10** was highly active against lung cancer cells, which induced apoptosis at a much lower dose than the original heptapeptides. Yet, the same compound had no anti-cancer activity before the click reaction (compound **9**). The chemical modifications before click reaction actually affected the original heptapeptides **1** negatively. All products were less soluble in water; therefore, they were dissolved in a small volume of dimethyl sulfoxide (DMSO) and then diluted with water for in vitro assays. Interestingly, the products **21** and **22** had no anti-cancer activities; this result shows that the selection of suitable segments for Ugi-4CR has an important role in the biological activity of products.

The biological activities of products **1** (the unmodified heptapeptide), **9**, **10**, **21**, **22**, and **27** were determined by their effects on A549, human lung cancer cell line. The results (Fig. 1) clearly indicate that cyclopeptide **10**, the final product in Scheme 9, shows significant anti-cancer activity. The cyclization of our original heptapeptides by this method has improved its biological activity up to 20-fold. The dose–response assay (Fig. 2) shows that 0.5 µg of compound **10** had the same or greater anti-cancer activity than 10 µg of compound **1** (Fig. 1). The biological activity of compound **10** was further examined by its effect on different cell lines: A549 used was a well-characterized human lung carcinoma cell line, known to have mutated ras oncogene; C26, an aggressive colorectal cancer cell line that contains constitutively activated ras oncogene; PC3 is a cell line characteristic of prostatic small cell carcinoma that also has mutated ras oncogene; and noncancerous CHO, Chinese hamster ovary cell line that does not have mutated ras oncogene. Cyclopeptide **10** showed great specificity against these cell lines. The results (Fig. 3) show that **10** had significant anti-cancer activity against all the three different cancer cell lines that have mutated ras oncogene. Meanwhile, it had little or no effect on noncancerous CHO cells and normal human leukocytes.

In conclusion, we have designed a hypothetical heptapeptides specifically to overcome the neoplastic activity of ras oncogene. This peptide showed the potential to be druggable by inducing apoptosis in some specific cancer cells known to have mutated ras oncogene. To improve

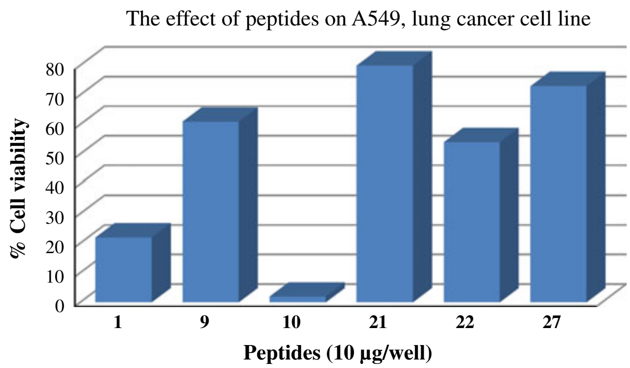


Fig. 1 The anti-cancer activities of synthesized peptides against human lung carcinoma cells, A549

the anti-cancer activity of this peptide, we chemically modified it by cyclization reaction. The reaction could be categorized as a sequential Ugi/Huisgen 1,3-Dipolar cyclization reaction. We have now constructed a cyclopeptide that contains a triazole motif. The existence of a triple bond opens an avenue to a diversity of subsequent compounds accessible by different reactions. The anti-cancer activity of all products was examined in vitro and only cyclopeptide **10** with triazole skeleton showed significant anti-cancer activity against cancer cells with mutated ras oncogene such as A549, PC3 and C26 cells. Cyclopeptide **10** had little or no activity on Chinese hamster cells, CHO that does not express ras oncogene. This study clearly shows the importance of triazole skeleton in biological activity of the peptides. It might be possible to overcome the difficulties involved in making complex peptides by employing this elegant chemistry.

Experimental section

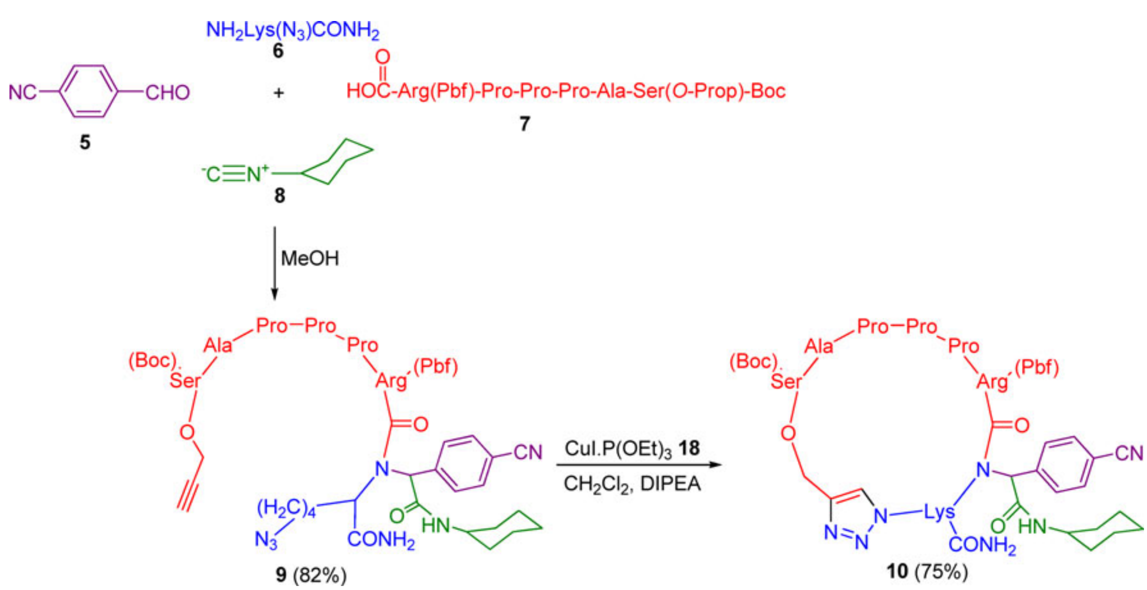
General

Commercially available chemicals were used as received unless otherwise stated. Flash column chromatography was carried out using silica Gel 60 (particle size 0.04–0.06 mm/230–400 mesh). The abbreviations are given in separate place. The mass spectra were recorded by EI-mass (70 eV), mass (ESI-triple quadrupole), mass (ESI-ion trap), HRMS (ESI-FT-ICR), HRMS (MALDI-FT-ICR). The purification of peptides was done using preparative HPLC (column C18, 7 μm). NMR spectra were recorded at 500, 300 MHz in $CDCl_3$, DMSO-d_6 and D_2O.

Synthetic procedures

*General procedure for the synthesis of heptapeptides-COOH (**1–4**)*

Synthesis was carried out using 2-chlorotrityl chloride resin (1.0 mmol/g) following the standard Fmoc strategy. Fmoc-Lys(Boc)-OH (4.687 g, 10 mmol) was attached to the 2-CTC resin (5.000 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. Then, it was 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



Scheme 9 Sequential Ugi ligation/Huisgen 1,3-Dipolar reaction to construct cyclopeptide **10**

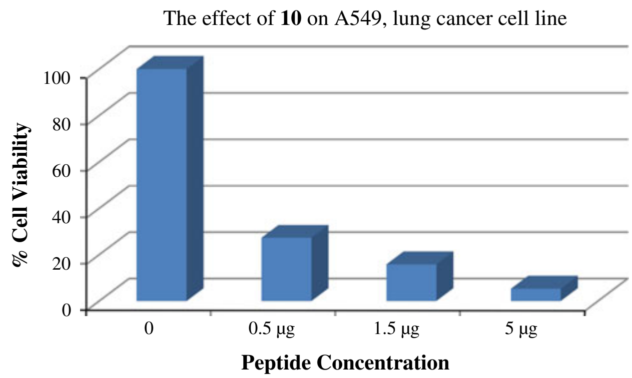


Fig. 2 The dose-dependent anti-cancer activity of cyclopeptide **10** against human lung carcinoma cells, A549

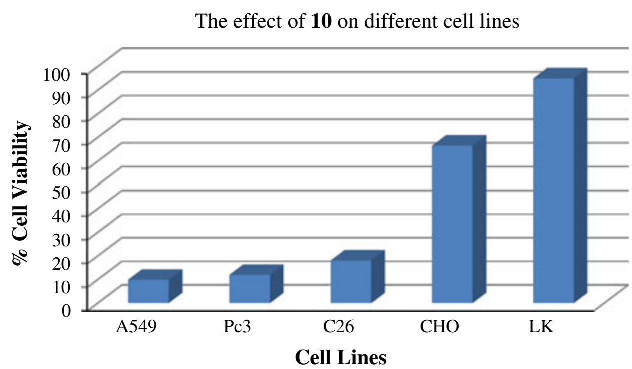


Fig. 3 The specificity of cyclopeptide **10** against different cell lines, A549, PC3, C26, CHO, and LK

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-Arg(Pbf)-OH (4.866 g, 7.5 mmol), TBTU (2.407 g, 7.5 mmol), and 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). The coupling was repeated as in the same way as 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). The produced heptapeptide 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 precipitate was filtered and dried. Final deprotection was done using TFA (95 %) and reagent K (TFA/TES/Water 95:2.5:2.5). The excess TFA/DCM was removed under reduced pressure. The desired peptide was precipitated in diisopropyl ether. The purification was done using preparative HPLC (Column C18). The same procedure was used for the synthesis of peptide **2–4**.

This procedure was used for the synthesis of hexapeptide **7**, only Fmoc-Ser(*O*-prop)-OH was used instead of Fmoc-Ser(^{t}Bu)-OH.

HRMS (ESI) heptapeptides

1 *m/z* $[M+H]^+$ Calcd for $C_{33}H_{58}N_{11}O_9$ 752.44189, Found 752.44183. $[M+Na]^+$ Calcd for $C_{33}H_{57}N_{11}NaO_9$ 774.42409, Found 774.42400.

2 *m/z* $[M+H]^+$ Calcd for $C_{27}H_{45}N_{10}O_{10}$ 669.33284, Found 669.33266.

3 *m/z* $[M+H]^+$ Calcd for $C_{29}H_{49}N_{10}O_9$ 681.36893, Found 681.36879.

4 *m/z* $[M+H]^+$ Calcd for $C_{31}H_{52}N_{13}O_9$ 750.40298, Found 750.40269.

HRMS (ESI-FT-ICR)

HRMS (ESI-FT-ICR) 7 *m/z*: $[M+H]^+$ Calcd for $C_{48}H_{72}N_9O_{13}S$ 1,014.49667, Found 1,014.49665, $[M+Na]^+$ Calcd for $C_{48}H_{71}N_9NaO_{13}S$ 1,036.47904, Found 1,036.47899, $[M+K]^+$ Calcd for $C_{48}H_{71}KN_9O_{13}S$ 1,052.45305, Found 1,052.45299.

Fmoc-Lys(Boc)-CONH$_2$ **12**

A solution of Fmoc-Lys(Boc)-OH **11** (4.396 g, 9.4 mmol) and ammonium chloride (1.069 g, 20 mmol) in *N*-methyl-2-pyrrolidinone (3 mL) was magnetically stirred and then TBTU (4.815 g, 15 mmol) in *N*-methyl morpholine (5.5 mL, 50 mmol) was added to the mixture. The mixture was stirred for 12 h at room temperature.

A yellow solution was formed. The reaction progress was monitored using thin layer chromatography (1:2:10, H_2O:MeOH:ethyl acetate). The reaction was completed after 12 h. Then, by slow addition of 70 mL H_2O, a yellow discretion was formed. The deposition was filtered and dried. Mass of discretion was 4.830 g (9.4 mmol) with 94 % yield (Arabanian et al. 2010).

m.p. 158–161 °C; ^{1}HNMR (300 MHz, DMSO-d_6) $\delta = 1.26$–1.28 (m, 2H, CH_2), 1.35 (s, 9H, ^{t}Bu), 1.56–1.59 (m, 2H, CH_2), 1.89 (quin, 2H, CH_2), 2.85–2.90 (m, 2H, CH_2NH), 3.28 (t, 1H, $J = 7.0$ Hz, CH fluorene), 4.20–4.29 (m, 3H, CHα and CH_2O), 6.76 (brs, 1H, N*H*–CO–CH_2fluorenyl), 6.96 (brs, 1H, N*H*-Boc), 7.31 (t, 1H, $J = 7.3$ Hz, H–Ar), 7.32–7.37 (m, 2H, H–Ar), 7.40 (t, 1H, $J = 7.0$ Hz, H–Ar), 7.71 (d, 1H, $J = 6.3$ Hz, H–Ar), 7.82 (d, 1H, $J = 7.5$ Hz, H–Ar), 7.87 (d, 2H, $J = 7.3$ Hz, H–Ar) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 17.2$, 22.9 (CH_2), 28.3 (C(CH_3)$_3$), 29.2 (CH_2), 31.5 (CH_2NH),46.7 (CH fluorene), 54.4 (CH^α–CONH$_2$), 65.6 (CH_2O–), 77.3

(–O*C*(CH_3)$_3$), 120.1, 125.3, 127.0, 127.6, 140.7, 143.8, 143.9 (C Ar), 155.5 (fluorenyl-CH_2-*C*ONH-), 155.9 (–O*C*O^{t}Bu), 173.9 (*C*ONH$_2$) ppm; IRν_{max}(neat) 3,313, 3,055 (NH), 1,687 (C=O), 1,511 cm^{-1}; MS (70 eV): *m/z* (%):467 (10) [M$^+$], 394 (74) [M$^+$-C_4H_9O], 366 (67) [M$^+$-$C_5H_9NO_2$].

Fmoc-Lys-CONH$_2$ **13**

Fmoc-Lys(Boc)-CONH$_2$ **12** (2.935 g, 8.0 mmol) was dissolved in 50 % (v/v) TFA in CH_2Cl_2 (100 mL). Then triethylsilane (1.4 mL, 9.0 mmol) was added to the mixture as scavenger. The reaction mixture was stirred for 3 h at room temperature. Then, the solvent was removed under the reduced pressure, the pH of the mixture was adjusted via addition of NaOH (1 N). The precipitate was filtered and washed with water. 2.490 g of product was achieved. The yield at this step was 93 % (Diaz-Mochon et al. 2005).

^{1}HNMR (300 MHz, DMSO-d_6) δ = 1.32–1.34 (m, 2H, CH_2), 1.43–1.50 (m, 2H, CH_2), 1.89 (quin, 2H, CH_2), 2.67–2.87 (m, 2H, C*H*$_2$–NH$_2$), 3.29 (t, 1H, J = 7.0 Hz, CH fluorene), 3.50–3.81 (brs, 2H, NH$_2$), 4.20–4.27 (m, 3H, –CH$_2$O, CHα) 6.26 (s, 1H, CONH), 6.95 (m, 2H, CON*H*, fluorenyl CH$_2$CON*H*), 7.31 (t, 1H, J = 7.3 Hz, H–Ar), 7.32–7.37 (m, 2H, H–Ar), 7.40 (t, 1H, J = 7.0 Hz, H–Ar), 7.71 (d, 1H, J = 6.2 Hz, H–Ar), 7.82 (d, 1H, J = 7.5 Hz, H–Ar), 7.87 (d, 2H, J = 7.4 Hz, H–Ar) ppm; ^{13}C NMR(75 MHz, DMSO-d_6) δ = 17.2, 22.1, 28.9 (CH_2), 30.1 (CH_2–NH_2), 33.8 (CH fluorene), 53.9 (CH$^\alpha$–CONH$_2$), 109.7, 111.2, 115.2, 119.1, 119.9, 121.3, 123.3, 127.3, 128.9, 137.4, 139.4, 142.5 (C Ar), 173.9 (fluorenyl-CH$_2$-*C*ONH-), 176.7 (CONH$_2$) ppm; IRν_{max} (neat) 3,055, 2,984 (NH), 2,854 (CH), 1,681 (CO) cm^{-1}; MS (70 eV): *m/z* (%): 366 (6) [M$^+$], 351 (16) [M$^+$-NH$_2$], 337 (12) [M$^+$-(H$_2$C=NH$_2$)].

Imidazole-1-sulfonyl azide hydrochloride **14**

A round bottle containing suspension of sodium azide (1.001 g, 15.4 mmol) and acetonitrile (20 mL) was placed in ice bath. When the reaction mixture was cooled, sulfuryl chloride (1.25 mL, 15.4 mmol) was dropped slowly. Then, ice bath was removed. And reaction mixture was stirred for 20 h at room temperature. The reaction mixture was cooled to 0 °C (ice bath) again and imidazole (2.000 g, 29.3 mmol) was added over 10 min. The reaction mixture was stirred for 4 h at room temperature. Then it was diluted with EtOAc (20 mL) and washed with H_2O (2 × 20 mL). The organic phase was washed by saturated $NaHCO_3$ solution (2 × 20 mL). The organic phase was separated and dried with anhydrous $MgSO_4$ and cooled to 0 °C (ice bath). Acetyl chloride (2.098 mL, 29.4 mmol) was added dropwise to ice cold EtOH (5 mL) over 10–15 min. After stirring for 10 min at 0 °C, the solution was added to the EtOAc solution over 20 min. After stirring for 10 min, the resulting suspension was filtered and the precipitate washed with EtOAc (4 × 10 mL) and dried under suction for 30 min affording the title compound as a white solid (2.250 g, 70 %) with spectral characteristics in accordance with literature data (Goddard-Borger and Stick 2007).

^{1}H NMR(300 MHz, D_2O) δ = 7.46 (dd, 1H, J = 2.1 Hz, J = 1.2 Hz, CH^4), 7.88 (t, 1H, J = 2.1 Hz, CH^5), 9.15 (t, 1H, J = 1.2 Hz, CH^2) ppm; ^{13}C NMR (75 MHz, D_2O) δ = 119.8 (C^4), 121.8 (C^5), 137.0 (C^2) ppm; IRν_{max} (KBr) 3,111 (NH), 2,167 (N_3), 1,428 (SO_2) cm^{-1}.

Fmoc-Lys(N_3)-CONH$_2$ **15**

A suspension of Fmoc-Lys-CONH$_2$ **13** (2.910 g, 7.9 mmol) with potassium carbonate (2.722 g, 19.7 mmol) and copper sulfate pentahydrate (0.017 g, 0.07 mmol) in methanol (50 mL) was prepared in a proper round bottle. The imidazole-1-sulfonyl azide hydrochloride (2.000 g, 9.5 mmol) was added to the suspension slowly. The above mixture was stirred for 20 h at room temperature. After completion of reaction, solvent was removed under vacuum, then 120 mL H_2O was added to reaction mixture and pH was diminished to 2 by consumption of concentrated HCl. The achieved acidic solution was extracted by ethylacetate (3 × 20 mL) and finally the whole organic phase was washed by brine. Organic phase was dried by anhydrous $MgSO_4$ and the solvent was distilled. Yellow oil (2.000 g, 65 %) was formed.

^{1}H NMR(300 MHz, DMSO-d_6) δ = 1.29–1.37 (m, 2H, CH_2), 1.42–1.59 (m, 2H, CH_2), 1.59–1.71 (m, 2H, CH_2), 3.32 (t, 2H, J = 6.9 Hz, CH_2N_3), 3.68–3.73 (m, 1H, CH fluorene), 3.85–3.92 (m, 1H, C*H*$^\alpha$–CONH$_2$), 4.20–4.25 (m, 2H, -fluorenyl-C*H*$_2$-O), 6.99 (brs, 1H, NH-COOCH$_2$fluorenyl), 7.28–7.35 (m, 3H, H–Ar), 7.36–7.50 (m, 3H, H–Ar and CONH$_2$), 7.59 (brs, 1H, CONH$_2$), 7.72 (d, 1H, J = 7.2 Hz, H–Ar), 7.78 (d, 2H, J = 7.4 Hz, H–Ar) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ = 22.5, 28.3, 31.7 (CH_2), 47.1 (CH fluorene), 51.1 (CH_2N_3), 56.8 (*C*H$^\alpha$–CONH$_2$), 67.2 (CH_2–O–), 120.1, 125.1, 127.1, 127.8, 141.3, 143.6, 143.8 (C–Ar), 156.3 (*C*OOCH$_2$fluorenyl), 177.0 (CONH$_2$) ppm; IRν_{max} (neat) 3,380, 3,351, 2,943 (NH), 2,105 (N_3), 1,775, 1,681 (C=O) cm^{-1}; MS(70 eV): *m/z* (%):393 (70) [M$^+$].

H$_2$N-Lys(N_3)-CONH$_2$ **6**

Fmoc-Lys(N_3)-CONH$_2$ **15** (2.001 g, 5.1 mmol) was dissolved in ethylacetate (8 mL) and then piperidine (2.0 mL, 20 mmol) was added. The reaction mixture was stirred at room temperature. After 3 h, the reaction mixture was extracted by water (3 × 10 mL). The aqueous phases were washed by fresh ethylacetate (1 × 10 mL) again. Aqueous phase was dried. Yellow viscous oil (0.850 g, 98 %) was obtained.

^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 1.32$–1.45 (m, 2H, CH_2), 1.47–1.60 (m, 4H, 2CH_2), 2.92 (m, 3H, CH-NH_2 and CH^{α}CONH$_2$), 3.29 (t, 2H, $J = 6.7$ Hz, CH_2N_3), 6.80 (brs, 2H, $CONH_2$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 22.5$, 23.9, 28.3 (CH_2), 43.9 (CH_2N_3), 53.6 (CH^{α}), 178.2 (C=O) ppm; IRν_{max} (neat) 3,485, 3,469, 3,420 (NH), 2,098 (N_3), 1,697 (C=O) cm^{-1}; MS (70 eV): *m/z* (%): 170 (5) [M^+-H], 127 (14) [M^+-$CONH_2$], 85 (90) [M^+-CH_2N_4O], 56 (94) [$C_2H_4NO^+$].

Boc-Ser(*O*-Prop)-OH17

N-tert-butoxycarbonyl-L-serine **16** (2.000 g, 10 mmol) was dissolved in DMF (5 mL) and the solution was placed in ice bath. Sodium hydride [0.880 g, 22 mmol, 60 % (w/w) dispersion in mineral oil] was added slowly and the reaction mixture stirred for 1 h at 0 °C. Then, propargyl bromide (1.3 mL, 11 mmol) was added dropwise to the mixture; yellow solution was formed. The solution was placed in ice bath for 1 h. Then, ice bath was removed and the solution was stirred at room temperature for 4 h. The progress of reaction was followed by thin layer chromatography (30:20:1, petroleum ether:ethylacetate:acetic acid). Water (15 mL) was added to the mixture which was then washed with diethylether (3 × 10 mL). The aqueous phase was acidified to pH 3 by adding 10 % HCl. The solution was extracted from acidic solution with ethylacetate (3 × 25 mL). The organic phase was dried using magnesium sulfate and the solvent was removed under vacuum. Orange viscous oil was achieved and purified by column chromatography (30:20:1, petroleum ether:ethylacetate:acetic acid). The solvent was evaporated affording a pale yellow, viscous oil (1.820 g, 75 %), with spectral characteristics in accordance with literature data (Brink et al. 2006; Jacobsen et al. 2011).

^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 1.44$ (s, 9H, ^{t}Bu), 2.45 (t, 1H, $J = 2.6$ Hz, CCH, rotamer 1), 2.49 (t, 1H, $J = 2.4$ Hz, CCH, rotamer 2) 3.78 (dd,1H, $J = 9.4$ Hz, $J = 3.6$ Hz, CH_2 serine), 3.98 (dd,1H, $J = 9.5$ Hz, $J = 3.3$ Hz, CH_2 serine), 4.14 (d, 2H, $J = 2.3$ Hz, O–CH_2CCH, rotamer 1), 4.49–4.51(m, 1H, CH^{α}), 4.75 (d, 2H, $J = 2.4$ Hz, O–CH_2CCH, rotamer 2), 5.38 (d, 1H, $J = 8.6$ Hz, NH) ppm; IRν_{max} (neat) 3,440 (CO*OH*), 3,294 (NH), 1,715, 1,692 (C=O) cm^{-1}.

Copper(I) iodide triethyl phosphate **18**

Copper iodide (1.000 g, 5.3 mmol) prepared by Nishizawa method was added to a round bottle containing triethylphosphite (0.9 mL, 5.3 mmol) and toluene (5.5 mL) in 10 min. The round bottle was sealed to protect from light; after 1 h stirring at room temperature, the reaction mixture was passed through Celite and was concentrated in vacuum. Further purification was done using recrystallization in toluene and hexane; the white crystal (1.100 g, 57 %) was formed (Langille and Jamison 2006).

^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 4.11$ (quin, 2H, CH_2), 1.29 (t, 3H, $J = 7.0$ Hz, CH_3) ppm. [Compare to P (OEt)$_3$: ^{1}H NMR (500 MHz, $CDCl_3$) 3.88 (6H, q, $J = 7.0$), 1.28 (9H, t, $J = 7.0$)].

General procedure for the synthesis of peptide **9** via Ugi-4CR

A solution of Fmoc-Lys(N_3)-$CONH_2$ **6** (0.260 g, 1.5 mmol) and 4-cyano-benzaldehyde (0.200 g, 1.5 mmol) in MeOH (5 mL) was added for the formation of imine. After 1 h, Boc-Ser(*O*-Prop)-Ala-Pro-Pro–Pro-Arg(Pbf)-COOH **7** (1.520 g, 1.5 mmol) was added, this reaction followed with addition of cyclohexyl isocyanide (0.2 mL, 1.5 mmol). The mixture was stirred for 48 h. After completion of the reaction, solvent was evaporated in vacuum. The crude oil was purified by flash column chromatography. The product **9** was obtained as a white solid (1.730 g, 82 %).

HRMS (ESI-FT-ICR) *m/z*: $[M+Na]^+$ Calcd for $C_{69}H_{98}N_{16}NaO_{14}S$, 1,430.72662, Found 1,430.72659.

General procedure for the synthesis of cyclopeptide **10** through Huisgen 1,3-Dipolar cycloaddition reaction

Freshly prepared **9** (1.500 g, 1.07 mmol) was dissolved in CH_2Cl_2 (1,000 mL). *N,N*-Diisopropylethylamine (0.55 mL, 3.41 mmol) and copper(I) iodide triethylphosphite **18** (0.370 g, 1.61 mmol) were added to the reaction mixture. The reaction mixture was stirred and protected from light for 5 days at room temperature. The solvent was evaporated. The residue was redissolved in CH_2Cl_2 and purified by flash column chromatography (14:1:1 CH_2Cl_2:acetone:MeOH) affording the title compound as a yellow solid (1.130 g, 75 %). HRMS (ESI-FT-ICR) *m/z*: $[M+Na]^+$ Calcd for $C_{69}H_{98}N_{16}NaO_{14}S$ 1,430.57978; Found 1,430.57972.

Fmoc-Lys-OH **23**

A 50 % (v/v) solution of TFA in CH_2Cl_2 (16 mL) was added to Fmoc-Lys(Boc)-OH **11** (4.680 g, 10 mmol) and then triethylsilane (1.6 mL, 10 mmol) as a scavenger was added to reaction mixture. The mixture was stirred for 2 h at room temperature. The solvent and bulk of excess TFA were removed under vacuum. The solution of sodium hydroxide (1 M) was added to the residue; the product was precipitated at pH 6. The precipitate was filtered and washed with water and dried affording a white powder (3.500 g, 95 %).

mp: 247–249 °C; ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 1.41–1.83$ (m, 4H, $2CH_2$), 1.94 (quin, 2H, CH_2), 3.01–3.10 (m, 2H, CH_2NH_2), 3.36 (t, 1H, $J = 7.1$ Hz, CH fluorene),4.18 (t, 1H, $J = 6.5$ Hz, *CH*COOH), 4.35–4.40 (m, 2H, $–OCH_2$), 4.76 (brs, 2H, NH_2), 5.94 (d, 1H, $J = 7.6$ Hz, N*H*$COOCH_2$fluorenyl), 7.28 (t, 2H, $J = 7.3$ Hz, H–Ar), 7.37 (t, 2H, $J = 7.3$ Hz, H–Ar), 7.73 (d, 2H, $J = 7.4$ Hz, H–Ar), 7.57 (d, 2H, $J = 6.9$ Hz, H–Ar) ppm; IRν_{max} (KBr): 3,330 (NH, COOH), 3,052 (CH aromatic), 2,940 (CH aliphatic), 1,689 (C=O), 1,596 (C=C) cm^{-1}.

Fmoc-Lys(N_3)-OH **24**

A suspension of Fmoc-Lys-OH **23** (2.947 g, 8 mmol) with potassium carbonate (2.730 g, 19.7 mmol) and copper sulfate pentahydrate (0.017 g, 0.07 mmol) in methanol (50 mL) was prepared in a proper round bottle. The imidazole-1-sulfonyl azide hydrochloride (2.000 g, 9.5 mmol) was added to the suspension slowly. The mixture was stirred for 20 h at room temperature. After completion of reaction, the solvent was removed under vacuum. Then 120 mL H_2O was added to reaction mixture and the pH was diminished to 2 by consumption of concentrated HCl. The achieved acidic solution was extracted by ethylacetate (3 × 20 mL) and finally the whole organic phase was washed by brine. The organic phase was dried by magnesium sulfate and the solvent was distilled. Yellow oil (2.360 g, 75 %) was formed (Sabido 2009).

mp: 224–228 °C; ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 1.27–1.73$ (m, 6H, $3CH_2$), 3.26 (t, 2H, $J = 6.5$ Hz, CH_2N_3), 4.22 (t, 1H,$J = 7.0$ Hz, CH fluorene), 4.43 (d, 2H, $J = 6.8$ Hz, $–OCH_2$), 4.50–4.54 (m, 1H, CH^{α}COOH), 5.58 (d, 1H, $J = 8.2$ Hz, fluorenylCH_2OOCN*H*), 7.32 (t, 2H, $J = 7.3$ Hz, H–Ar), 7.41 (t, 2H, $J = 7.3$ Hz, H–Ar), 7.55 (d, 1H, $J = 7.3$ Hz, H–Ar), 7.60 (d, 1H, $J = 7.3$ Hz, H–Ar), 7.76 (d, 2H, $J = 7.3$ Hz, H–Ar) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 22.9$, 27.8, 30.3 (CH_2), 50.5 (CH fluorene), 54.9 (CH_2N_3), 55.5 (*C*H^{α}COOH), 61.1(OCH_2), 120.0, 120.1 121.2, 123.9, 125.3, 127.1, 127.6, 129.5, 135.3, 140.7, 143.8, 143.9 (C–Ar), 156.2 (CONH), 171.8 (COOH) ppm; IRν_{max} (KBr): 3,455 (NH, COOH), 3,152 (CH aromatic), 2,089 (N_3), 1,743 (C=O carboxylic acid), 1,670 (*CO*NH) cm^{-1}.

General procedure for preparation of dipeptide **25**

The synthesis of dipeptide was carried out using 2-chlorotrityl chloride resin (1.0 mmol/g) following the standard Fmoc strategy. Fmoc-Lys(N_3)-OH (0.790 g, 2 mmol) was attached to the 2-CTC resin (1.000 g) with DIPEA (1.37 mL, 8 mmol) in anhydrous DCM:DMF (10 mL, 1:1) at room temperature for 2 h. After filtration, the resin was capped by a solution of DCM/MeOH/DIPEA (17:2:1, 24 mL) for 30 min. Then, it was filtered and washed thoroughly with DCM (1 × 7 mL), DMF (4 × 7 mL). The resin-bound Fmoc-amino acid was treated with 25 % piperidine in DMF (15 mL) for 30 min and the resin was washed with DMF (3 × 7 mL). Then a solution of Fmoc-Arg(Pbf)-OH (0.971 g, 1.5 mmol), TBTU (0.480 g, 1.5 mmol), and DIPEA (0.6 mL, 3.5 mmol) in 7 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 × 7 mL) and DCM (3 × 7 mL). The produced dipeptide was cleaved from resin by treatment of TFA (1 %) in DCM (55 mL) and neutralization with pyridine (4 %) in MeOH (17 mL). The solvent was removed under reduced pressure and precipitated in water and dried affording a powder (1.202 g).

Mass (ESI-triple quadrupole) *m/z*: $[M+H]^+$ Found for $C_{40}H_{51}N_8O_8S$ 803.10000.

Amidation of C-terminal of dipeptide **26**

The dipeptide **25** (1.200 g, 1.5 mmol) and *N*-methyl morpholine (0.8 mL, 7.5 mmol) were added to a solution of TBTU (0.740 g, 2.3 mmol) and NH_4Cl (0.160 g, 3.0 mmol) in NMP (3 mL). The mixture was stirred overnight. The dipeptide was precipitated in water and the C-terminal amidated dipeptide **26** was dried affording a yellow powder (1.040 g, 87 %).

General procedure for Fmoc deprotection of amidated dipeptide **19**

Purified amidated dipeptide **26** (0.96 g, 1.2 mmol) was added to ethylacetate (2 mL), and then piperidine (0.5 mL, 5 mmol) was added. The reaction mixture was stirred at room temperature. After 3 h, reaction mixture was extracted by water (3 × 10 mL). The aqueous phases were washed by fresh ethylacetate (1 × 10 mL) again. Aqueous phase was dried using natrium sulfate. Yellow viscous oil (0.62 g, 90 %) was obtained.

Mass (ESI-triple quadrupole) *m/z*: $[M+H]^+$ Found for $C_{25}H_{42}N_9O_5S$ 580.10000.

General procedure for the synthesis of pentapeptides-COOH **20**

The synthesis of pentapeptide **25** was done using the standard Fmoc SPPS strategy. At first, Fmoc-Pro-OH was loaded on the surface of resin. The two times Fmoc-Pro-OH, Fmoc-Ala-OH, and Boc-Ser(*O*-Prop)-OH were loaded on the surface of resin. The peptide was removed from the surface of 2-CTC resin using 1 % TFA based on the known procedure.

HRMS (ESI-FT-ICR) $m/z = C_{29}H_{44}N_6O_9$ $[M+H]^+$ Found 606.31390, Calc. 606.31399, $C_{29}H_{43}N_6NaO_9$ $[M+Na]^+$ Found 628.29535, Calc. 628.29536, $C_{29}H_{44}KN_6O_9$ $[M+K]^+$ Found 644.26965, Calc. 644.26971.

General procedure for the synthesis of peptide **21** via Ugi-4CR

A solution of H_2N-Arg(Pbf)-Lys(N_3)-NH_2 **19** (0.500 g, 0.86 mmol) and 4-cyano-benzaldehyde (0.120 g, 0.86 mmol) in MeOH (3 mL) was added for the formation of imine. After 1 h, Boc-Ser(*O*-Prop)-Ala-Pro-Pro–Pro-COOH **20** (0.520 g, 0.86 mmol) was added, this reaction was followed with addition of cyclohexyl isocyanide (0.11 mL, 0.86 mmol). The mixture was stirred for 48 h. Further purification was done using flash column chromatography. The desired product was achieved as yellow oil (1.030 g, 85 %).

Mass (ESI-ion trap) *m/z*: $[M+Na]^+$ Found for $C_{69}H_{98}N_{16}NaO_{14}S$ 1430.33.

General procedure for the synthesis of cyclopeptide **22** via Huisgen 1,3-Dipolar cycloaddition reaction

Freshly prepared **21** (1.100 g, 0.8 mmol) was dissolved in CH_2Cl_2 (900 mL). Then, *N,N*-diisopropylethylamine (0.4 mL, 2.4 mmol) and copper(I) iodide triethylphosphite (0.272 g, 1.2 mmol) were added to reaction mixture. The reaction mixture was stirred and protected from light for 5 days at room temperature. The solvent was evaporated. The residue was redissolved in CH_2Cl_2 and purified by flash column chromatography (14:1:1 CH_2Cl_2:acetone:MeOH) affording the title compound as a yellow solid (0.820 g, 73 %).

Mass (ESI-ion trap) *m/z*: $[M+H]^+$Found for $C_{69}H_{98}N_{16}O_{14}S$ 1408.13.

HRMS (ESI-FT-ICR) *m/z*: $[M-(C_6H_{10}N_3)]^+$ Calcd for $C_{63}H_{88}N_{13}O_{14}S$ 1,282.55259, Found 1,282.55248; $[M-(C_{18}H_{29}N_3O_2S)]^+$ Calcd for $C_{51}H_{69}N_{12}O_{10}$ 1009.49428, Found 1,009.49420; $[M-(C_{31}H_{50}N_8O_4S)]^+$ Calcd for $C_{38}H_{48}N_8O_{10}$ 776.24264, Found 776.24257.

General procedure for the synthesis of heptapeptides-COOH (**27**)

The synthesis of heptapeptide **27** was carried out using the general Fmoc SPPS strategy using 2-CTC. At first, Fmoc-Lys (N_3)-OH **24** was attached on the surface of resin and then Fmoc-Arg(Pbf)-OH, three times Fmoc-Pro-OH, Fmoc-Ala-OH and finally Fmoc-Ser(*O*-Prop)-OH were added on the surface. The peptide was removed from the surface of resin using TFA (1 %) based on the known procedure.

HRMS (MALDI-FT-ICR) *m/z*: $[M+H]^+$ Calcd for $C_{54}H_{82}N_{13}O_{14}S$ 1168.58514, Found 1,168.58489; $[M+Na]^+$ Calcd for $C_{54}H_{81}N_{13}NaO_{14}S$ 1,190.57059, Found 1,190.57048; $[M+K]^+$ Calcd for $C_{54}H_{81}KN_{13}O_{14}S$ 1,206.52828, Found 1,206.52901.

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 units/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 the 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 solved by addition of 100 µL dimethylsulfoxide (DMSO). The absorbance was read using an automatic multiwell 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 Meanwhile, I certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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