1. Introduction

Facing emerging bacterial infections has become more challenging worldwide due to the increasing number of multidrug-resistant (MDR) microbes.\(^5\)–\(^7\) This indicates the crucial need to develop new efficient anti-bacterial agents. Many factors contribute to mutations in microbial genomes leading to resistance to known antibiotics. For instance, it is broadly confirmed that the abuse of antibiotics can significantly increase the development of resistant-genotypes.\(^8\)–\(^10\) As the number of infectious diseases and multidrug-resistant bacterial strains continues to increase, researchers are prompted to develop novel anti-microbial molecules.\(^11\)

From a medicinal chemistry prospective, creating new generation of therapeutic molecules with improved pharmacological properties and drug-tolerance profile, as well as fewer side effects, is an ultimate goal.\(^12\) Hence, libraries with privileged heterocyclic scaffolds are frequently utilized in the development of new potent drugs.\(^13\) For instance, hybrids from 1,2,4-triazole derived compounds usually hold a series of pharmacological properties such as anticancer,\(^14,15\) antiviral,\(^16\) antitubercular,\(^17,18\) antifungal,\(^19\) antileishmanial\(^20\) and antibacterial\(^21\) activities. However, only few reports about fused systems of 1,2,4-triazolo[1,5-a]pyrimidines were reported in literature with pronounced antibacterial activities.\(^22\) In addition, coupling with simple amino acids, e.g., glycine and others, has been frequently attracted the interest of medicinal chemists due to its improving ability for the physicochemical and drug-likeness properties.\(^23,24\) In addition, glycine and its derivatives appear to be promising safe antimicrobial agents.\(^25\)

Being analogues of DNA purine bases, 1,2,4-triazolo[1,5-a] pyrimidines can be regarded as plausible substrates for enzymatic biochemical processes.\(^26\) In particular, derivatives of [1,2,4]triazolo-[4,3-a]pyrimidines have recently been reported as potential antibacterials.\(^27\)–\(^29\) It was reported that series of 1,2,4-triazolo[1,5-a]pyrimidines carboxamide derivatives attributed good narrow-spectrum antibacterial activity against \textit{E. faecium}\(^\text{a}\) and possessed metabolic stability with low intrinsic clearance. Macromolecular synthesis assays revealed cell-wall biosynthesis as the target of these compounds.\(^30\) It is worth mentioning that recently, several 1,2,4-triazolo[1,5-a]pyrimidines were synthesized and screened for their antibacterial derivatives as DNA
gyrase inhibitors. Some compounds possessed high activity against Gram-positive and Gram-negative bacteria with MIC values ranging from 0.25–2.0 μg mL⁻¹. In addition they showed good toxicity profile against human kidney and red blood cell.³¹

Accordingly and as a continuation of our efforts to discover diverse chemotypes for potential antibacterial agents,³² we aim at preparing new triazolo[1,5-a]pyrimidine derivatives coupled with amino acids. The synthetic process comprises green conditions, e.g., using lemon juice as green catalyst and aqueous medium as green solvent.

2. Results and discussion

Chemistry

In continuation of our research program on the utility of heterocyclic moieties to find out novel antibacterial agents,²⁰,³¹,³² we are going here to report an efficient and facile synthesis of some [1,2,4]triazolo[1,5-a]pyrimidine derivatives starting from 2-(3-amino-5-(2-hydroxyphenyl)-1H-1,2,4-triazol-4(5H)-yl)propionic acid derivatives 1a-d. The tree component reaction of compound 1a-d with aromatic aldehyde (4-chlorobenzaldehyde) and acetylacetone or ethyl acetoacetate in a one-pot reaction under green conditions [lemon juice, water–ethanol (8 : 2)] afforded the corresponding 2-(1,2-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-3(5H)-yl)propanoic acid derivatives 2a–d and 3a–d respectively, in good to excellent yield, Scheme 1.

It worth mentioning that some [1,2,4]triazolo[1,5-a]pyrimidine derivatives synthesized through one-pot multicomponent reactions using a low viscous and acid-functionalized ionic liquid. The results showed that new ionic liquid can act as a green solvent and acid catalyst due to low viscosity and acid functionality.³³

Structures of the newly obtained compounds were confirmed based upon their IR, ¹H-NMR, ¹³C-MR, MS spectral data, and elemental analyses. The IR spectra of compound 2a exhibited the presence of broad band at 3455 cm⁻¹ corresponding to two OH groups, another characteristic band at 1685 cm⁻¹ corresponding to the α,β-unsaturated carbonyl group. The ¹H-NMR spectrum of compound 2a revealed the presence of a broad band at δ 12.24 ppm characterized to the OH of the carboxyl group, a singlet at δ 9.22 ppm corresponding to the phenolic OH group, another singlet at δ 8.12 ppm for NH group, a multiplet between δ 6.92–7.60 ppm attributed to the aromatic protons, a singlet at δ 5.24 ppm corresponding to CH (triazole), a quartet at 2.92 ppm corresponding to CH–COOH, a singlet at 2.33 ppm for δ CH₃ group, a doublet at δ 2.23 ppm attributed to (CH₃ alanine) and a singlet at δ 2.12 ppm characteristic for CH₃ CO group.¹³CMR spectrum of compound 2a showed the following signals: 9.88 (CH₃–CH), 20.12 (CH₃), 22.02 (CH₂CO), 41.80 (CH–COOH), 53.12 (CHpyrimidine), 57.80 (CHtriazole), 118.12, 119.21, 122.20, 123.41, 124.50, 126.54, 127.32, 128.62, 134.55, 136.01, 138.12, 143.21 (ArC), 152.1 (C=N), 178.10 (C=O), 192.54 (C=O).

Scheme 1 Synthesis of [1,2,4]triazolo[1,5-a]pyrimidine derivatives.
Similarly, the reaction of compound 1b with cyclic 1,3-dicarbonyl compounds *viz.* meldrum's acid, barbituric acid, cyclohexane-1,3-dione and/or dimedone under the same experimental conditions [lemon juice, water–ethanol (8 : 2)] afforded the corresponding [1,2,4]triazolo[1,5-α]pyrimidine derivatives 4–7 respectively, Scheme 2.

On continuation of our work, 2-(3-amino-5-(2-hydroxyphenyl)-1H-1,2,4-triazol-4(5H)-yl)propanoic acid derivatives 1a–d were allowed to react with 4-chlorobenzaldehyde and malononitrile or ethyl cyanoacetate under the same experimental conditions, where the corresponding [1,2,4]triazolo[1,5-α]pyrimidine derivatives 8a–d and 9a–d were obtained in excellent yields, Scheme 3.

Finally, the reaction of compound 1a–d with α-cyanoketene-S,S-dithioacetal namely: 2-(bis(methylthio)methylene)malononitrile under green condition gave a product which was precipitated during the course of reaction and was identified as 2-(6-cyano-2-(2-hydroxyphenyl)-7-imino-5-(methylthio)-1,2-dihydro-1,2,4-triazolo[1,5-α]pyrimidin-3(7H)-yl)propanoic acid 10a–d, as shown in Scheme 4.

The analytical and spectral data of all compounds were found to be accordance with the structures assigned to these compounds.

Scheme 2  Synthesis of [1,2,4]triazolo[1,5-α]pyrimidine derivatives.
Antimicrobial screening

**Antimicrobial inhibitory activity of synthesized compounds.**
The antimicrobial activity of the newly synthesized heterocyclic compounds was listed in Table 1 were tested applying agar diffusion method\(^{(34,35)}\) against the following microorganisms: Gram-positive bacteria \([S. aureus \,(ATCC 25923) \mbox{and} \, S. pyogenes (ATCC 19615)]\) and Gram-negative bacteria \([P. phaseolicola (GSPB 2828) \mbox{and} \, P. \, fluorescens \,(S 97)]\) beside the yeast like fungi \(C. \, albicans.\) Compounds \(2c, \, 2d, \, 3c, \, 3d, \, 8c, \, 8d, \, 9c\) and \(9d\) were found to be active against Gram-positive bacteria, where compounds \(2b, \, 3a, \, 3b, \, 5, \, 6, \, 7, \, 8a, \, 8b, \, 9a\) and \(9b\) are active against Gram-negative bacteria. The rest of the tested compounds showed weak to moderate sensitivity towards test bacteria. Moreover, none of the test compounds showed good activity against \(C. \, albicans\) compared to clotrimazole as reference standard, shown in Table 1.

**Minimal inhibitory concentration (MIC) of active compounds against MDR bacteria.** Compounds which
1. Introduction

Antimicrobial resistance (AMR) is a major global public health concern, as bacteria evolve strategies to withstand the effects of antibiotics.

2. Results

The synthesized derivatives were tested for their antibacterial activity against S. aureus, S. pyogenes, P. phaseolicola, and P. fluorescens. The MIC and MBC values for each compound are reported in Table 1. The compounds demonstrated varied levels of inhibitory activity, with some showing promising results.

3. Methods

Chemistry

All melting points were determined on a Kofler melting point apparatus and are uncorrected. 1H-NMR and 13C NMR spectra were recorded on a Bruker avance 400 MHz spectrometer using TMS as internal reference (chemical shifts in δ, ppm), and IR spectra were obtained on a Nicolet 710 FT-IR spectrometer (KBr, v_{max} in cm⁻¹). Mass spectra were recorded on a GC-MSQP 1000EX Shimadzu at the Microanalytical laboratory, Cairo University, Cairo, Egypt. Elemental analyses were recorded on Vario El Fab-Nr elemental analyzer (Cairo University).

General procedure for the synthesis of [1,2,4]triazolo[1,5-a]pyrimidine derivatives 2a-d and 3a-d

An equimolar mixture of 2-(3-amino-5-(2-hydroxyphenyl)-1H-1,2,4-triazol-4(5H)-yl)propanoic acid derivative 1a-d (0.001

Table 1  In vitro antibacterial activities of the synthesized compounds against standard bacteria

| Comp. no. | Diameter of zone inhibition in mm |
|-----------|----------------------------------|
|           | Gram-positive bacteria            | Gram-negative bacteria | Fungi |
|           | S. aureus (ATCC 25923) | S. pyogenes (ATCC 19615) | P. phaseolicola (GSPB 2828) | P. fluorescens (S 97) | C. albicans |
|           | 10 μg mL⁻¹ | 15 μg mL⁻¹ | 10 μg mL⁻¹ | 15 μg mL⁻¹ | 10 μg mL⁻¹ | 15 μg mL⁻¹ | 10 μg mL⁻¹ | 15 μg mL⁻¹ |
| 2a        | 10      | 16      | 12      | 17      | 18      | 28      | 16      | 25      | 8       | 9       |
| 2b        | 12      | 17      | 10      | 18      | 16      | 32      | 15      | 29      | 6       | 8       |
| 2c        | 19      | 32      | 18      | 30      | 11      | 23      | 12      | 22      | 6       | 9       |
| 2d        | 20      | 33      | 20      | 32      | 12      | 22      | 14      | 21      | 9       | 12      |
| 3a        | 11      | 20      | 12      | 22      | 18      | 32      | 16      | 30      | 6       | 8       |
| 3b        | 12      | 25      | 14      | 24      | 19      | 33      | 18      | 30      | 10      | 12      |
| 3c        | 19      | 33      | 18      | 32      | 10      | 21      | 8       | 23      | 6       | 7       |
| 3d        | 18      | 30      | 17      | 31      | 11      | 23      | 10      | 19      | 9       | 10      |
| 4         | 10      | 24      | 8       | 22      | 7       | 18      | 12      | 25      | 9       | 12      |
| 5         | 14      | 26      | 11      | 25      | 17      | 30      | 15      | 28      | 7       | 9       |
| 6         | 15      | 25      | 10      | 24      | 18      | 29      | 19      | 33      | 7       | 8       |
| 7         | 15      | 25      | 12      | 20      | 19      | 33      | 18      | 32      | 10      | 11      |
| 8a        | 13      | 20      | 12      | 21      | 18      | 30      | 17      | 29      | 10      | 13      |
| 8b        | 12      | 22      | 16      | 26      | 19      | 34      | 18      | 32      | 8       | 10      |
| 8c        | 18      | 30      | 19      | 33      | 14      | 24      | 13      | 23      | 8       | 11      |
| 8d        | 16      | 29      | 17      | 31      | 14      | 24      | 12      | 22      | 6       | 7       |
| 9a        | 14      | 26      | 12      | 27      | 18      | 30      | 17      | 30      | 6       | 9       |
| 9b        | 15      | 26      | 16      | 25      | 19      | 31      | 16      | 28      | 6       | 8       |
| 9c        | 18      | 32      | 19      | 34      | 10      | 22      | 11      | 20      | 9       | 11      |
| 9d        | 20      | 35      | 19      | 32      | 10      | 18      | 12      | 23      | 7       | 8       |
| Cephalothin | 28      | 30      | NT      | NT      | 25      | 30      | NT      | NT      | 36      | 44      |
| Chloramphenicol | NT      | NT      | 25      | 30      | 36      | 44      | 36      | 44      |
| Clotrimazole    | NT      | NT      | NT      | NT      | NT      | NT      | NT      | NT      |

- Less active: 6–12 mm; moderately active: 13–19 mm; highly active: 20–30 mm; no inhibition or inhibition less than 5 mm; NT – not tested. Each result represents the average of triplicate readings.
- S. aureus (ATCC 25923) = Staphylococcus aureus (ATCC 25923); S. pyogenes (ATCC 19615) = Streptococcus pyogenes (ATCC 19615); P. phaseolicola (GSPB 2828) = Pseudomonas phaseolicola (GSPB 2828); P. fluorescens (S 97) = Pseudomonas fluorescens (S 97); C. albicans = Candida albicans.

Exhibited encouraging inhibition zones were further screened for their inhibitory effect against MDR clinical isolates. K. pneumoniae and methicillin-resistant S. aureus. Results revealed that 3c and 9d attributed remarkable activity against methicillin-resistant S. aureus. On the other hand, it appears that electron-donating amino group in 8a played an important role to increase the activity against MDR K. pneumoniae as shown in Table 2.

Determination of compounds 3c, 8a, and 9d. Compounds 3c, 8a, and 9d were tested for their minimum bactericidal concentric (MBC) and calculate MIC index (MBC/MIC) to check whether they are bactericidal (MIC index <4) or bacteriostatic (MIC index >4) against the growth bacteria. Results revealed that test compounds have MIC index >4 and they have bacteriostatic effect, Table 3. It maybe speculated that these compounds revealed cell-wall biosynthesis as the target of their action according to published similar compounds.

In vitro cytotoxicity assay. The cytotoxicity of the most active compounds 2c, 2d, 3c, 8a, 8b, 9a, 9b, 9c and 9d was tested in a VERO cell line as reported earlier. The 50% cytotoxic concentration (CC₅₀) values represent the concentration of compound required to kill 50% of the cells (Table 4). Table 4 indicates that the compounds have greater selectivity towards antimalarial activity against K. pneumoniae and MRSA compared to mammalian cells, thus showing a good toxicity profile.
| Comp. no. | Diameter of zone inhibition in mm (15 μg mL⁻¹) | MIC (μg mL⁻¹) | Diameter of zone inhibition in mm (15 μg mL⁻¹) | MIC (μg mL⁻¹) |
|----------|---------------------------------------------|---------------|---------------------------------------------|---------------|
| 2b       | 25                                          | 50            | 8                                           | —             |
| 2c       | 8                                           | —             | 26                                          | 25            |
| 2d       | 6                                           | —             | 28                                          | 25            |
| 3a       | 24                                          | 50            | 10                                          | —             |
| 3b       | 27                                          | 50            | 12                                          | —             |
| 3c       | 9                                           | —             | 24                                          | 12.5          |
| 3d       | 6                                           | —             | 6                                           | —             |
| 5        | 23                                          | 50            | 8                                           | —             |
| 6        | 22                                          | 50            | 10                                          | —             |
| 7        | 20                                          | 50            | 6                                           | —             |
| 8a       | 28                                          | 12.5          | 6                                           | —             |
| 8b       | 27                                          | 25            | 8                                           | —             |
| 8c       | 12                                          | —             | 28                                          | 50            |
| 8d       | 10                                          | —             | 25                                          | 50            |
| 9a       | 27                                          | 25            | 5                                           | —             |
| 9b       | 29                                          | 25            | 7                                           | —             |
| 9c       | 13                                          | —             | 27                                          | 25            |
| 9d       | 11                                          | —             | 29                                          | 12.5          |
| Cephalothin | —                                  | —             | —                                           | —             |
| Chloramphenicol | —                                  | —             | —                                           | —             |

Table 3 Minimum bactericidal concentration (MBC) of compounds 3c, 8a and 9d

| Comp. no. | CC₅₀a  | MICa  | MBC  | MICa  | MBC  |
|-----------|--------|-------|------|-------|------|
| 3c        | 125    | —     | —    | 12.5  | 100  |
| 8a        | 125    | 12.5  | 100  | —     | —    |
| 9d        | 500    | —     | —    | 12.5  | 100  |

a CC₅₀ is the concentration at which 50% of the cells survive.

Table 4 CC₅₀ values of the most active compounds against normal VERO cells and their selectivity index

| Comp. no. | CC₅₀a  | MICa  | Slb  | MICb  | Slb  |
|-----------|--------|-------|------|-------|------|
| 2c        | 250    | —     | —    | 25    | 10   |
| 2d        | 500    | —     | —    | 25    | 20   |
| 3c        | 250    | —     | —    | 12.5  | 20   |
| 8a        | 125    | 12.5  | 10   | —     | —    |
| 8b        | 125    | 25    | 5    | —     | —    |
| 9a        | 500    | 25    | 20   | —     | —    |
| 9b        | 250    | 25    | 10   | —     | —    |
| 9c        | 250    | —     | —    | 25    | 10   |
| 9d        | 500    | —     | —    | 12.5  | 40   |

a CC₅₀ is the concentration at which 50% of the cells survive and MIC is the minimum concentration that inhibits bacterial growth reported in μg mL⁻¹. b Sl is the selectivity index regarding antimicrobial activity against K. pneumoniae and MRSA1; SI = CC₅₀/MIC.
Fig. 1  CC50 of the test compound (blue), their MIC against K. pneumoniae (red) and MRSA (green).

1H-NMR (DMSO-d6), δ ppm: 1.48 (d, 3H, J = 7.20 Hz, CH3), 2.12 (s, 3H, COCH3), 2.22 (s, 3H, CH3), 2.32 (s, 3H, CH3), 3.76 (q, 1H, J = 7.14 Hz, CH–COOH), 5.35 (s, 1H, CHpyrimidine), 6.90–7.59 (m, 8H, 2ArH), 8.01 (s, 1H, NH, exchangeable by D2O), 9.05 (s, 1H, OH exchangeable by D2O), 12.23 (br, 1H, OH, exchangeable by D2O).

13CMR (DMSO-d6), δ ppm: 17.22, 20.50, 22.37, 28.43, 41.88, 54.10, 57.67, 118.65, 122.11, 123.42, 124.67, 127.23, 128.25, 133.16, 134.29, 136.27, 139.18, 141.16, 151.20, 178.14, 191.89. MS (m/z, %): 468 (M+ − 1, 0.30%).

2-(6-Acetyl-5-(4-chlorophenyl)-2-(2-hydroxyphenyl)-7-methyl-1,2-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-3(5H)-yl)-3-(1H-indol-3-y1)propanoic acid 2c. Yield (70%), yellow brown crystals, mp: 212–214 °C. anal. data: (C32H30ClN5O4, 584.06), calc.: C, 65.26; H, 4.91; N, 12.88; Cl, 5.41. Found: C, 65.07; H, 4.76; N, 12.68; Cl, 6.08. IR (vmax, cm⁻¹): 3460 (br, 2OH), 3212, 3175 (2NH), 3062 (CHarom), 2938 (CHaliph), 1690 (CO), 1621 (COOasy), 1583 (COOsy), 811 (C-Cl). 1H-NMR (DMSO-d6), δ ppm: 2.10 (s, 3H, COCH3), 2.31 (s, 3H, CH3), 3.12 (d, 2H, J = 7.6 Hz, CH3), 3.76 (q, 1H, J = 7.14 Hz, CH–COOH), 5.23 (s, 1H, CHtriazole), 5.38 (s, 1H, CHpyrimidine), 6.76 (s, 1H, CHindole), 6.90–7.66 (m, 12H, 3ArH), 8.05 (s, 1H, NH, exchangeable by D2O), 9.05 (s, 1H, OH exchangeable by D2O), 10.24 (s, 1H, NHindole), 12.23 (br, 1H, OH, exchangeable by D2O). 13CMR (DMSO-d6), δ ppm: 20.50, 22.36, 28.40, 36.44, 41.80, 54.12, 57.60, 107.88, 117.44, 118.59, 122.15, 122.20, 123.06, 124.32, 126.67, 125.44, 127.23, 128.25, 133.16, 134.29, 135.75, 136.27, 138.19, 141.16, 144.33, 151.20, 178.14, 191.89. MS (m/z, %): 570 (M+ − 1, 0.2%).

2-(5-(4-Chlorophenyl)-6-(ethoxycarbonyl)-2-(2-hydroxyphenyl)-7-methyl-1,2-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-3(5H)-yl)propanoic acid 3a. Yield (80%), pale yellow crystals, mp: 180–182 °C. anal. data: (C23H26Cl2N6O4, 484.92), calc.: C, 59.39; H, 5.15; N, 11.54; Cl, 7.32. Found: C, 59.22; H, 4.90; N, 11.28; Cl, 7.04. IR (vmax, cm⁻¹): 3458 (br, 2OH), 3176 (NH), 3053 (CHarom), 2948 (CHaliph), 1698 (CO), 1621 (COOasy), 1583 (COOsy), 810 (C-Cl). 1H-NMR (DMSO-d6), δ ppm: 1.14 (t, 3H, J = 7.2 Hz, CH3), 1.48 (d, 3H, J = 7.20 Hz, CH3), 2.30 (s, 3H, CH3), 3.76 (q, 1H, J = 7.14 Hz, CH–COOH), 4.02 (q, 2H, J = 7.2 Hz, CH2), 5.24 (s, 1H, CHtriazole), 5.36 (s, 1H, CHpyrimidine), 6.94–7.52 (m, 8H, 2ArH), 8.02 (s, 1H, NH, exchangeable by D2O), 9.12 (s, 1H, OH exchangeable by D2O), 12.24 (br, 1H, OH, exchangeable by D2O). 13CMR (DMSO-d6), δ ppm: 14.27, 18.25, 28.55, 44.10, 53.78, 57.88, 117.36, 118.47, 121.50, 122.30, 128.35, 128.66, 129.85, 134.11, 136.51, 138.45, 139.94, 143.10, 151.22, 178.66, 192.73. MS (m/z, %): 583 (M+ − 1, 0.35%).

2-(5-(4-Chlorophenyl)-6-(ethoxycarbonyl)-2-(2-hydroxyphenyl)-7-methyl-1,2-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-3(5H)-yl)propanoic acid 3b. Yield (78%), yellow crystals, mp: 196–198 °C. anal. data: (C23H26Cl2N6O4, 498.96), calc.: C, 60.12; H, 5.41; N, 11.23; Cl, 7.11. Found: C, 59.92; H, 5.23; N, 11.01; Cl, 7.05. IR (vmax, cm⁻¹): 3450 (br, 2OH), 3180 (NH), 3058 (CHarom), 2940 (CHaliph), 1692 (CO), 1622 (COOasy), 1580 (COOsy), 811 (C-Cl). 1H-NMR (DMSO-d6), δ ppm: 1.14 (t, 3H, J = 7.2 Hz, CH3), 1.48 (d, 3H, J = 7.20 Hz, CH3), 2.21 (s, 3H, CH3), 2.30 (s, 3H, CH3), 3.72 (q, 1H, J = 7.14 Hz, CH–COOH), 4.01 (q, 2H, J = 7.2 Hz, CH2), 5.38 (s, 1H, CHpyrimidine), 6.90–7.50 (m, 8H, 2ArH), 8.02 (s, 1H, NH, exchangeable by D2O), 9.12 (s, 1H, OH exchangeable by D2O), 12.34 (br, 1H, OH, exchangeable by D2O).
2ArH), 8.01 (s, 1H, NH, exchangeable by D2O), 9.10 (s, 1H, OH exchangeable by D2O), 12.20 (br, 1H, OH, exchangeable by D2O).

13CMR (DMSO-d6), δ ppm: 14.20, 18.23, 22.56, 28.50, 44.08, 53.75, 57.80, 117.30, 118.41, 121.52, 122.19, 123.55, 124.20, 127.25, 128.70, 134.33, 136.50, 138.81, 143.02, 151.25, 178.10, 193.18.

2-[5-(4-Chlorophenyl)-6-(ethoxycarbonyl)-2-(hydroxyphenyl)-7-methyl-1,2-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-3(5H)-yl]-3-(1H-indol-3-yl)propanoic acid 3c. Yield (75%), brownish powder, mp: 232–234 °C, anal. data: (C25H25ClN5O6, 512.94): C, 55.70; H, 4.10; Cl, 7.13; N, 17.65. IR (ν_{max}, cm⁻¹): 3396 (ν_{COOH}), 3294 (ν_{CHpyrimidine}), 56.54 (C_{triazole}), 104.90, 117.38, 119.08, 120.60, 129.36, 131.32, 136.63, 135.17, 141.38, 142.89, 149.31 (m, 2ArC), 157.74 (C_{Cl}), 176.15 (C_{COOH}), 178.57 (C=O), 181.09 (C=O).

2-(5-(4-Chlorophenyl)-2-(hydroxyphenyl)-2,8,8-trimethyl-6-methyl-6-oxo-1H-[1,3]dioxin-5,4-e]-[1,2,4]triazolo[1,5-a]pyrimidin-3(2H,5H,6H)-yl)propanoic acid 4. Yield (57%), brown crystals, mp 208–210 °C, anal. caleed for (C_{22}H_{23}ClN_{6}O_{2}, 512.94): C, 58.54; H, 4.91; N, 10.92; Cl, 4.91. Found: C, 58.24; H, 5.12; N, 10.97; Cl, 6.98. IR (ν_{max}, cm⁻¹): 3392 (ν_{OH}), 3199 (ν_{NH}), 3067 (ν_{CHaromatic}), 2978 (ν_{CHaliphatic}), 1675 (C=O_{median}), 1616 (ν_{COO}), 816 (C=Cl). 1H-NMR (DMSO-d6), δ ppm: 2.22 (d, 3H, CH_{3}), 2.32 (3H, CH_{3}), 2.63 (3H, C-CH_{3}), 2.65 (3H, C-CH_{3}), 3.07 (m, 1H, CH-{COOH}), 5.53 (s, 1H, CH_{pyrimidine}), 6.53–8.25 (m, 8H, 2ArH), 10.66 (s, 2H, 2OH, exchangeable by D2O), 11.50 (s, 1H, NH, exchangeable by D2O). 13C NMR (DMSO-d6), δ ppm: 14.51 (CH_{3}), 15.57 (CH_{3}), 18.87 (CH_{2}), 26.43 (CH_{3}). 1621 (COO_{sy}), 1583 (COO_{sy}), 817 (C=Cl). 1H-NMR (DMSO-d6), δ ppm: 2.14, 28.46, 36.48, 41.81, 53.12, 57.66, 107.86, 117.40, 118.62, 121.67, 122.15, 122.24, 123.02, 124.40, 126.41, 125.41, 127.25, 128.20, 130.32, 134.25, 135.70, 136.21, 141.18, 141.30, 151.12, 178.25, 191.80.

Reaction of compounds 1a–d with cyclic-1,3-dicarbonyl compounds: synthesis of 2-[[1,2,4]triazolo[1,5-a]pyrimidin-3(2H,5H,6H)-yl]propanoic acid derivatives 4–7.

General procedures. A mixture of 2-(3-amino-5-(2-hydroxyphenyl)-1H-1,2,4-triazolo-4(5H)-yl)propanoic acid derivative 1a–d (0.001 mol), 4-chlorobenzaldehyde (0.14 g, 0.001 mol) and cyclic 1,3-dicarbonyl compounds (0.001 mol) viz. meldrum’s acid, barbituric acid, cyclohexane-1,3-dione and/or dimedone under the same experimental conditions [lemon juice, water–ethanol (8:2)] was mixed in water (8 mL)–ethanol (2 mL) then treated with 1 mL of fresh lemon juice. The reaction mixture was heated under reflux for 5–8 h, then left to cool. The formed precipitates were collected by filtration, washed thoroughly with water and then recrystallized from ethanol to give the corresponding 4–7 derivatives.

2-(5-(4-Chlorophenyl)-2-(hydroxyphenyl)-2,8,8-trimethyl-6-oxo-1H-[1,3]dioxin-5,4-e]-[1,2,4]triazolo[1,5-a]pyrimidin-3(2H,5H,6H)-yl)propanoic acid 7. Yield (58%), pale yellow crystals, mp 257-
An equimolar mixture of 2-(3-amino-5-(2-hydroxyphenyl)-1-cyanoacetate (0.001 mol) was mixed in water (8 mL)-ethanol (2 mL) with water and then recrystallized from ethanol to give the corre-

yield (67%), yellow crystals, mp 114–120 °C. Anal. calcd for (C12H12ClO5, 551.98): C, 63.04; H, 3.98; Cl, 6.43; N, 17.75. Found: C, 62.91; H, 3.72; Cl, 6.42; N, 17.41. IR (νmax cm⁻¹): 3430 (OH), 3400 (OH), 3312 (NHpyrimidine), 3230 (NH), 3065 (CH_2), 3043 (CH_2), 2922 (CH_2), 1764 (C=O), 1697 (C=O), 1147 (C–C). 

**2-[(4-Chlorophenyl)-6-cyano-2-(2-hydroxyphenyl)]-7-imino-1,2-dihydro-[1,2,4]triazolo[1,5-α]pyrimidin-3(7H)-yl]propanoic acid 8d.** Yield (67%), yellow crystals, mp 130 °C. Anal. calcd for (C_22H_19ClN_6O_3, 450.89): C, 57.28; H, 3.42; N, 17.72. Found: C, 57.02; H, 4.42; Cl, 8.03; N, 17.41. IR (νmax cm⁻¹): 3430 (OH), 3400 (OH), 3312 (NHpyrimidine), 3230 (NH), 3065 (CH_2), 3043 (CH_2), 2922 (CH_2), 1764 (C=O), 1697 (C=O), 1147 (C–C).
NMR (DMSO-d$_6$), $\delta$ ppm: 1.57 (d, 3H, CH$_3$), 3.52 (q, 1H, CH–COOH), 5.23 (s, 1H, CH$_{triazole}$), 6.94–7.59 (m, 8H, 2ArH), 8.03 (s, 1H, NH, exchangeable by D$_2$O), 10.02 (s, 1H, OH, exchangeable by D$_2$O), 12.22 (s, 1H, OH, exchangeable by D$_2$O). $^{13}$C NMR (DMSO-d$_6$), $\delta$ ppm: 19.91 (CH$_3$), 44.55 (CH–COOH), 53.53 (CH$_{triazole}$), 107.44 (C–(C=N)), 116.86, 119.00, 120.84, 121.87, 123.56, 127.20, 129.14, 131.46, 131.94, 133.98, (m, ArC), 140.81 (C–(C=N)), 157.92 (C–Cl), 167.14 (C–OH), 177.96 (C–(C=N)), 178.20 (C–O).

2-(5-(4-Chlorophenyl)-2-hydroxy-2-methyl-7-oxo-1,2-dihydro-[1,2,4]triazolo[1,5-$\alpha$]pyrimidin-3(7$\beta$)-yl)propanoic acid 9b. Yield (64%), yellow crystals, mp 177–182 °C, anal. calc'd for (C$_{25}$H$_{20}$ClN$_6$O$_4$, 451.56): C, 59.66; H, 3.81; N, 15.43. Found: C, 59.62; H, 4.02; N, 15.30. IR (r$_{\text{max}}$ cm$^{-1}$): 3450 (OH), 3182 (NH), 3056 (CH$_{aromatic}$), 2949 (CH$_{aliphatic}$), 2218 (C=O), 1675 (CO), 1600 (CO$_2$$_{as}$), 1533 (CO$_2$$_{sy}$), 757 (C=Cl). $^1$H-NMR (DMSO-d$_6$), $\delta$ ppm: 1.54 (d, 3H, CH$_3$), 2.23 (s, 3H, CH$_3$), 3.54 (q, 1H, CH–COOH), 5.21 (s, 1H, CH$_{triazole}$), 6.92–7.56 (m, 8H, 2ArH), 8.02 (s, 1H, OH, exchangeable by D$_2$O), 9.24 (s, 1H, OH, exchangeable by D$_2$O), 12.18 (s, 1H, OH, exchangeable by D$_2$O). $^{13}$C NMR (DMSO-d$_6$), $\delta$ ppm: 19.87 (CH$_3$), 18.12, 44.23 (CH–COOH), 80.65 (CH$_{triazole}$), 107.44 (C–(C=N)), 116.86, 119.00, 120.84, 121.87, 123.56, 127.20, 129.14, 131.46, 131.94, 133.98, (m, ArC), 140.81 (C–(C=N)), 153.96 (C–(C=N)), 157.92 (C–Cl), 167.14 (C–OH), 178.15 (C–O).

2-(5-(4-Chlorophenyl)-2-hydroxy-2-methyl-7-oxo-1,2-dihydro-[1,2,4]triazolo[1,5-$\alpha$]pyrimidin-3(7$\beta$)-yl)-3-(1H-indol-3-yl)propanoic acid 9c. Yield (75%), yellow crystals, mp 188–190 °C, anal. calc'd for (C$_{27}$H$_{22}$ClN$_7$O$_3$, 525.52): C, 62.93; H, 3.79; Cl, 6.41; N, 15.97. Found: C, 62.60; H, 3.52; Cl, 6.41; N, 15.01. IR (r$_{\text{max}}$ cm$^{-1}$): 3430 (OH), 3405 (OH), 3230 (NH), 3176 (NH$_{indole}$), 3060 (CH$_{aromatic}$), 2952 (CH$_{aliphatic}$), 2206 (C=CN), 1623 (CO$_2$$_{sy}$), 1578 (CO$_2$$_{as}$), 765 (C=Cl). $^1$H-NMR (DMSO-d$_6$), $\delta$ ppm: 3.12 (t, 1H, CH–COOH), 3.56 (d, 2H, CH$_2$), 5.25 (s, 1H, CH$_{triazole}$), 6.74 (s, 1H, NH, exchangeable by D$_2$O), 6.80–8.12 (m, 13H, 3ArH), 8.40 (s, 1H, OH, exchangeable by D$_2$O), 10.25 (s, 1H, NH, exchangeable by D$_2$O), 12.23 (s, 1H, OH, exchangeable by D$_2$O). $^{13}$C NMR (DMSO-d$_6$), $\delta$ ppm: 34.80, 53.83, 66.67, 107.77, 130.70, 119.32, 119.84, 121.60, 123.60, 123.96, 124.84, 128.31, 128.95, 129.12, 129.43, 130.05, 131.38, 131.50, 131.71, 141.20, 153.76, 157.16, 165.81, 168.33, 178.41.

2-(5-(4-Chlorophenyl)-6-cyano-2-hydroxy-2-methyl-7-oxo-1,2-dihydro-[1,2,4]triazolo[1,5-$\alpha$]pyrimidin-3(7$\beta$)-yl)-3-(1H-indol-3-yl)propanoic acid 9d. Yield (72%), brown needles, mp 202–205 °C, anal. calc'd for (C$_{25}$H$_{20}$ClN$_6$O$_4$, 566.99): C, 63.49; H, 4.05; Cl, 6.26; N, 14.83. Found: C, 63.33; H, 3.92; Cl, 6.01; N, 14.55. IR (r$_{\text{max}}$ cm$^{-1}$): 3436 (2OH), 3236 (NH), 3180 (NH$_{indole}$), 3059 (CH$_{aromatic}$), 2948 (CH$_{aliphatic}$), 2212 (C=CN), 1625 (CO$_2$$_{as}$), 1575 (CO$_2$$_{sy}$), 771 (C=Cl). $^1$H-NMR (DMSO-d$_6$), $\delta$ ppm: 2.23 (d, 3H, CH$_3$), 3.13 (t, 1H, CH–COOH), 3.58 (d, 2H, CH$_2$), 6.75 (s, 1H, NH, exchangeable by D$_2$O), 6.83–8.12 (m, 13H, 3ArH), 8.38 (s, 1H, OH, exchangeable by D$_2$O), 10.23 (s, 1H, NH, exchangeable by D$_2$O), 12.25 (s, 1H, OH, exchangeable by D$_2$O). $^{13}$C NMR (DMSO-d$_6$), $\delta$ ppm: 24.13, 34.80, 53.83, 66.75, 107.76, 117.30, 119.30, 119.97, 121.60, 123.54, 123.96, 124.84, 128.31, 128.90, 129.13, 129.45, 130.05, 131.38, 131.50, 131.73, 141.25, 153.16, 157.12, 165.07, 168.27, 178.48.

Reaction of compound 1a–d with 2-(bis(methylthio)methylene)malononitrile

An equimolar mixture of 2-(3-amino-5-(2-hydroxyphenyl)-1H-1,2,4-triazol-4(5H)-yl)propanoic acid derivative 1a–d (0.001 mol) and 2-(bis(methylthio)methylene)malononitrile (0.17 g, 0.001 mol) was mixed in water (8 mL)–ethanol (2 mL) then was treated with 1 mL of fresh lemon juice. The reaction mixture was heated under reflux until evolution of methyl mercaptan was ceased (lead acetate, 10–12 h), then left to cool. The formed precipitates were collected by filtration, washed thoroughly with water and then recrystallized from ethanol to give the corresponding 2-(6-cyano-2-hydroxyphenyl)-7-imino-5-(methylthio)-1,2-dihydro-[1,2,4]triazolo[1,5-$\alpha$]pyrimidin-3(7$\beta$)-yl)propanoic acid derivatives 10a–d respectively.
2-(6-Cyano-2-(2-hydroxyphenyl)-7-imino-2-methyl-5-(methylthio)-1,2-dihydroy-[1,2,4]triazolo[1,5-a]pyrimidin-3(7H)-yl)-3-(1H-indol-3-yl)propanoic acid 10d. Yield (65%), orange crystals, mp 235–237 °C, anal. calcd for (C_{25}H_{23}N_{7}O_{3}S, 501.56): C, 59.81; H, 4.58; N, 19.53; S, 6.38. Found: C, 58.56; H, 4.33; N, 19.26; S, 5.35. IR (ν_{max} cm⁻¹): 3412 (O-H), 3280 (NH, pyrimidine), 3188, 3141 (2NH), 3059 (CH_{aromatic}), 2956 (CH_{aliphatic}), 1588 (COO\_sy), 1588 (COO\_sy), 3059 (CH_{aromatic}), 2956 (CH_{aliphatic}), 1588 (COO\_sy), 1588 (COO\_sy). 1H-NMR (DMSO-d$_{6}$), δ ppm: 2.58 (s, 3H, S–CH$_{3}$), 3.48 (m, 1H, CH–COOH), 3.56 (d, 3H, CH$_{3}$), 6.81 (s, 1H, NH exchangeable by D$_{2}$O), 7.30–7.62 (m, 8H, ArH), 8.05 (s, 1H, NH, exchangeable by D$_{2}$O), 9.20 (s, 1H, OH, exchangeable by D$_{2}$O), 12.24 (s, 1H, OH, exchangeable by D$_{2}$O).

Antimicrobial screening

Agar diffusion method. The newly synthesized heterocyclic compounds were tested for their antimicrobial activity against Gram positive bacteria (Staphylococcus aureus and Streptococcus pyogenes) and Gram negative bacteria (Pseudomonas phaseolica and Pseudomonas fluorescens) for yeast-like fungi C. albicans. For all bacteria (nutrient medium), consisting of (g L\(^{-1}\)) peptone, 5 and meat extract, 3. pH was adjusted for 18 h. MIC was the lowest concentration of each compound that inhibited the growth of the bacteria under test.

Minimal inhibitory concentration (MIC) of active compounds against MDR bacteria. A mixture of 80 μl of sterile Mueller–Hinton broth; 20 μl tween 80 and 100 μl of each test compound were serially diluted using two fold dilution in 96-well microtiter plate. Each well was inoculated with 100 μl of 0.5 McFarland standard bacterial suspensions equivalent to 1.5 × 10⁶ CFU mL\(^{-1}\). The plates were covered and incubated at 35 ± 2 °C for 24 h. MIC was the lowest concentration of each compound that inhibited the growth of the bacteria under test.\(^{26,27}\)

Determination of minimum bactericidal concentration (MBC) and MIC index of compounds 3e, 8a and 9d. The most active compound against MRSA (3e & 9d) and against K. pneumoniae were subjected to MC test. The highest dilution of each compound not exhibiting bacterial growth was taken as the MIC. After estimation of the MICs, 20 μl aliquots from each well were plated onto Müller–Hinton agar plates and incubated at 35 ± 2 °C for 18 h. The lowest dilution not exhibiting bacterial growth was recorded as the minimal bactericidal concentration (MBC).

MIC index (MBC/MIC) was calculated for the antibacterial agent to determine whether the agent was bactericidal (MBC/MIC < 4) or bacteriostatic (MBC/MIC > 4) against the growth of the tested bacteria. The range of MIC index values greater than 4 and less than 32 were considered to be bacteriostatic.\(^{19}\)

In vitro cytotoxicity assessment: MTT assay. The cytotoxicity of the compounds was tested in the Vero (African Green Monkey-kidney cells) cell line using the Mosmann method, with certain modifications, as described in the literature.\(^{37,40}\) African green monkey kidney (Vero) cells were purchased from American type culture collection (ATCC, Manassas, VA, USA). All media, serum and other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Vero cells was maintained in minimum essential medium (MEM) (Eagle) with non-essential amino acids, with 10% fetal bovine serum in a humidified atmosphere at 37 °C with 5% CO\(_{2}\). The cell line was maintained in their growing phase at 70% confluence with regular passaging. The prepared compounds were tested for its cytotoxicity by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Vero cells were seeded in their respective culture medium (200 μl, 1 × 10⁴ cells per well) in a 96-well plate and incubated at 37 °C for 24 h with 5% CO\(_{2}\) supply. After incubation, the control wells were replenished with fresh medium and the test wells were treated with 62.5, 125, 250 and 500 μg mL\(^{-1}\) of synthesized compounds. The cells were further incubated for 72 h maintaining the same conditions. After the treatment incubation period, medium in each well was replenished with 200 μl of fresh medium plus 20 μl of MTT (0.5 mg mL\(^{-1}\)). The plate was then incubated for 4 h in the same conditions after which the absorbance was measured at 570 nm using ELISA reader. Measurements were performed and the concentration required for a 50% inhibition of viability (IC\(_{50}\)) was determined graphically. Standard Graph was plotted by taking concentration of the drug in X axis and relative cell viability in Y axis.

Cell viability (%) = mean optical density/control optical density × 100%

4. Conclusion

A new series of [1,2,4]-triazole derivatives were obtained under green reaction conditions. The obtained candidates showed promising antibacterial screening namely, 2b–d, 3a–d, 5, 6, 7, 8a–d and 9a–d. They were further subjected to a screening against MRD (multi drug resistant) clinical isolates and showed promising antibacterial activity. The most active compounds 2e, 2d, 3c, 8a, 8b, 9a, 9b, 9c and 9d showed a high selectivity index towards antimicrobial activity against K. pneumoniae and MRSA1 compared to mammalian cells, revealed a good safety profile. Moreover, in contrast to the reference drugs cephalothin and chloramphenicol, compounds 3e, 8a and 9d exhibited significant better MIC values towards the tested MDR strains. The MIC index of these compound suggesting bacteriostatic mechanism of action.

Conflicts of interest

The authors confirm that the content of this article contains no conflict of interest.

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