We report a new synthetic aromatic ε-amino acid containing a triazole moiety with antimicrobial potential against Gram-positive, Gram-negative and pathogenic bacteria including *Vibrio cholerae*. Structure–property relationship studies revealed that all the functional groups are essential to enhance the antimicrobial activity. The 1-(2-aminophenyl)-1H-1,2,3-triazole-4-carboxylic acid was synthesized by click chemistry. From X-ray crystallography, the amino acid adopts a kink-like structure where the phenyl and triazole rings are perpendicular to each other and the amine and acid groups maintain an angle of 60°. The agar diffusion test shows that the amino acid has significant antibacterial activity. The liquid culture test exhibits that the minimum inhibitory concentration (MIC) value for *Bacillus subtilis* and *Vibrio cholerae* is 59.5 µg ml$^{-1}$. FE-SEM experiments were performed to study the morphological changes of bacterial shape after treatment with compound 1. The antimicrobial activity of the amino acid was further studied by DNA binding and degradation study, protein binding, dye-binding assay and morphological analysis. Moreover, the amino acid does not have any harmful effect on eukaryotes.

1. Introduction

Infectious diseases are responsible for a large number of human deaths worldwide. However, the rise in the bacterial resistance to popular antibiotics poses a serious threat to public health [1]. So, new drugs with improved efficacy are desired. The
medicinal chemists have shorted out that the development of more powerful antibiotics is not the only solution to this problem [2]. Rather, developing new antibiotics that act through mechanisms different from those of existing drugs will be more effective [3]. In this regard, it is very important to develop novel, efficient antimicrobial agents which have different functionalities from common antibiotics and which may have clinically unexploited modes of action [4]. Antimicrobial peptides are ubiquitous in nature, found in almost every multicellular organism [5]. They have attracted considerable attention because of their broad-spectrum activity and relatively low bacterial resistance [6]. The θ-defensins RTD-1 and RTD-2 from Rhesus macaque [7], the Protegrin-1 (PG-1) from porcine leucocytes [8], polyphemusin-1 (PM-1) from horseshoe crab haemocytes [9] and BTD-2 from baboon [10] are interesting as natural antimicrobial peptides with a cyclic backbone. Robinson and co-workers have used PG-1 as a starting point for the development of a potent and specific lead compound with nanomolar activity against Pseudomonas aeruginosa [11]. Chemical modification or substitution at single (or multiple) sites of these natural amino acids are emerging strategies to modulate antimicrobial activity of such host defence peptides [12]. On the other hand, information gleaned from the study of these peptides has been used in the design of antimicrobial agents using synthetic building blocks [13]. Particularly, conformationally constrained new amino acids with desired functionalities are very much important because of their immunity towards enzymatic degradation. In this context, we are developing antimicrobial amino acid building blocks [14]. Here, we have incorporated a triazole moiety [15] in an amino acid backbone. Triazole with an N-N entity is promising as a versatile bioactive heterocycle [16]. Triazoles have a wide presence in many synthetic drugs of several infective diseases such as tuberculosis [17], malaria [18], inflammation [19], fungal and bacterial infection [20].

We are developing designer amino acids, peptides and analogues with desired functionalities [21]. Recently, we have described the synthesis of 2-acetyl amino-3-[4-(2-amino-5-sulfo-phenylazo)phenyl]-propionic acid starting from phenylalanine, as a hen egg white lysozyme (HEWL) amyloid inhibitor [22]. Here, we report the synthesis and antimicrobial activities of an aromatic ε-amino acid containing a triazole moiety. 1-(2-aminophenyl)-1H-1,2,3-triazole-4-carboxylic acid was synthesized by click chemistry using copper(II) sulphate pentahydrate and sodium ascorbate as catalyst [23]. The X-ray crystallography sheds some light on the atomic-level structure of the reported ε-amino acid. Based on the agar diffusion test, liquid culture test, DNA binding and degradation study, dye-binding assay and morphological analysis, we conclude that the new amino acid acts as a broad-spectrum antibiotic against Gram-positive, Gram-negative and pathogenic bacteria but does not have any harmful effect on eukaryotes.

2. Material and methods

2.1. General

All chemicals were purchased from Sigma chemicals.

2.2. Amino acid synthesis

2.2.1. Preparation of 2-phthalimidonitrobenzene

For the preparation, 14.18 g of phthalic anhydride (100 mmol) and 13.18 g of 2-nitroaniline were mixed together and heated at 215°C for 2 h and the reaction mixture was allowed to cool at room temperature, and 50 ml of glacial acetic acid was added into it. The resulting solution was refluxed for another half an hour to dissolve the unreacted phthalimide. After 5 min, the reaction mixture was slowly cooled and the resulting mixture was filtered and the precipitate was washed with diethyl ether until a yellow-coloured crystalline solid appeared (19.16 g, 72%): mp 195–196°C. 1H NMR (DMSO- d6, 400 MHz, δ in ppm): 8.25–8.23 [d, J = 8 Hz, 1H, Aromatic proton], 8.05–8.02 [m, 2H, Aromatic protons], 7.98–7.95 [ m, 3H, Aromatic protons], 7.80–7.76 [m, 2H, Aromatic protons]. 13C NMR (DMSO- d6, 100 MHz, δ in ppm): 166.1, 145.5, 135.3, 134.7, 131.2, 130.3, 125.4, 124.7, 123.9.

2.2.2. Preparation of 2-phthalimidoaniline (2)

To a solution of 10.73 g of 2-phthalimidonitrobenzene, 200 ml of acetone, 32 ml of glacial acetic acid, 32 ml of water and 26.81 g of iron powder (480.0 mmol) were added. The reaction mixture was heated to reflux. After 8 h, the reaction mixture was cooled and filtered through a pad of Celite. The filtrate was
concentrated under vacuum. From the filtrate the compound was obtained as a yellow-coloured solid after column chromatography using ethyl acetate and hexane as the mobile phase and silica as the solid phase (7.04 g, 74%): ¹H NMR (CDCl₃, 400 MHz, δ in ppm): 7.93–7.91 [m, 2H, Aromatic protons], 7.78–7.74 [m, 2H, Aromatic protons], 7.25–7.20 [m, 1H, Aromatic proton], 7.1–7.07 [m, 1H, Aromatic proton], 6.88–6.84 [t, J = 8 Hz, 2H, Aromatic proton], 4.32 [br, 2H, NH₂ protons] ¹³C NMR (CDCl₃, 100 MHz, δ in ppm): 167.8, 143.1, 134.8, 132.4, 130.0, 129.6, 124.2, 119.7, 118.4.

2.2.3. Preparation of 2-phthalimidophenyl azide (3)
For this preparation, 5.52 g of 2 (25 mmol) was suspended in 160 ml of glacial acetic acid and 160 ml of water. The mixture was cooled to 0°C, and 2.42 g of NaNO₂ (35.0 mmol) was added in one portion. After 3 h, the remaining undissolved starting 2 was isolated by filtration. To the filtrate, 2.44 g of NaN₃ (37.5 mmol) was added slowly and during the addition a small portion of ethyl ether was added to stop excessive foaming. After half an hour, the reaction mixture was filtered to get 3 as a light yellow solid (6.37 g, 99%): ¹H NMR (CDCl₃, 400 MHz, δ in ppm): 7.95–7.93 [m, 2H, Aromatic protons], 7.79–7.78 [m, 2H, Aromatic protons], 7.51–7.48 [m, 1H, Aromatic protons], 7.31–7.24 [m, 3H, Aromatic protons]. ¹³C NMR (CDCl₃, 100 MHz, δ in ppm): 167.4, 138.3, 134.8, 132.4, 131.1, 125.8, 124.3, 123.0, 119.8.

2.2.4. Synthesis of 2-aminophenyl azide (4)
For this synthesis, 5.55 g of 2-phthalimidophenyl azide (21.0 mmol) was suspended in 14 ml of methanol. To this slurry, 1.5 ml of hydrazine hydrate (31.5 mmol) was added in one portion. The resulting reaction mixture was stirred rapidly. After 20 min, the mixture was very thick and into it another 10 ml of methanol was added, and stirring was continued for another 1 h. To the resulting paste, 40 ml of 1(M) aqueous solution of NaOH was added. The mixture was diluted with 30 ml of water and 60 ml of DCM. The resulting two phases were separated and the aqueous extract was separated with another 2 × 30 ml of DCM. The combined organic phase was washed with 1 × 30 ml of distilled water and 1 × 30 ml of brine. The resulting organic solution was dried over Na₂SO₄ and the homogeneous mixture was concentrated under vacuum. The compound was purified by column chromatography using ethyl acetate and hexane as the mobile phase and silica gel as the solid phase (1.76 g, 62%): ¹H NMR (DMSO-d₆, 400 MHz, δ in ppm): 7.02–6.99 [m, 1H, Aromatic proton], 6.90–6.87 [m, 1H, Aromatic proton], 6.65–6.60 [m, 2H, Aromatic protons], 4.95 [br, 2H, NH₂ proton]. ¹³C NMR (DMSO-d₆, 100 MHz, δ in ppm): 139.9, 125.7, 123.0, 118.6, 116.7, 115.0.

2.2.5. Synthesis of 1-(2-aminophenyl)-1H-1,2,3-triazole-4-carboxylic acid (1)
To a 1:1 mixture of 2-aminophenyl azide (1.34 g, 10 mmol) and propiolic acid (0.7 g, 10 mmol) in a 1:1 ethanol and water solvent in a 250 ml round bottom flask, 0.2 mmol sodium ascorbate and 0.05 mmol CuSO₄ were added, and this was covered with an aluminium foil followed by stirring at room temperature for 12h. The resulting mixture was concentrated under vacuum, and amino acid was obtained after separating the mixture by column chromatography using ethyl acetate and hexane as the mobile phase and silica gel as the solid phase (1.59 g, 78%): ¹H NMR (DMSO-d₆, 400 MHz, δ in ppm): 8.92 [s, 1H, Triazole CH proton], 7.24–7.21 [m, 2H, Aromatic protons], 6.92–6.90 [d, J = 8 Hz, 1H, Aromatic proton], 6.69–6.66 [t, J = 6 Hz, 1H, Aromatic proton], 5.40 [br, 2H, NH₂ proton]. ¹³C NMR (DMSO-d₆, 100 MHz, δ in ppm): 161.7, 142.9, 139.9, 130.6, 130.08, 126.2, 121.4, 116.6, 115.9.

2.2.6. NMR experiments
All NMR studies were carried out on a Jeol 400 MHz spectrometer at 278 K. Compound concentrations were in the range of 1–10 mM in CDCl₃ and (CD₃)₂SO.

2.2.7. FT-IR spectroscopy
All reported solid-state FT-IR spectra were obtained with a Perkin Elmer Spectrum RX1 spectrophotometer with the KBr disc technique.

2.2.8. UV/vis spectroscopy
Absorption spectra were recorded on a Perkin Elmer spectrophotometer.
2.2.9. Field emission scanning electron microscopy

Morphological changes of bacterial shape after treatment with compound 1 were investigated using field emission-scanning electron microscopy (FE-SEM). Aliquots of 10 ml of the liquid culture of each E. coli (E. coli XL10), Bacillus subtilis and Vibrio cholerae O395 bacteria were incubated and allowed to grow for 8 h in the presence of compound 1; 5 ml aliquots from each culture were centrifuged at 5000 r.p.m. for 5 min. The supernatants were discarded. The pellets were washed with water two times and then with 1% PBS buffer two times. The resultant solutions for each set of bacteria were incubated at RT for 30 min and then at 4°C overnight. The suspensions were washed with PBS buffer three times. Then the pellets were collected by centrifugation. Then each sample was dehydrated using 200 µl of different grades of ethanol like 30%, 50%, 70%, 80%, 90% and 100%, respectively. The samples were incubated for 1 h, and 5 µl from each set for each sample was drop cast on a glass slide and dried under vacuum. Similarly, samples were prepared for the control experiment, that is bacteria in the absence of compound 1. The materials were gold-coated, and the micrographs were taken in an FE-SEM apparatus (Jeol Scanning Microscope-JSM-6700F).

2.3. Single crystal X-ray diffraction study

Crystallographic data of compound 1: C₉H₈N₄O₂, Mw = 236.24, monoclinic, space group P 1 21/n 1, a = 10.7631(4), b = 4.5960(2), c = 22.5078(9) Å, α = 90°, β = 94.127(4), γ = 90°, V = 13637.9(9) Å³, Z = 4, dm = 1.413 Mg m⁻³, T = 100 K, R1 0.0422 and wR2 0.1082 for 1952 data with I > 2σ(I). Intensity data were collected with MoKα radiation using a Bruker APEX-2 CCD diffractometer. Data were processed using the Bruker SAINT package and the structure solution and refinement procedures were performed using SHELX97. The data for compound 1 have been deposited at the Cambridge Crystallographic Data Centre with reference number CCDC 1543560.

2.4. Agar diffusion test

A small amount of bacteria (E. coli XL10 and Bacillus subtilis) culture was spread over the whole agar plate that contained some tiny pores; inside the pores, the aqueous solution of the test compound and the reference were placed separately and the plate was left for incubation for 10 h at a temperature of 37°C; the zone of inhibition has been calculated. For the agar diffusion test on Bacillus subtilis, 150 µl of an aqueous solution of compound 1 with concentration 20 mg ml⁻¹ and 20 µl of an aqueous solution of kanamycin with a concentration of 50 mg ml⁻¹ were used for the test and the reference, respectively. A 35 µl solution of compound 1 with a concentration of 20 mg ml⁻¹ and a 14 µl aqueous solution of kanamycin with a concentration of 50 mg ml⁻¹ were used for the agar diffusion test of E. coli.

2.5. MIC test

Bacteria are allowed to grow in different identical sets of liquid media (LB media) containing variable concentrations of compound 1. The growth of bacteria for each set is measured by recording the optical density of the solution at 600 nm after each one hour time interval until the bacterial growth has become saturated. Then the optical density versus time is plotted for each set to get the growth curve for all the bacteria. From that growth curve, the MIC value has been calculated. In a conical tube containing 10 ml of LB media, E. coli bacteria (E. coli XL10) are incubated for 8 h at 37°C and the growth of E. coli are measured by recording the optical density of the solution at 600 nm. Then 200 µl of the bacterial suspension is added into each conical tube containing the test compound with final concentrations of 34, 42.5, 51, 59.5, 68, 76.5 and 85 µg ml⁻¹ in 10 ml of LB media, respectively. The parallel control experiment is done by adding the same 200 µl of the bacterial suspension to each conical tube containing 10 ml of LB media alone and 30 µg ml⁻¹ kanamycin in 10 ml of LB media, respectively. The growth of bacteria for each conical tube is measured by recording the optical density of the solution at 600 nm after each one hour time interval until the bacterial growth becomes saturated. The optical density versus time are plotted together. The black sigmoidal growth curve is for only culture that is in the absence of any test compound.

2.6. DNA-binding assay

A 10 ml aliquot of the liquid culture of E. coli bacteria was incubated at 37°C and allowed to grow for 8 h. Then 1.5 ml of culture was centrifuged under 5000 r.p.m. and the supernatant was discarded. The
pellets were resuspended in 567 µl of TE (Tris-EDTA) buffer followed by addition of 30 µl of 10% SDS and 3 µl of 20 mg ml⁻¹ proteinase K in order to make a final concentration of 100 µg ml⁻¹ proteinase K in 0.5% SDS. The resultant solution was mixed thoroughly and allowed to incubate for 1 h at 37°C. Then 100 µl of 5 M NaCl was added to the solution with thorough mixing followed by the addition of 80 µl of CTAB (cetyl trimethyl ammoniumbromide)/NaCl solution. This resultant mixture was mixed thoroughly and incubated for 10 min at 65°C. These two steps are very vital because the addition of 100 µl of 5 M NaCl keeps the salt concentration above 0.5 M. At this salt concentration, cell wall debris, denatured protein and polysaccharides formed a complex with CTAB, while the nucleic acid remained unaffected. To extract CTAB-cell wall debris/denatured protein and polysaccharides complex, 0.7 ml of chloroform/isoamyl alcohol was added and the solution was mixed thoroughly and centrifuged at 5000 r.p.m. for 5 min. A white interface containing the CTAB-cell wall debris/denatured protein and polysaccharides complex was found to appear; this interface was discarded and the viscous supernatant was transferred to a microcentrifuge tube containing an equal volume of phenol/chloroform/isoamyl alcohol. The resultant solution was centrifuged under 5000 r.p.m. for 5 min. The supernatant was transferred to a fresh tube and 0.6 volume of isopropanol was added to precipitate the nucleic acid. The tube was shaken gently till the white nucleic acid precipitate appeared. The nucleic acid precipitate was dissolved in 70% ethanol. Similarly, the nucleic acid was collected from compound 1-treated E. coli.

2.7. Protein isolation

A 10 ml aliquot of the liquid culture of only E. coli bacteria and compound 1-treated E. coli bacteria were incubated separately and allowed to grow for several hours; 1.5 ml of the control culture and 4.5 ml of the test culture were centrifuged at 5000 r.p.m. for 5 min. The supernatant was discarded. Then each of the pellets was suspended in 200 µl of Tris-NaCl buffer (10 mM Tris and 50 mM NaCl) followed by addition of 4× Sample Loading Buffer (SLB compositions). The solutions were boiled and sonicated and again boiled. Now, on a Whatman filter paper 15 squares, each of 1.5 × 1.5 cm, were drawn. During the experiment, it was ensured that the filter paper was not touched with any surface from which it could absorb any protein. A standard protein solution of a known amount (ranging from 1 µg to 4 µg) was drop cast on the centre of each small square in triplicate. Similarly, the test sample and the control sample were drop cast in triplicate. The paper was allowed to air-dry and then washed with methanol to remove the interfering substances during dye binding for 10–20 min, and allowed to dry again. The paper was stained in 200 ml of 0.5% Coomassie brilliant blue G in 7% acetic acid for 45 min at room temperature with gentle shaking. Then the destaining of the filter paper was done in 7% acetic acid until the background became adequately reduced. The filter paper was air-dried. After complete drying, each small square was cut and put into a 2 ml microtube; 1 ml of the extraction buffer (66% methanol, 33% water, 1% ammonium hydroxide) was added and the tube was shaken thoroughly. This buffer extracts the proteins from the paper. The optical density of the solution was recorded in a spectrophotometer at a 610 nm wavelength. The optical densities of the sample were plotted against the amount of protein (in µg). From the plot, we can quantify the amount of protein in the control sample and the test sample. Equal amounts of protein from the control sample and the test sample are allowed to run by Protein-gel electrophoresis under a 15 mA current for 90 min. The gel is stained in 200 ml of 0.5% Coomassie brilliant blue G in 7% acetic acid for 45 min at room temperature with gentle shaking followed by destaining in 7% acetic acid.

3. Results and discussion

For compound 1, we have incorporated a triazole moiety to enhance the antimicrobial activity [24]. The 1-[2-(aminophenyl)]-1H-1,2,3-triazole-4-carboxylic acid was synthesized by click chemistry (scheme 1) between 2-aminophenyl azide and propiolic acid using copper(II) sulfathe pentahydrate and sodium ascorbate as catalyst [23]. Compound 1 was purified and characterized by 1H-NMR, 13C-NMR and FT-IR analysis. X-ray crystallography confirmed the formation of compound 1.

To investigate the conformational preference of compound 1, solid-state FT-IR spectroscopy was performed. From FT-IR spectra, the bands at 3427 cm⁻¹ indicate that all NH groups are not hydrogen-bonded (electronic supplementary material, figure 1). The broad peak around 3300 cm⁻¹ also suggests that the compound has extensively hydrogen-bonded network structures.
Colourless monoclinic crystals of compound 1 were obtained from a methanol–water solution by slow evaporation. The X-ray crystallography shows that the asymmetric unit contains one molecule of compound 1 and a methanol molecule. The ORTEP diagram (figure 1a) shows that compound 1 adopts a kink-like conformation. The phenyl and triazole rings are perpendicular to each other. Moreover, the amine and acid groups maintain an angle of 60° like o-aminobenzoic acid moiety. There is an intramolecular N-H…N hydrogen bond between amine NH and triazole N. There exists another O–H…O hydrogen bond between acid OH and methanol oxygen (figure 1a).

From the packing diagram, it can be seen that the individual amino acid building blocks are themselves regularly interlinked through methanol-mediated intermolecular hydrogen-bonding interactions, O3–H3A…N4, and thereby form a 14-member ring-like structure along the crystallographic a direction. In higher-order packing, compound 1 forms a complex sheet-like structure through multiple intermolecular hydrogen bonds and π–π stacking interaction (minimum C-N distance 3.72 Å) (figure 1c) along the crystallographic c direction. The hydrogen-bonding parameters are listed in table 1.

The agar diffusion test is a well-known procedure to check the bacterial sensitivity because of its simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents together, and ease of interpretation of the test results [25]. Here, we used compound 1 as the test compound and kanamycin as the reference. The result shows that the trizole-based compound 1 has significant antibacterial activity against *E. coli* and *Bacillus subtilis* (electronic supplementary material, figure 2).

Inspired by this result, we tried to check the antibacterial effect of the compound quantitatively. The strength of an antibacterial agent is expressed in terms of the minimum inhibitory concentration (MIC). The results indicate that as the compound concentration increases, bacterial growth is gradually inhibited, which is reflected from the gradual lowering of the OD (optical density) for each set, and at a compound concentration of 85 µg ml⁻¹ the bacterial growth becomes fully diminished like that of kanamycin (electronic supplementary material, figure 3).

We have also investigated the effect of compound 1 on other species of bacteria like *Bacillus subtilis*. The experimental data show that the MIC of the amino acid for *Bacillus subtilis* is 59.5 µg ml⁻¹ (figure 2a). Further, we are interested to know whether compound 1 is active on pathogenic bacteria like *Vibrio cholerae* or not. The results show that with increase in compound 1 concentration the growth of *Vibrio*
Figure 1. (a) The ORTEP diagram of compound 1. Ellipsoids are drawn at the 50% probability level. Hydrogen bonds are shown as dotted lines. (b) Solvent-mediated dimer of compound 1. (c) The complex sheet-like structure of compound 1.

Table 1. Hydrogen-bonding parameters of compound 1\(^a\) (symmetry equivalent).

| D–H...A | D...H (Å) | H...A (Å) | D...A (Å) | D–H...A (°) |
|---------|-----------|-----------|-----------|-------------|
| N1–H1A...N3 | 0.86 | 2.56 | 3.147(2) | 126\(a\) |
| N1–H1B...N2 | 0.86 | 2.54 | 2.890(2) | 106 |
| N1–H1B...N3 | 0.86 | 2.52 | 2.935(2) | 111 |
| N1–H1B...N1 | 0.86 | 2.46 | 3.115(2) | 134\(a\) |
| O2–H2...O3 | 0.82 | 1.74 | 2.552(2) | 169 |
| O3–H3A...O2 | 0.88 | 2.59 | 3.087(2) | 117\(b\) |
| O3–H3A...N4 | 0.88 | 1.94 | 2.808(9) | 169\(b\) |

\(a\) = \(3/2 - x, -1/2 + y, 3/2 - z\).
\(b\) = \(2 - x, 1 - y, 1 - z\).

the MIC value is 59.5 µg ml\(^{-1}\) (figure 2b). For a potential antibiotic, the compound should be active only on bacteria, and should not have a harmful effect on eukaryotes. The result (figure 2c) indicates that the growth of the yeast is not inhibited significantly by compound 1 even at very high concentration.
Figure 2. (a) Growth curve of *Bacillus subtilis* against compound 1 and kanamycin as the reference showing that the growth significantly decreases. (b) Growth curve of *Vibrio cholerae* against compound 1 and kanamycin as the reference exhibiting the growth of *Vibrio cholerae* gradually decreases with increasing compound concentration. (c) Growth curve of yeast against compound 1 and kanamycin as the reference showing the growth of yeast is not inhibited significantly by compound 1 even at a very high concentration.

To study the morphological changes of bacterial shape after treatment with compound 1, FE-SEM experiments were performed. FE-SEM images (figure 3) show the images of healthy bacterial cells in the control experiment. However, upon treating the bacteria with compound 1, the *E. coli*, *Bacillus subtilis* and *Vibrio cholerae* bacterial cells become completely deformed. These results reveal that compound 1 significantly affects the bacteria.

Moreover, to study the effect of compound 1 on bacteria, *in vitro* experiments were carried out. For the *in vitro* experiments, only *E. coli* bacteria was used. The interactions of DNA with other chemical and biological functionalities lead to a diversity of binding and reaction patterns [26,27]. Previously, peptide-based molecules as well as iron-containing complexes have been reported as DNA-binding agents [28,29]; however, even some of them have been reported to damage the DNA [30]. Inspired from the above reports, we wanted to study whether compound 1 has the ability to damage or degrade DNA. For that purpose, we have isolated the bacterial genome from the control experiment and compound 1-treated bacterial cell by the standard procedure and compared them. Then gel electrophoresis was performed with the extracted DNA materials. Figure 4a indicates that no extra DNA band appears for the compound 1-treated bacterial DNA. In both the test sample and the control the band positions are the same. This result indicates that no bacteria DNA degradation occurs by treatment with 1-(2-aminophenyl)-1H-1,2,3-triazole-4-carboxylic acid.

Many drug molecules have been known to bind with protein and make drug–protein adducts [31]. Bacterial cell division can be inhibited by targeting the specific protein [32]. Now, to investigate whether 1-(2-aminophenyl)-1H-1,2,3-triazole-4-carboxylic acid can bind or damage some bacterial protein or not, we need to isolate the bacterial protein from the control experiment and compound 1-treated bacteria and compare them. The proteins were isolated from the bacterial cell using the standard procedure. The results (figure 4b) indicate that the intensity of the protein band marked by the arrow are going to be reduced in the compound 1-treated sample compared to the control one, while the intensity of the other
bands remain the same in both samples. Hence, compound 1 significantly interacts with some bacterial proteins and degrades them, causing bacterial death.

4. Conclusion

In conclusion, we have designed and synthesized a new \( \epsilon \)-amino acid, namely 1-(2-aminophenyl)-1H-1,2,3-triazole-4-carboxylic acid by click reaction. From X-ray crystallography, the amino acid adopts a kink-like structure where the phenyl and triazole rings are perpendicular to each other and the amine and acid groups maintain an angle of 60°. The amino acid has significant antibacterial activity. The MIC value of the compound for \( \textit{Bacillus subtilis} \) and \( \textit{Vibrio cholerae} \) is 59.5 µg ml\(^{-1}\). However, the amino acid does not have any harmful effect on eukaryotes. The activity of the amino acid on bacteria was further studied by DNA and protein binding and degradation study, dye-binding assay and morphological analysis, which show that the amino acid significantly interacts with bacterial proteins and degrades them, causing

Figure 3. (a) FE-SEM image of \( \textit{E. coli} \), (b) FE-SEM image of compound 1-treated \( \textit{E. coli} \). (c) FE-SEM image of \( \textit{Bacillus subtilis} \), (d) FE-SEM image of compound 1-treated \( \textit{Bacillus subtilis} \). (e) FE-SEM image of \( \textit{Vibrio cholerae} \), (f) FE-SEM image of compound 1-treated \( \textit{Vibrio cholerae} \).
bacterial death. The results presented here show that the synthetic amino acid may eventually be effective against pathogens, but additional studies are required to reach this goal.

Ethics. The bio assay was done in the laboratory of Dr Sankar Maiti, Department of Biological Sciences, Indian Institute of Science Education and Research Kolkata, Mohanpur-741246, India. We have obtained the necessary licences and approvals from the institutional animal ethics committee before conducting the research.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions. K.M. performed the experiments and collected data. D.H. conceived and designed the synthesis, coordinated the study and wrote the manuscript. Both the authors gave their final approval for publication.

Competing interests. We have no competing interests.

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