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Synthesis of novel ricinoleic acid-based 1,2,3-triazoles and their anticancer activity

Y Mohini[a#], K R Kunduru[a], Madiga HariKrishna[a], M S L Karuna*[a], Y Poornachandra[b], C Chandrasekhar[c] & Podha Sudhakar[d]

[a] Centre for Lipid Science and Technology, [b] Medicinal Chemistry and Pharmacology Division,

[c] Organic Synthesis and Process Chemistry Division, CSIR-Indian Institute of Chemical Technology, Uppal Road, Hyderabad 500 007, India

[d] Department of Biotechnology, Acharya Nagarjuna University, Guntur 522 510, India

E-mail: karuna@iict.res.in, karuna@csiriict.in

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A series of novel chiral 1,4-disubstituted-1*H*-1,2,3-triazole derivatives **8a-i** has been accomplished using (*Z*)-methyl-12-azidooctadec-9-enoate, which is a derivative of castor oil fatty acid ester, methyl ricinoleate. The 1,2,3-triazole analogues have been synthesized regioselectively by the Cu-catalysed azide-alkyne cycloaddition (CUAAC) *via* Huisgen "Click Chemistry". The target 1,2,3-triazole derivatives' structure has been characterized by FT-IR, ^{1}H and ^{13}C NMR, and mass spectroscopy. The dipole moment of the triazole derivatives are in the range of 4.8 –5.6 Debye units, indicating their stability towards different environments. The synthesized compounds **8 a-i** have been evaluated for their anticancer activity against four different cancer cell lines such as human lung cancer cell line (A549), human cervical cancer cell line (HeLa), human prostate cancer cell line (DU145), and human breast cancer cell line (MDA-MB-231) and doxorubicin is used as a standard reference drug. All the compounds exhibit moderate anticancer activity against the four cancer cell lines except compound **8g** bearing phenylalanine. Among these compounds, **8c**, *i.e.*, valine substituted-1,2,3-triazole displays superior anticancer activity against A549 (IC_{50} 12.3 ± 0.24µM); DU145 (IC_{50} 15.6±0.24µM); MDA-MB-231 (IC_{50} 17.8±0.20µM) cancer cell line compared to the other tested compounds. Further, the triazole derivatives are found to be quite safe towards the normal cell, as they do not exhibit any activity towards HLF-Human lung fibroblast.

Keywords: Castor oil, L-Amino acid, Click reaction, CUAAC, 1,2,3-Triaozle, Anticancer activity

Cancer is the name given to a collection of related diseases due to the uncontrolled growth of cells that interfere with the development of healthy cells. Cancer can start almost anywhere in the human body, which is made up of trillions of cells, and it differs from most other diseases that it can develop at any stage in life and any body organ. Various cancer cells such as human breast cancer, cervical cancer, lung cancer, and prostate cancer cells are targeted to control their growth during the treatment. Keeping this in mind, cancer drugs have been designed to slowly act on the cancerous cells and halt their progression by suppressing them through various molecular mechanisms[1-3].

The knowledge of the molecular basis of carcinogenesis has paved the way for discovering new, more selective, and less toxic chemo-preventive agents. Nitrogen-containing five-membered heterocyclic molecules have received much attention due to their use as synthetic intermediates. Amongst these biologically active heterocyclic compounds, 1,2,3-triazoles and their derivatives have shown therapeutic applications in day-to-day life[4]. 1,2,3-Triazole, a planar and highly nitrogen-rich five-membered heterocyclic compound, exhibits interesting biological systems. Some of these 1,2,3-Triazole compounds have shown significant anticancer activity against different cancer lines such as prostate, lung, and breast cancers[5]. This heterocyclic unit is stable to metabolic degradation, which can be used to bind bimolecular targets and solubility[6,7]. These are pharmacologically active molecules and can be utilized in material science and chemical biology[8]. It is an important class of heterocyclic ring compounds found in numerous industrial applications such as dyestuffs, corrosion inhibitors, and photo stabilizers of polymers[9,10]. In addition, 1,2,3-triazole ring is chemically inert against hydrolysis, oxidation, and reduction under acidic and basic conditions[11].

Several methodologies have been developed to control the regioselectivity involved in using transition metals and improve the reaction conditions

Dr. D. S. Kothari Postdoctoral Fellow, School of Chemistry, University of Hyderabad, Gachibowli, Hyderabad 500 046, India

for forming 1,2,3-triazoles. Among these, Cu (I) catalysed azide-alkyne cycloaddition (CUAAC) reaction is the well-documented method described by varying catalysts and ligands used, including the mechanistic aspects[12,13]. In 2001, Sharpless[14] and Meldal[15] developed the copper-catalysed azide-alkyne cycloaddition reaction (CuAAC) independently, allowing the selective formation of chiral 1,4-disubstituted regioisomers in the presence of copper (I) catalyst at RT. The CuAAC reaction is an essential class in the chemistry of 1,2,3-triazoles due to 100% atom economy, regioselectivity, and mild reaction conditions[16]. The CUAAC reaction is the most widely used "click chemistry" method given by Sharpless due to its simplicity, highly reliable, and high selectivity[17-19]. Consequently, this protocol has increased its applications in various disciplines such as drug discovery, organic, polymer, material, and medicinal chemistry[11].

The approach has been used to synthesize pharmacologically active molecules by regioselective synthesis of 1,2,3-triazoles. The broad range of CuAAC is being used in several areas of life and material sciences, such as drug discovery[20], DNA labelling[21], oligonucleotide synthesis[22], and bioconjugation[23], biochemistry[24], and dendrimer[25]. These can be considered an essential class of *N*-containing heterocycles in the field of organic and medicinal chemistry due to their wide range of biological applications, including antibacterial[26], antifungal[27], anticancer[28], anti-tuberculosis[29], antimalarial[30] anti-HIV[31] and α-glycosidase inhibitor[32,33].

India is the second-largest producer of castor oil (11.26 Lakh tons, 2018) obtained from *Ricinus communis* seeds (Euphorbiaceous family). It is one of the oldest medicinal oils primarily used as a purgative/laxative to counter constipation[34]. Castor oil is classified as non-edible oil. It contains around 87-90% of ricinoleic acid [(9Z, 12R)-12-hydroxyoctadec-9-enoic acid; C18:1-OH], present in the castor oil. The fatty acid present in castor oil is homochiral, with the hydroxyl group in a homoallylic position that seems unusual and unique. Due to the unique structure of ricinoleic acid makes it a beneficial chemical entity in the transformation of chemical and biochemical processes to produce several bioactive molecules[35-37]. In the recent years most of the researchers have focused on the ricinoleic acid-based derivatives and their therapeutic applications[38]. On the other hand, L-Amino acids are also essential components in the living system. The amine and carboxyl functional groups of amino acids are beneficial for producing novel compounds that can exhibit specific properties. Given the positive aspects of ricinoleic acid and amino acids, the present study is designed to synthesize a novel series of ricinoleic acid-based-amino acid substituted chiral 1,4-disubstituted-1,2,3-triazole derivatives **8 (a-i)** and their evaluation for anticancer activities against four cancer cell lines. As most of the cancer drugs have adverse effect on normal cells, the synthesized triazoles have been tested for cytotoxicity towards normal cell line. The retrosynthetic pathway for the formation of ricinoleic acid based-chiral 1,4-disubstituted- 1,2,3-triazole derivatives is shown in Fig. 1.

Experimental Section

All the chemicals such as methane sulfonyl chloride (MsCl), sodium azide (NaN_3), di-*tert*-butyl dicarbonate (BOC anhydride), 4-Dimethylamino pyridine (DMAP), sodium hydroxide (NaOH), sodium bicarbonate ($NaHCO_3$), potassium hydrogen sulphate ($KHSO_4$), and imidazole were purchased from SRL chemicals (Mumbai, India). Propargyl alcohol, copper iodide (CuI), N, N-isopropyl diimide (DIPEA), N, N'-dicyclohexylcarbodiimide (DCC), trifluoroacetic acid (TFA), *tert*-butyldimethylsilyl chloride (TBDMS-Cl) and L-amino acids namely glycine, alanine, phenylalanine, valine, leucine, isoleucine, serine, methionine, tyrosine, and tryptophan were purchased from Sigma-Aldrich Chemicals Co., without any further purification. All

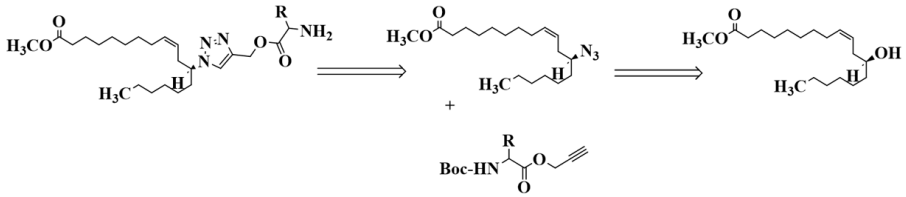


Fig. 1 — The retro synthetic pathway for formation of ricinoleic acid based-chiral 1,4-disubstituted-1,2,3-triazole derivatives

the solvents like chloroform ($CHCl_3$), dichloromethane (DCM), ethanol (EtOH), dimethyl sulfoxide (DMSO), 1,4-dioxane, dry dimethylformamide (DMF), dry tetrahydrofuran (THF), tetra-N-butyl ammonium fluoride (TBAF) and acetic acid (AcOH) were purchased from M/s S. D. Fine Chemicals (Mumbai, India) and those are the highest-grade purity. Castor oil was purchased from M/s Ramcharan Industries Pvt. Ltd., Hyderabad. Methyl ricinoleate was prepared from castor oil and was purified by column chromatography in the laboratory (99%).

Analytical methods

All the synthesized 1,2,3-triazole derivatives were purified by silica gel (60-120 mesh) column chromatography (Acme Synthetic Chemicals, Mumbai, India), and fractions containing the pure compounds were identified by thin-layer chromatography (TLC) performed on precoated silica gel 60 F_{254} plates from Merck (Darmstadt, Germany). Infrared spectra (IR) were recorded in chloroform on a Perkin-Elmer FT-IR spectrum BX instrument (Model: Spectrum BX; Perkin-Elmer, Connecticut, USA). Nuclear magnetic resonance ^1H and ^{13}C NMR spectral data were recorded on Avance 300 MHz, 500 MHz, and 75 MHz spectrometers (^1H at 300 & 500 MHz and ^{13}C at 75 MHz) in deuterated chloroform ($CDCl_3$) solution and Tetramethylsilane was used as internal standard. The chemical shift values were expressed in units of δ (ppm) downfield from tetramethylsilane. The standard abbreviations s, d, t, q, m, and bs refer to a singlet, doublet, triplet, quartet, multiplet, and broad singlet, respectively. The coupling constant (J) values were measured in Hertz (Hz). Mass spectra were recorded using Micromass-Quatromicro electron spray ionization on ESI/MS using Waters e2695 separators module (Waters, Milford, MA, USA) mass spectrometer. All the reactions were conducted on the magnetic stirrer. The static dipole moment of the molecules was measured by Density Functional Theory (DFL). These values were obtained with a finite field approach implemented in the DFT program ALLCHEM.

Synthetic procedures

N-BOC protection of L-amino acids 2a-i

L-Amino acids (5 mM) and 1.0 N NaOH (6 mL) were taken in a round bottom flask. BOC anhydride (7 mM) was added dropwise to the mixture at 0 ºC for 30 min under magnetic stirring. After the addition, the reaction mixture was allowed to reach RT, and the reaction was continued for 10-12 h (Fig. 2). The reaction course was monitored by TLC using the $CHCl_3$: MeOH: H_2O (65: 24: 4, v/v/v) solvent system. The formation of the product was confirmed by a negative ninhydrin test, *i.e.*, the disappearance of the pink colour. The reaction mixture was dissolved in hexane and extracted with water (2 × 25 mL) followed by aqueous saturated sodium carbonate (2 × 25 mL). The combined aqueous layer was adjusted to *p*H 1 using potassium hydrogen sulphate ($KHSO_4$), then extracted with ethyl acetate and washed with water (3 × 25 mL). The ethyl acetate layer was passed through Na_2SO_4 and concentrated on a rota evaporator to obtain the corresponding *N*-Boc-protected L-amino acids. The yields and structure of *N*-Boc-protected L-amino acids are given in Table 1. These compounds are used for the following reaction without any further purification.

Synthesis of propargyl esters of N-Boc-protected L-amino acids 3a-i

A solution of *N*-Boc-protected amino acid (5 mM), DMAP (0.5 mM) in 20 mL DCM was taken in a round bottom flask and cooled to 0ºC under magnetic stirring. To this solution, propargyl alcohol (5 mM) was slowly added to the reaction mixture under N_2 atmosphere. A solution of DCC (5 mM) was dissolved in 15 mL DCM dropwise under magnetic stirring for 30 minutes. During the addition of DCC to the reaction mixture, by-product DCU formation was observed at the time of response. The reaction mixture was raised to RT and continued reaction for 12-15 h

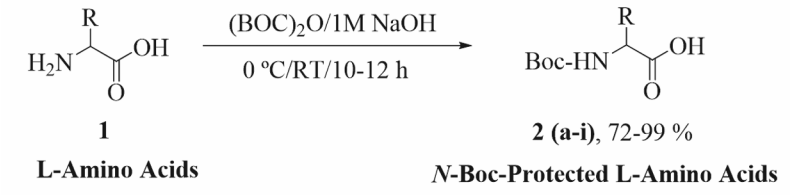
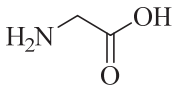
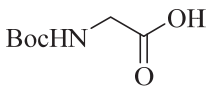
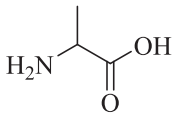
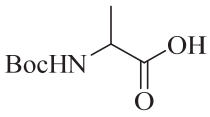
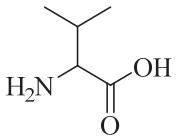
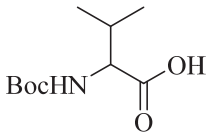
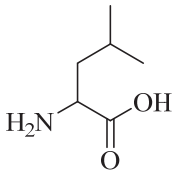
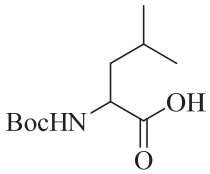
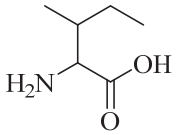
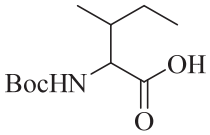
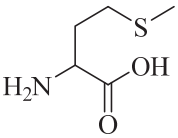
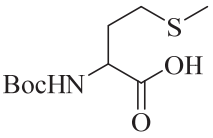
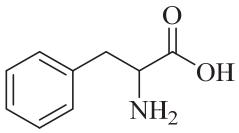
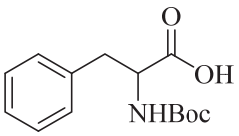
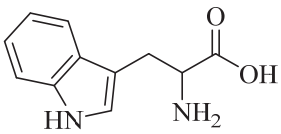
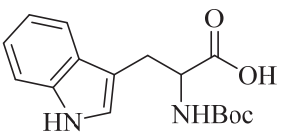
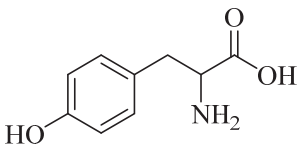
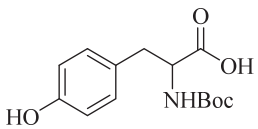


Fig. 2 — *N*-Boc-protection of L-amino acids

Table 1 — *N*-Boc protected L-amino acids and their yields

	L-Amino acid **1a-i**		*N*-Boc-protected L-amino acid **2a-i**	Yield (%)
1a	(Glycine)		**2a** 	99
1b	(Alanine)		**2b** 	92
1c	(Valine)		**2c** 	99
1d	(Leucine)		**2d** 	98
1e	(Isoleucine)		**2e** 	99
1f	(Methionine)		**2f** 	80
1g	(Phenylalanine)		**2g** 	98
1h	(Tryptophan)		**2h** 	95
1i	(Tyrosine)		**2i** 	72

until the esterification was completed. The reaction was monitored by TLC using the $CHCl_3$: MeOH (95:5, v/v) solvent system. The by-product N, N'-dicyclohexylurea (DHU), was filtered off, and the filtrate was washed with water and 5% acetic acid solution and passed through anhydrous Na_2SO_4. The solvent layer was evaporated under reduced pressure to yield the corresponding N-Boc-protected propargyl amino acid esters **3 (a-i)** with good yields (Fig. 3). The structure and yields of the propargyl ester compounds are shown in Table 2.

The remaining reaction steps are explained taking tyrosine based triazole as a representative.

N-Boc-OTBDMS-L-Tyrosine-OH, 2i′

N-Boc-L-Tyrosine (1.5 g, 5.3 mM) and imidazole (1.08 g, 16 mM) dissolved in dry DMF (10 mL) were taken in a 50 mL round bottom flask and cooled to 0° C under magnetic stirring. Tert-Butyldimethylsilyl chloride TBDMS-Cl (1.19 g, 8 mM) dissolved in 10 mL dry DMF was added slowly to the reaction mixture for 15 min and then heated to RT, and the

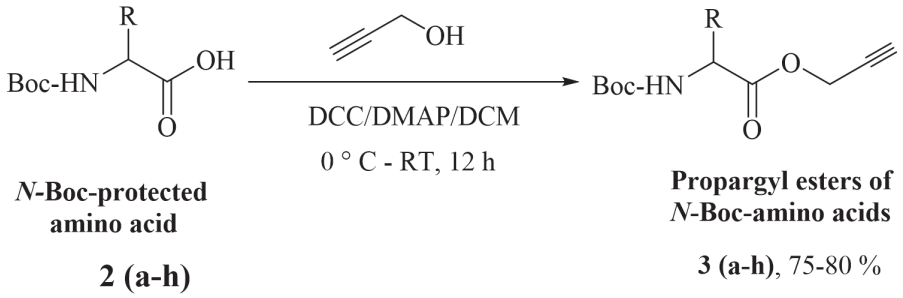
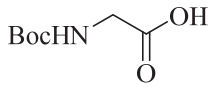
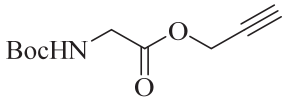
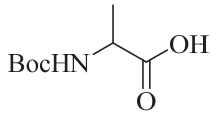
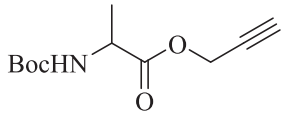
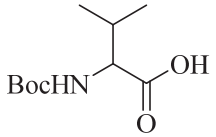
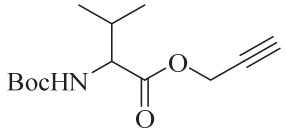
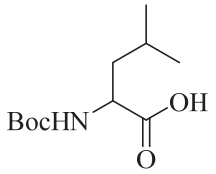
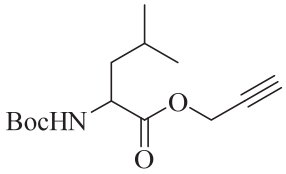
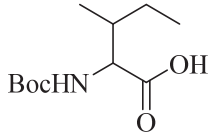
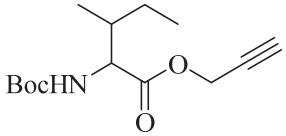
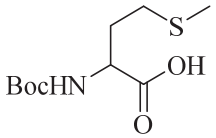
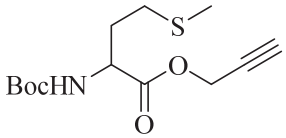
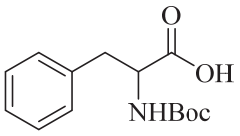
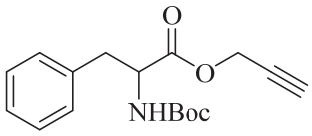
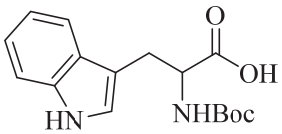
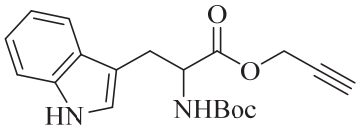
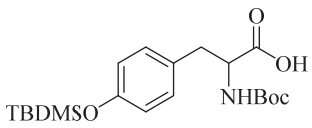
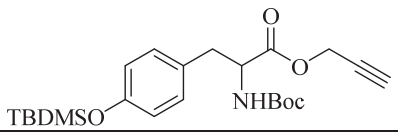


Fig. 3 — Synthesis of propargyl esters of *N*-Boc-amino acids

Table 2 — Propargyl esters of *N*-Boc-protected L-amino acids

	N-Boc-protected L-amino acid	N-Boc-protected propargyl amino acid ester	Yield (%)
2a		**3a** 	98
2b		**3b** 	72
2c		**3c** 	92
2d		**3d** 	50
2e		**3e** 	92
2f		**3f** 	96
2g		**3g** 	91
2h		**3h** 	62
2i'		**3i'** 	92

reaction was continued for 18 h. After that, 10 mL distilled water was added to the reaction mixture and extracted with diethyl ether and chloroform. The ether layer was dried over anhydrous Na_2SO_4 and evaporated to obtain 1.92 g of *N*-Boc-OTBDMS-L-Tyrosine-OH (Ref. 39), shown in Fig. 4.

Propargyl ester of N-Boc-OTBDMS-L-Tyrosine, 3i′

Propargyl ester of *N*-Boc-OTBDMS-L-Tyrosine was synthesized by taking *N*-BOC-OTBDMS-L-tyrosine-OH (1.15 g, 3 mM) and DMAP (0.04 g, 0.3 mM) dissolved in 15 mL DCM taken in a 50 mL round bottom flask and cooled to 0° C. Propargyl alcohol (0.17 g, 3 mM) was added to the reaction mixture was added at 0° C. During the addition of DCC, the by-product DCU formation was observed. The reaction was continued for 12 h at RT. The reaction progress was monitored by TLC using the $CHCl_3$: MeOH (95:5, v/v) solvent system. The by-product, DCU, was separated and concentrated the DCM layer. After concentrating the DCM, hexane was added to the crude mixture to separate DCU as it was not soluble in hexane. Filtered off the DCU and concentrated the hexane layer to obtain 1.22 g (yield 92%) propargyl ester of *N*-Boc-OTBDMS-L-tyrosine (Fig. 5). The product was confirmed by ^{1}H NMR and mass spectral studies.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.19 (s, 6H), 0.97 (s, 9H), 1.42 (s, 9H), 2.50 (t, J = 2.4 Hz, 1H), 3.04 (m, 2H), 4.56 (m, 1H), 4.70 (m, 2H), 4.93 (d, J = 8.1 Hz, 1H), 6.76 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.3 Hz, 2H); ESI-MS: *m/z* 433 $[M+H]^+$, 456 $[M+Na]^+$.

Synthesis of 5 and 6

Synthesis of (Z)-methyl-12-(methan sulfonyloxy) octadec-9-enoate (**5**) and (Z)-methyl-12-azidooctadec-9-enoate (**6**) were prepared as per the literature procedure[35,36].

General procedure for the synthesis of N-Boc-1,2,3-triazoles from propargyl esters of N-Boc-L-amino acids 7a-i′

N-Boc-protected 1,2,3-triazole derivatives were prepared from an equimolar ratio of propargyl ester of *N*-Boc-L-amino acids (1 mM), and (*Z*)-methyl-12-azidooctadec-9-enoate (1 mM) dissolved in 10 mL DCM was taken in a 25 mL round bottom flask at RT under magnetic stirring. CuI (0.01 mM) and DIPEA (0.02 mM) were added to the reaction mixture. To this, 0.1 mL of ACOH was added during the reaction. However, the colour change was observed in the reaction mixture. The progress of the reaction was monitored by TLC eluting hexane: ethyl acetate (70:30 v/v) as a solvent system. After the maximum conversion of the reaction, added 10 mL distilled water, extracted the reaction mixture with DCM, and washed the product with aq. $NaHCO_3$. The DCM layer was passed through anhydrous Na_2SO_4 and concentrated by a rotary evaporator. The obtained product was purified by silica gel column chromatography to obtain a 72-95% yield of the corresponding *N*-Boc-1,2,3-triazole derivatives (Fig. 6).

The structure of the compounds was thoroughly characterized by ^{1}H NMR and mass spectral studies. The prepared compounds 7 (a-i') spectral data are given below.

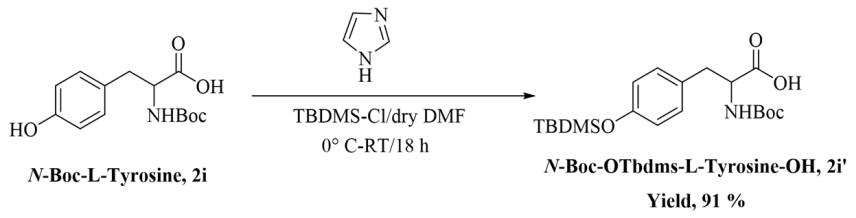


Fig. 4 — TBDMS protection of *N*-Boc-L-Tyrosine-OH.

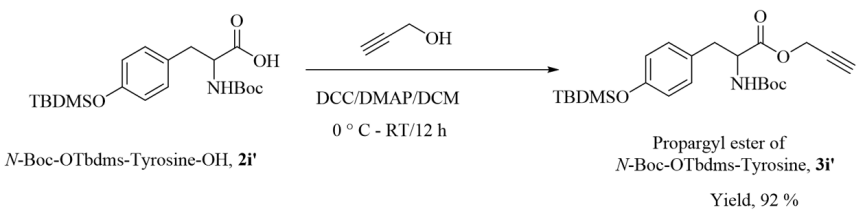


Fig. 5 — Propargyl ester of *N*-Boc-OTBDMS-L-Tyrosine-OH.

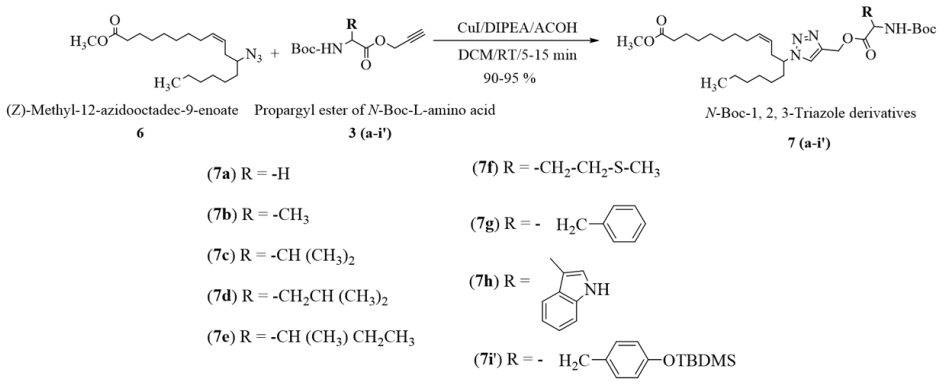


Fig. 6 — Synthesis of *N*-Boc-1,2,3-triazole derivatives.

(Z)-Methyl-12-(4-((((tert-butoxycarbonyl) glycyl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 7a: Mol. Formula: $C_{29}H_{50}N_4O_6$. Yield 82%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.85 (t, *J* = 6.8 Hz, 3H), 1.26 (m, 16H), 1.45 (s, 9H), 1.60-1.90 (m, 6H), 2.30 (t, *J* = 7.6 Hz, 2H), 2.60 (t, *J* = 7.1 Hz, 2H), 3.66 (s, 3H), 3.94 (d, *J* = 5.6 Hz, 2H), 4.46 (m, 1H), 4.98 (m, 1H), 5.19 (m, 1H), 5.30 (s, 2H), 5.45 (m, 1H), 7.55 (s, 1H); ESI-MS: *m/z* 574 $[M+H+Na]^+$.

(Z)-Methyl-12-(4-((((tert-butoxycarbonyl) alanyl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 7b: Mol. Formula: $C_{30}H_{52}N_4O_6$. Yield 85%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.85 (t, *J* = 7.1 Hz, 3H), 1.25 (m, 16H), 1.35 (d, 3H), 1.43 (s, 9H), 1.59-1.89 (m, 6H), 2.30 (t, *J* = 7.4 Hz, 2H), 2.59 (t, *J* = 7.0 Hz, 2H), 3.66 (s, 3H), 4.32 (m, 1H), 4.45 (m, 1H), 4.97 (d, *J* = 7.5 Hz, 1H), 5.18 (m, 1H), 5.29 (s, 2H), 5.44 (m, 1H), 7.54 (s, 1H); ESI-MS: *m/z* 565 $[M+H]^+$, 587 $[M+Na]^+$.

(Z)-Methyl-12-(4-((((tert-butoxycarbonyl) valyl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 7c: Mol. Formula: $C_{32}H_{56}N_4O_6$. Yield 91%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.79-0.91 (m, 9H), 1.26 (m, 16H), 1.44 (s, 9H), 1.62-1.91 (m, 6H), 2.09 (m, 1H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.59 (t, *J* = 6.9 Hz, 2H), 3.67 (s, 3H), 4.23 (m, 1H), 4.46 (m, 1H), 4.99 (d, *J* = 8.8 Hz, 1H), 5.16 (m, 1H), 5.29 (q, 2H), 5.44 (m, 1H), 7.55 (s, 1H); ESI-MS: *m/z* 594 $[M+2]^+$, 616 $[M+H+Na]^+$.

(Z)-Methyl-12-(4-((((tert-butoxycarbonyl) leucyl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 7d: Mol. Formula: $C_{33}H_{58}N_4O_6$. Yield 94%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.83-0.97 (m, 9H), 1.27 (m, 16H), 1.43 (s, 9H), 1.61-1.90 (m, 9H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.60 (t, *J* = 6.9 Hz, 2H), 3.66 (s, 3H), 4.30 (m, 1H), 4.46 (m, 1H), 4.89 (d, *J* = 9.0 Hz, 1H), 5.18 (m, 1H), 5.28 (s, 2H), 5.44 (m, 1H), 7.56 (s, 1H); ESI-MS: *m/z* 608 $[M+2]^+$, 629 $[M+Na]^+$.

(Z)-Methyl-12-(4-((((tert-butoxycarbonyl) isoleucyl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl) octadec-9-enoate, 7e: Mol. Formula: $C_{33}H_{58}N_4O_6$. Yield 95%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.83-0.88 (m, 9H), 1.26 (m, 16H), 1.43 (s, 9H), 1.63 (m, 2H), 1.71 (m, 3H), 1.89 (m, 4H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.59 (t, *J* = 7.5 Hz, 2H), 3.67 (s, 3H), 4.27 (m, 1H), 4.46 (m, 1H), 4.98 (d, *J* = 7.5 Hz, 1H), 5.18 (m, 1H), 5.29 (q, 2H), 5.44 (m, 1H), 7.55 (s, 1H); ESI-MS: *m/z* 608 $[M+2]^+$, 629 $[M+Na]^+$.

(Z)-Methyl-12-(4-((((tert-butoxycarbonyl) methionyl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl) octadec-9-enoate, 7f: Mol. Formula: $C_{32}H_{56}N_4O_6S$. Yield 80%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.85 (t, *J* = 7.5 Hz, 3H), 1.26 (m, 16H), 1.43 (s, 9H), 1.60-1.90 (m, 8H), 2.06 (s, 3H), 2.30 (t, *J* = 7.6 Hz, 2H), 2.49 (t, *J* = 7.7 Hz, 2H), 2.60 (t, *J* = 6.8 Hz, 2H), 3.67 (s, 3H), 4.44 (m, 2H), 4.96 (d, *J* = 7.5 Hz, 1H), 5.14 (m, 1H), 5.29 (s, 2H), 5.44 (m, 1H), 7.55 (s, 1H); ESI-MS: *m/z* 647 $[M+Na]^+$.

(Z)-Methyl-12-(4-((((tert-butoxycarbonyl) phenylalanyl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl) octadec-9-enoate, 7g: Mol. Formula: $C_{36}H_{56}N_4O_6$. Yield 85%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.85 (t, *J* = 7.5 Hz, 3H), 1.27 (m, 16H), 1.41 (s, 9H), 1.61-1.90 (m, 6H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.60 (t, *J* = 7.5

Hz, 2H), 3.06 (m, 2H), 3.66 (s, 3H), 4.45 (m, 1H), 4.59 (m, 1H), 4.97 (d, J = 7.5 Hz, 1H), 5.18 (m, 1H), 5.26 (s, 2H), 5.45 (m, 1H), 7.09-7.24 (m, 5H), 7.45 (s, 1H); ESI-MS: *m/z* 641 $[M+H]^+$.

(Z)-Methyl-12-(4-((((tert-butoxycarbonyl) tryptophyl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl) octadec-9-enoate, 7h: Mol. Formula: $C_{38}H_{57}N_5O_6$. Yield 86%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.85 (m, 3H), 1.26 (m, 16H), 1.42 (s, 9H), 1.60-1.89 (m, 6H), 2.30 (t, J = 7.5 Hz, 2H), 2.59 (t, J = 6.9 Hz, 2H), 3.27 (d, J = 5.2 Hz, 2H), 3.67 (s, 3H), 4.42 (m, 1H), 4.67 (m, 1H), 5.07 (m, 1H), 5.20 (s, 2H), 5.44 (m, 1H), 6.96 (s, 1H), 7.09-7.36 (m, 4H), 7.57 (d, J = 7.7 Hz, 1H), 8.13 (s, 1H); ESI-MS: *m/z* 702 $[M+Na]^+$.

(Z)-Methyl-12-(4-(((2-((tert-butoxycarbonyl) amino)-3-(4-((tert-butyldimethylsilyl)oxy)phenyl) propanoyl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl) octadec-9-enoate, 7i′: Mol. Formula: $C_{42}H_{70}N_4O_7Si$. Yield 86%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.17 (s, 6H), 0.85 (t, J = 6.9 Hz, 3H), 0.97 (s, 9H), 1.26 (m, 14H), 1.40 (s, 9H), 1.61 (m, 4H), 1.90 (m, 4H), 2.30 (t, J = 7.5 Hz, 2H), 2.60 (t, J = 6.9 Hz, 2H), 2.99 (m, 2H), 3.66 (s, 3H), 4.43-4.57 (m, 2H), 4.93 (d, J = 8.3 Hz, 1H), 5.18-5.44 (m, 2H), 5.25 (s, 2H), 6.73 (d, J = 8.3 Hz, 2H), 6.95 (d, J = 8.4 Hz, 2H), 7.45 (s, 1H); ESI-MS: *m/z* 771 $[M+H]^+$, 794 $[M+Na]^+$.

Deprotection of TBDMS group in N-Boc-tyrosine-1,2,3-triazole, 7i

0.87 mL of a 1M solution of tetra butyl ammonium fluoride (TBAF) in anhydrous THF were slowly added to a solution of *N*-Boc-OTBDMS-Tyrosine-1,2,3-triazole (0.673 g, 0.87 mmol) in 10 mL of dry THF at 0° C. The resulting mixture was stirred at RT for 1 h. The progress of the reaction was monitored by TLC eluted with hexane-ethyl acetate (70:30 v/v) as a solvent system. To this, 10 mL of water was added. The organic phase was extracted, dried over Na_2SO_4, evaporated to dryness, and purified by silica gel column chromatography (hexane/ethyl acetate 70:30, v/v) to obtain 0.52g (yield, 93%) of *N*-Boc-Tyrosine-1,2,3-triazole as a colourless liquid (Fig. 7). The structure of the product **7i** was confirmed by ^{1}H NMR and mass spectral studies.

Mol. Formula: $C_{36}H_{56}N_4O_7$. Yield 93%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.85 (t, 3H, J = 6.7 Hz), 1.25 (m, 14H), 1.42 (s, 9H), 1.59 (m, 4H), 1.90 (m, 4H), 2.31 (t, J=7.5 Hz, 2H), 2.61 (t, J = 7.5 Hz, 2H), 2.98 (m, 2H), 3.68 (s, 3H), 4.44-4.55 (m, 2H), 4.97 (d, J = 8.3 Hz, 1H), 5.18-5.49 (m, 2H), 5.25 (s, 2H), 6.73 (d, J = 8.3 Hz, 2H), 6.94 (d, J = 8.3 Hz, 2H), 7.48 (s, 1H); ESI-MS: *m/z* 656 $[M+H]^+$, 679 $[M+Na]^+$.

General procedure for the deprotection of N-Boc group in amino acid substituted-N-Boc-1,2,3-triazole derivatives, 8a-i

The synthesized amino acid substituted-*N*-Boc-1,2,3-triazole derivatives **7 (a-i)** (1 mM) were dissolved in CH_2Cl_2 (20 mL). TFA (2.5 Mm) in DCM was added slowly to this solution in an ice-cold solution. The reaction mixture was allowed to stir at RT for 8-10 h until the completion of the reaction. TFA was neutralized by the gradual addition of an aqueous NaOH solution. The product was extracted from the reaction mixture. The organic layer was washed with distilled water; the organic layer was passed through anhydrous Na_2SO_4, concentrated, and dried the product under reduced pressure. The resulting product was purified by column chromatography, eluted with $CHCl_3$: MeOH (95:5, v/v) to obtain 67-75% isolated yields of the corresponding amino acid substituted-1,2,3-triazole derivatives (Fig. 8).

The structure of the compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR, and mass spectral studies.

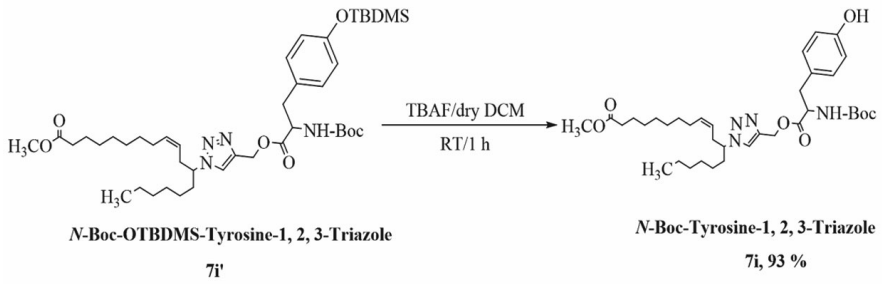


Fig. 7 — Synthesis of *N*-Boc-Tyrosine-1,2,3-triazole.

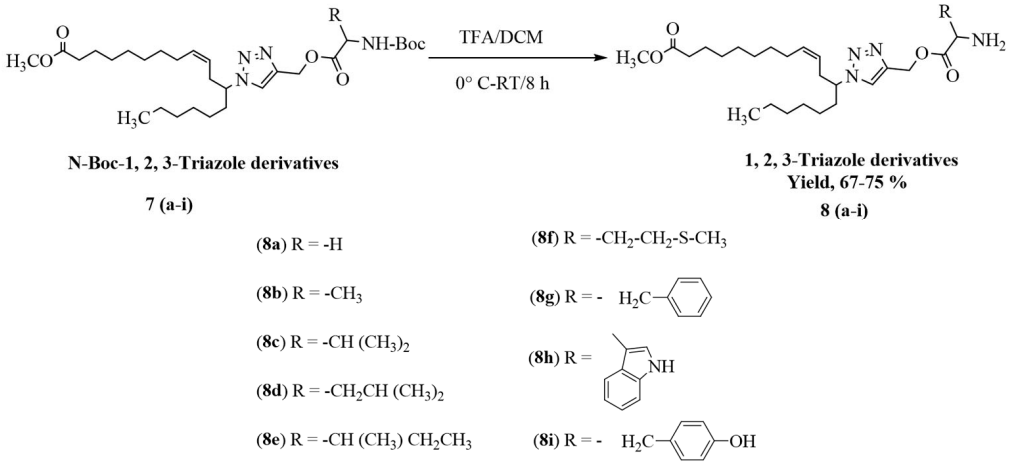


Fig. 8 — Synthesis of L-amino acid substituted- 1,2,3-Triazole derivatives.

The spectral data of the synthesized compounds **8a-i** are given below.

(Z)-Methyl-12-(4-((glycyloxy)methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 8a: Mol. Formula: $C_{24}H_{42}N_4O_4$. Yield 70%. IR ($CHCl_3$): 3338, 3003, 2923, 2852, 1739, 1437, 1357, 1200, 1050, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.85 (t, J = 6.9 Hz, 3H), 1.25 (m, 16H), 1.62 (m, 4H), 1.90 (m, 2H), 2.30 (t, J = 7.5 Hz, 2H), 2.60 (t, J = 6.9 Hz, 2H), 3.67 (s, 3H), 4.08 (m, 2H),4.48 (m, 1H), 4.79 (s, 2H), 5.16-5.48 (m, 2H), 7.47 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 22.4, 24.8, 27.1, 28.7, 29.2, 31.1, 33.4, 34.0, 34.7, 42.0, 51.4, 56.5, 62.2, 120.2, 123.4, 133.7, 146.9, 174.3, 175.5; ESI-MS: *m/z* 474 $[M+H+Na]^+$.

(Z)-Methyl-12-(4-((alanyloxy)methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 8b: Mol. Formula: $C_{25}H_{44}N_4O_4$. Yield 65%. IR ($CHCl_3$): 3338, 3003, 2923, 2852, 1739, 1437, 1357, 1200, 1050, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.85 (t, J = 6.9 Hz, 3H), 1.25 (m, 16H), 1.62 (m, 4H), 1.69 (s, 3H), 1.90 (m, 2H), 2.30 (t, J = 7.5 Hz, 2H), 2.39 (m, 1H), 2.60 (t, J = 6.9 Hz, 2H), 3.67 (s, 3H), 4.48 (m, 1H), 4.80 (d, J = 5.0 Hz, 2H), 5.16-5.48 (m, 2H), 7.47 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 18.2, 22.4, 24.8, 25.8, 27.1, 28.7, 31.4, 33.4, 33.9, 34.7, 50.1, 51.3, 56.4, 62.1, 120.1, 123.4, 133.7, 147.5, 174.2, 175.4; ESI-MS: *m/z* 488 $[M+H+Na]^+$.

(Z)-Methyl-12-(4-((valyloxy)methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 8c: Mol. Formula: $C_{27}H_{48}N_4O_4$. Yield 68%. IR ($CHCl_3$): 3383, 3007, 2929, 2857, 1737, 1458, 1339, 1172, 1049, 758 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 0.82 (d, J = 6.8 Hz, 3H), 0.85 (d, J = 7.1 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H), 1.25 (m, 16H), 1.61 (m, 4H), 1.91 (m, 2H), 2.00 (m, 1H), 2.30 (t, J = 7.4 Hz, 2H), 2.59 (t, J = 7.0 Hz, 2H), 3.32 (d, J = 4.8 Hz, 1H), 3.67 (s, 3H), 4.48 (m, 1H), 5.16 (m, 1H), 5.27 (q, 2H), 5.44 (m, 1H), 7.55 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 16.9, 19.1, 22.4, 24.8, 25.7, 27.1, 28.6, 28.9, 31.4, 31.9, 33.4, 33.9, 34.7, 51.4, 57.7, 59.6, 62.2, 120.0, 122.5, 123.2, 133.8, 142.1, 174.4, 175.4; ESI-MS: *m/z* 494 $[M+2]^+$, 516 $[M+H+Na]^+$.

(Z)-Methyl-12-(4-((leucyloxy)methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 8d: Mol. Formula: $C_{28}H_{50}N_4O_4$, Yield, 75%. IR ($CHCl_3$): 3382, 3007, 2927, 2957, 1730, 1438, 1361, 1170, 1050, 758 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.83-0.92 (m, 9H), 1.26 (m, 16H), 1.60 (m, 7H), 1.90 (m, 2H), 2.30 (t, J = 7.5 Hz, 2H), 2.60 (t, J = 7.1 Hz, 2H), 3.48 (m, 1H), 3.67 (s, 3H), 4.47 (m, 1H), 5.18 (m, 1H), 5.26 (s, 2H), 5.44 (m, 1H), 7.53 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 21.7, 22.4, 22.8, 24.6, 24.8, 25.7, 27.1, 28.6, 28.9, 29.2, 31.4, 33.4, 33.9, 34.6, 43.7, 120.1, 122.4, 123.2, 133.8, 142.0, 174.2, 176.4; ESI-MS: *m/z* 508 $[M+2]^+$, 530 $[M+H+Na]^+$.

(Z)-Methyl-12-(4-((isoleucyloxy)methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 8e: Mol. Formula: $C_{28}H_{50}N_4O_4$, Yield, 73%. IR ($CHCl_3$): 3338, 3008, 2927, 2957, 1730, 1440, 1361, 1168, 1050, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 0.83-0.88

(m, 9H), 1.26 (m, 16H), 1.59 (m, 6H), 1.74 (m, 1H), 1.89 (m, 2H), 2.30 (t, J = 7.6 Hz, 2H), 2.59 (t, J = 7.0 Hz, 2H), 3.36 (d, J = 5.0 Hz, 1H), 3.67 (s, 3H), 4.48 (m, 1H), 5.17 (m, 1H), 5.26 (q, 2H), 5.44 (m, 1H), 7.54 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.5, 13.9, 15.5, 22.4, 24.5, 25.7, 27.1, 27.1, 28.6, 31.4, 33.4, 33.9, 34.7, 39.0, 51.3, 57.7, 58.8, 62.2, 120.0, 122.4, 123.2, 133.8, 142.1, 174.1, 175.4; ESI-MS: *m/z* 508 $[M+2]^+$, 530 $[M+H+Na]^+$.

(Z)-Methyl-12-(4-(((S-methylcysteinyl)oxy) methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 8f: Mol. Formula: $C_{27}H_{48}N_4O_4S$, Yield, 70%;IR ($CHCl_3$): 3355, 2930, 2857, 1741, 1456, 1217, 1050, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.86 (t, J = 6.9 Hz, 3H), 1.27 (m, 16H), 1.62 (m, 4H), 1.91 (m, 6H), 2.05 (s, 3H), 2.30 (t, 7.5 Hz, 2H), 2.60 (t, J = 6.9 Hz, 2H), 3.67 (s, 3H), 4.45 (m, 1H), 4.81 (m, 1H), 5.12-5.50 (m, 2H), 5.30 (s, 2H), 7.55 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 15.2, 21.9, 22.5, 24.6, 25.4, 26.8, 27.6, 28.1, 29.8, 30.2, 31.6, 33.2, 34.6, 52.5, 57.2, 59.6, 62.4, 120.1, 122.9, 123.2, 133.8, 142.1, 174.2, 175.4; ESI-MS: *m/z* 525 $[M+H]^+$, 547 $[M+Na]^+$.

(Z)-Methyl-12-(4-((phenylalanyloxy) methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 8g: Mol. Formula: $C_{31}H_{48}N_4O_4$, Yield, 76%. IR ($CHCl_3$): 3364, 3007, 2927, 2856, 1722, 1498, 1365, 1166, 1051, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.85 (t, J = 7.5 Hz, 3H), 1.26 (m, 16H), 1.61 (m, 4H), 1.90 (m, 2H), 2.30 (t, J = 7.5 Hz, 2H), 2.60 (t, J = 7.5 Hz, 2H), 3.06 (m, 2H), 3.66 (s, 3H), 4.45 (m, 1H), 4.59 (m, 1H), 5.14-5.50 (m, 2H), 5.26 (s, 2H), 7.08-7.25 (m, 5H), 7.45 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 22.4, 24.8, 25.8, 28.1, 28.9, 31.4, 33.3, 33.9, 34.6, 38.0, 51.3, 54.3, 58.5, 62.2, 120.2, 122.3, 123.2, 126.8, 128.4, 129.2, 133.8, 135.7, 141.7, 142.3, 171.7, 173.2; ESI-MS: *m/z* 564 $[M+H+Na]^+$.

(Z)-Methyl-12-(4-((tryptophyloxy)methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 8h: Mol. Formula: $C_{33}H_{49}N_5O_4$, Yield, 69%; IR ($CHCl_3$): 3318, 3129, 2926, 2856, 1732, 1454, 1223, 1057, 762 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.85 (m, 3H), 1.27 (m, 16H), 1.61 (m, 4H), 1.90 (m, 2H), 2.31 (t, J = 7.5 Hz, 2H), 2.59 (t, J = 6.9 Hz, 2H), 3.29 (d, J = 5.2 Hz, 2H), 3.67 (s, 3H), 4.48 (m, 1H), 4.70 (m, 1H), 5.14 (m, 1H), 5.20 (s, 2H), 5.44 (m, 1H), 6.96 (s, 1H), 7.09-7.36 (m, 4H), 7.57 (d, J = 7.7 Hz, 1H), 8.13 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 22.6, 24.9, 25.2, 27.4, 28.7, 29.9, 31.8, 32.9, 33.2, 34.1, 38.0, 51.2, 55.5, 59.2, 62.2, 109.8, 111.2, 118.5, 119.6, 120.2, 121.7, 123.5, 127.5, 132.2, 133.8, 136.5, 142.6, 171.2, 173.5; ESI-MS: *m/z* 602 $[M+Na]^+$.

(Z)-Methyl-12-(4-((tyrosyloxy) methyl)-1*H*-1,2,3-triazol-1-yl)octadec-9-enoate, 8i: Mol. Formula: $C_{31}H_{48}N_4O_5$, Yield, 75%. IR ($CHCl_3$): 3333, 3137, 3015, 2929, 1730, 1437, 1251. 1200, 1057, 763 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.86 (t, J = 6.7 Hz, 3H), 1.25 (m, 16H), 1.60 (m, 4H), 1.90 (m, 2H), 2.31 (t, J = 7.5 Hz, 2H), 2.60 (t, J = 7.5 Hz, 2H), 2.97 (m, 2H), 3.68 (s, 3H), 4.50 (m, 1H), 4.97 (m, 1H), 5.19 (m, 1H), 5.25 (s, 2H), 5.44 (m, 1H), 6.73 (d, J = 8.3 Hz, 2H), 6.93 (d, J = 8.3 Hz, 2H), 7.47 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.0, 22.4, 23.9, 25.6, 27.6, 28.8, 31.2, 33.3, 34.0, 35.2, 38.0, 51.6, 54.3, 58.6, 62.2, 120.1, 122.9, 126.8, 128.5, 130.1, 133.8, 141.3, 142.9, 155.3, 171.3 173.2; ESI-MS: *m/z* 557 $[M+H]^+$, 579 $[M+Na]^+$.

Anticancer activity

Anticancer activity of the synthesized ricinoleic acid-amino acid substituted 1,2,3-triazole derivatives **8 (a-i)** were measured using MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] against different cancer cell lines obtained from American Type Culture Collection (ATCC), Manassas, VA, the USA, such as **A549**- human lung cancer cell line (ATCC No. CCL-185), **HeLa**- human cervical cancer cell line (ATCC No. CCL-2), **DU-145** -human prostate cancer cell line (ATCC No. HTB-81), and **MDA-MB-231**-human breast cancer cell line (ATCC HTB-26) according to the method of Mosmann[40].

Briefly, the cells (2×10^4) were seeded in each well containing 0.1 mL of medium in 96 well plates. These well plates were incubated overnight at 37°C in 5% CO_2. The cells were treated with 100 µl of test concentrations such as 0.1, 1, 5, and 10 µM of test compounds at identical conditions with three replicates each. The final test concentrations were equivalent to 10 to 100 µM. The cell viability was assessed after 24 h by adding 10 µL of MTT (5 mg/mL) per well. The plates were incubated at 37 °C for an additional 3 h. The medium was discarded, and the formazan blue formed in the cells was dissolved in 100 µL of DMSO. The absorbance was measured at 540 nm using the TRIAD multimode reader (Dynex Technologies, Inc., Chantilly, VA). The IC_{50} values (50% inhibitory concentration) were

calculated from the plotted absorbance data from the dose-response curves. The assay used doxorubicin as standard or positive control and 1% DMSO as vehicle control. To account for the toxicity of DMSO, the values obtained for the DMSO control were subtracted from those of the test compounds. The IC_{50} values (in μM) are expressed as the mean ± SD of three independent experiments.

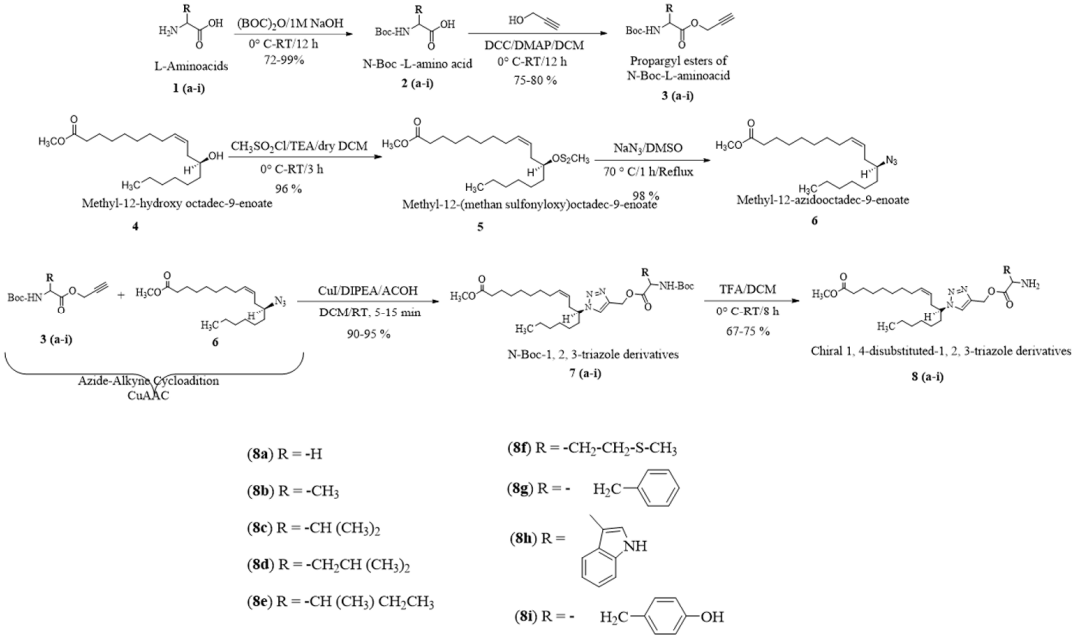
Results and Discussion

Heterocyclic scaffolds such as 1,2,3-triazoles with three nitrogen containing ring can interact with the biological targets by forming hydrogen bonds, have taken a leading role in drug discovery[39,41]. Keeping this in mind, the present study was focussed on the synthesis of ricinoleic acid-based chiral 1,4-disubstituted-1,2,3-triazole derivatives from naturally occurring L-amino acids, which were used as chiral synthons utilizing the strategy of acid-base Cu (I)-catalysed azide-alkyne cycloaddition (CuAAC) as shown in Scheme 1.

Initially, L-amino acids **1a-i** were converted into their corresponding propargyl esters of *N*-Boc-L-amino acids **3a-i** according to the literature procedure[42]. The second step involves the synthesis of the key intermediate; (*Z*)-methyl-12-azidooctadec-9-enoate[35,36] **(6)** from methyl ricinoleate (**4).** The third step involves the coupling of *N*-Boc-L-amino acids **3 (a-i)** and (*Z*)-methyl-12-azidooctadec-9-enoate (**6**) in the presence of CuI/DIPEA/ACOH[43] in DCM at RT for an overall 5-15 min to obtain the corresponding *N*-Boc-L-amino acid containing 1,2,3-triazole derivatives **7a-i** with 90-95% yields. Finally, the *N*-Boc-protected-1,2,3-triazole derivatives were deprotected in TFA/DCM, which resulted in the corresponding 1,2,3-triazole derivatives **8a-i** with 67-75% yields.

All the derivatives **8a-i** structures have been characterized by FT-IR, ^{1}H and ^{13}C NMR, and mass spectral studies. The ^{1}H NMR spectra of compound **8i** showed the disappearance of *N*-Boc protons in the spectra. The presence of two protons attached to the triazole ring appeared at δ 5.23. One proton in the triazole ring appeared at δ 7.53, which appeared as a singlet, and three protons in the -OCH_3 group as a singlet at δ 3.66, which are the characteristic peaks of methyl ester. The compound was also confirmed by its ESI-MS. The molecular ion peaks in the ESI-MS spectrum showed an m/z peak at 557 $[M+H]^+$ and 579 $[M+Na]^+$. Similarly, the structures of the remaining compounds **8a-h** were also confirmed by its ^{1}H and ^{13}C NMR and mass spectral studies. The corresponding NMR spectra are given in the supporting information.

The dipole moment measurements of the molecules as measured by Density Functional Theory (DFL) exhibited values, 4.8-5.6 Debye Units. The values indicated numerous useful properties such as high



Scheme 1 — Synthetic route of ricinoleic acid based 1,4-disubstituted-1,2,3-triazole derivatives 8 (a-i)

stability towards acidic and basic hydrolysis and high temperatures. The values also depict the strong hydrogen bonding ability as observed by most of the 1,2,3-triazole core containing marketed drugs such as anticonvulsant drug Rafinamide, broad spectrum cephalosporin antibiotic cefatrizine, anti-cancer drug carboxyamidotriazole, β-lactum antibiotic tazobactam, *etc*.[12,5]

Biology

Anticancer activity

The title compounds 1,2,3-triazole derivatives 8 (a-i) were tested for anticancer activity against A549 (human lung cancer cell line), HeLa (human cervical cancer cell line), DU-145 (human prostate cancer cell line), and MDA-MB-231 (human breast cancer cell line) at a concentration of 100 μM using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) assay[40]. The IC_{50} values (μM, concentration required to inhibit cancer cell proliferation by 50%) for each cell line is reported as an average of two independent experiments, and the results are given in Table 3. The results were compared with the standard reference anticancer drug; doxorubicin used as a positive control. The triazole derivatives were also tested for cytotoxicity towards normal cell line – Human lung fibroblast [HLF]. Unlike most of the anti-cancer drugs which have adverse effect towards the normal cells[44], the synthesized triazoles did not exhibit any activity, indicating their non-toxic behavior towards normal cell.

The triazoles exhibited good to moderate anticancer activity, of which compound **8c** derived from valine substituted-1,2,3-triazole showed good activity against A549 ($\mathbf{IC_{50}}$ 12.3 ± 0.24μM); DU145 ($\mathbf{IC_{50}}$ 15.6±0.24); MDA-MB-231 ($\mathbf{IC_{50}}$ 17.8±0.20) compared to the other tested compounds, which could be due to more electron-donating $–NH_2$ group attached to a shortest tertiary carbon-containing carboxylic acid, serving as hydrogen bond acceptor with active sites. A similar observation was seen in the anti-cancer activity of 1,2,3-triazoles synthesized by Han Luo, *et al*.[45] While, compound **8g,** phenylalanine substituted 1,2,3-triazole bearing an aromatic ring attached to $–CH_2$, not allowing the accumulation of electrons on $–NH_2$, did not show any anticancer activity against the tested cancer cell lines and compound **8i** tyrosine substituted-1,2, 3-triazole containing electron donating hydroxyl moiety on the aromatic ring exhibited moderate anticancer activity with IC_{50}, 18.9-25.4 μM. Compound **8f** derived from methionine substituted 1,2,3-triazole bearing sulphur group which is also electron donating, showed moderate anticancer activity ($\mathbf{IC_{50}}$, 18.2-24.2 μM) against all the tested cancer cell lines. Compounds **8a**, **8b**, **8d**, and **8c** derived from glycine, alanine, leucine, and isoleucine substituted-1,2,3-triazole derivatives with electron donating long chain hydrocarbon moieties also exhibited moderate anticancer activity against the tested cancer cell lines (Table 3). The anticancer activities of all the synthesized compounds **8a-i** were compared with the standard drug doxorubicin. Based on the above study, the synthesized ricinoleic acid-based-amino acid substituted-1,2,3-triazole molecules are worth pursuing further by molecular modelling studies to develop promising lead molecules.

Table 3 — Anticancer activity of amino acid substituted-1,2,3-triazole derivatives **8a-i**

R (Entry)	IC_{50} values in μM			
	A549	**HeLa**	**DU145**	**MDA-MB-231**
8a	34.2± 0.18	77.0 ± 0.52	>100	>100
8b	54.7 ± 0.26	>100	51.1 ± 0.14	33.9 ± 0.22
8c	12.3 ± 0.24	48.0 ± 0.34	15.6 ± 0.24	17.8 ± 0.20
8d	31.7 ± 0.36	38.0 ± 0.42	28.9 ± 0.24	34.2 ± 0.28
8e	48.6 ± 0.22	64.2 ± 0.31	44.3 ± 0.42	41.9 ± 0.28
8f	18.2 ± 0.11	24.2 ± 0.32	20.2 ± 0.09	23.1 ± 0.29
8g	>100	>100	>100	>100
8h	74.5 ± 0.25	54.2 ± 0.44	59.1 ± 0.22	>100
8i	21.6 ± 0.44	24.8 ± 0.34	18.9 ± 0.42	25.4 ± 0.26
Doxorubucin (Stadandard)	0.6 ± 0.21	0.7 ± 0.32	0.6 ± 0.22	0.8 ± 0.18

A549 – Human lung cancer cell line (ATCC No. CCL-185)
HeLa – Human cervical cancer cell line (ATCC No. CCL-2)
DU-145 – Human prostate cancer cell line (ATCC No. HTB-81)
MDA-MB-231 – Human breast cancer cell line (ATCC HTB-26)

Conclusions

In conclusion, a series of novel L-amino acid substituted-1,2,3-triazole derivatives of ricinoleic acid **8 (a-i)** were designed and synthesized for the first time using CuAAC catalysed 1, 3-dipolar cycloaddition. The structures of all the compounds were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, and Mass Spectral studies. The molecules are expected to be highly stable in different environments as indicated by their static dipole moments [4.8-5.6 Debye Units]. The target compounds were evaluated for their anticancer activity against four different cancer cell lines such as human lung cancer cell line (A549), human cervical cancer cell line (HeLa), human prostate cancer cell line (DU145), and human breast cancer cell line (MDA-MB-231). Among all structurally active perspective, the synthesized 1,2,3-triazole derivatives **8(a-i)** indicated that compound **8c**, *i.e*., valine substituted-1,2,3-triazole exhibited potent anticancer activity against A549, DU145, and MDA-MB-231 cancer cell lines with $\mathbf{IC_{50}}$, 12.3-17.8 μM. It was an interesting result that compound **8g** bearing phenylalanine did not show any anticancer activity, but compound **8i** bearing tyrosine containing hydroxyl group on phenyl moiety was found to exhibit moderate anticancer activity ($\mathbf{IC_{50}}$, 18.9-25.4 μM), which reveals that the hydroxyl group, an electron donating moiety could be affecting the bioactivity of the molecule. In general, all the synthesized 1,2,3-triazole molecules exhibited good to moderate anticancer activity against the tested cancer cell lines compared to the Doxorubucin standard reference drug. Further, they were found to be quite safe towards the normal cell as they did not show any activity towards the human lung fibroblast (HLF) used as normal cell line. Although triazoles are well known for their antifungal properties, we tried to explore whether these novel compounds can function as anticancer agents and understand their cytotoxicity. Further ricinoleic acid-based-amino acid substituted-1,2,3-triazole molecules by molecular docking and mechanistic studies can come out with promising lead molecules.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

CRediT authorship contribution statement

Y. Mohini, K. R. Kunduru, Madiga Hari Krishna, Y. Poornachandra, C. Chandrasekhar : Writing – original draft, Visualization, Validation, Methodology, Conceptualization. Podha Sudhakar: Validation, Supervision. M.S.L. Karuna: Supervision.

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