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Synthesis and antimicrobial activity of some novel 1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidines bearing amino acid moiety†

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A new series of [1,2,4]-triazole bearing amino acid derivatives **2a–d**–**9a–d** were synthesized under green chemistry conditions *via* multicomponent reaction using lemon juice as an acidic catalyst. The obtained compounds were characterized by different spectral and elemental analyses. The obtained candidates showed promising antibacterial activity against some standard bacteria and multidrug resistant (MDR) clinical isolates. In contrast to the reference drugs cephalothin and chloramphenicol, the tested compounds showed substantial better MIC values towards the tested MDR strains. The most active compounds **3c**, **8a** and **9d** against MDR bacteria were tested for MBC and MIC index, the results indicted the bacteriostatic activity of these compounds. The most active compounds **2c**, **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, suggesting a good safety profile.

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1. Introduction

Facing emerging bacterial infections has become more challenging worldwide due to the increasing number of multidrug-resistant (MDR) microbes.[1–7] 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 interst of medicinal chemists due to its improving ability for the physicochemical and drug-likeness properties.[20,23] In addition, glycine and its derivatives appear to be promising safe antimicrobial agents.[24,25]

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.[27–30] It was reported that series of 1,2,4-triazolo[1,5-*a*]pyrimidines carboxamide derivatives attributed good narrow-spectrum antibacterial activity against *E. faecium* and possessed metabolic stability with low intrinsic clearance. Macromolecular synthesis assays revealed cell-wall biosynthesis as the target of these compounds.[22] 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

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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.

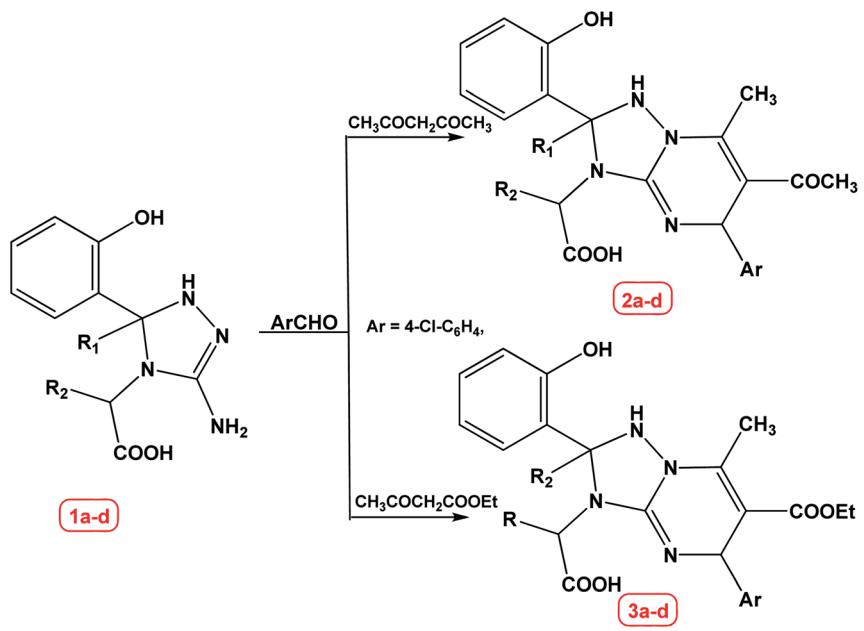
2. Results and discussion

Chemistry

In continuation of our research program on the utility of hetrocylic 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)-1*H*-1,2,4-triazol-4(5*H*)-yl)propanoic 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(5*H*)-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, ^{1}H-NMR, ^{13}C-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 ^{1}H-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 singlet at δ 4.50 ppm for CH (pyrimidine), a quartet at 2.92 ppm corresponding to CH–COOH, a singlet at 2.33 ppm for δ CH_3 group, a doublet at δ 2.23 ppm attributed to (CH_3 alanine) and a singlet at δ 2.12 ppm characteristic for CH_3 CO group. ^{13}CMR spectrum of compound **2a** showed the following signals: 9.88 (CH_3–CH), 20.12 (CH_3), 22.02 (CH_3CO), 41.80 (CH–COOH), 53.12 ($CH_{pyrimidine}$), 57.80 ($CH_{triazole}$), 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).



	a	b	c	d
R_1	H	CH_3	H	CH_3
R_2	CH_3	CH_3	3-indolylmethyl	3-indolylmethyl

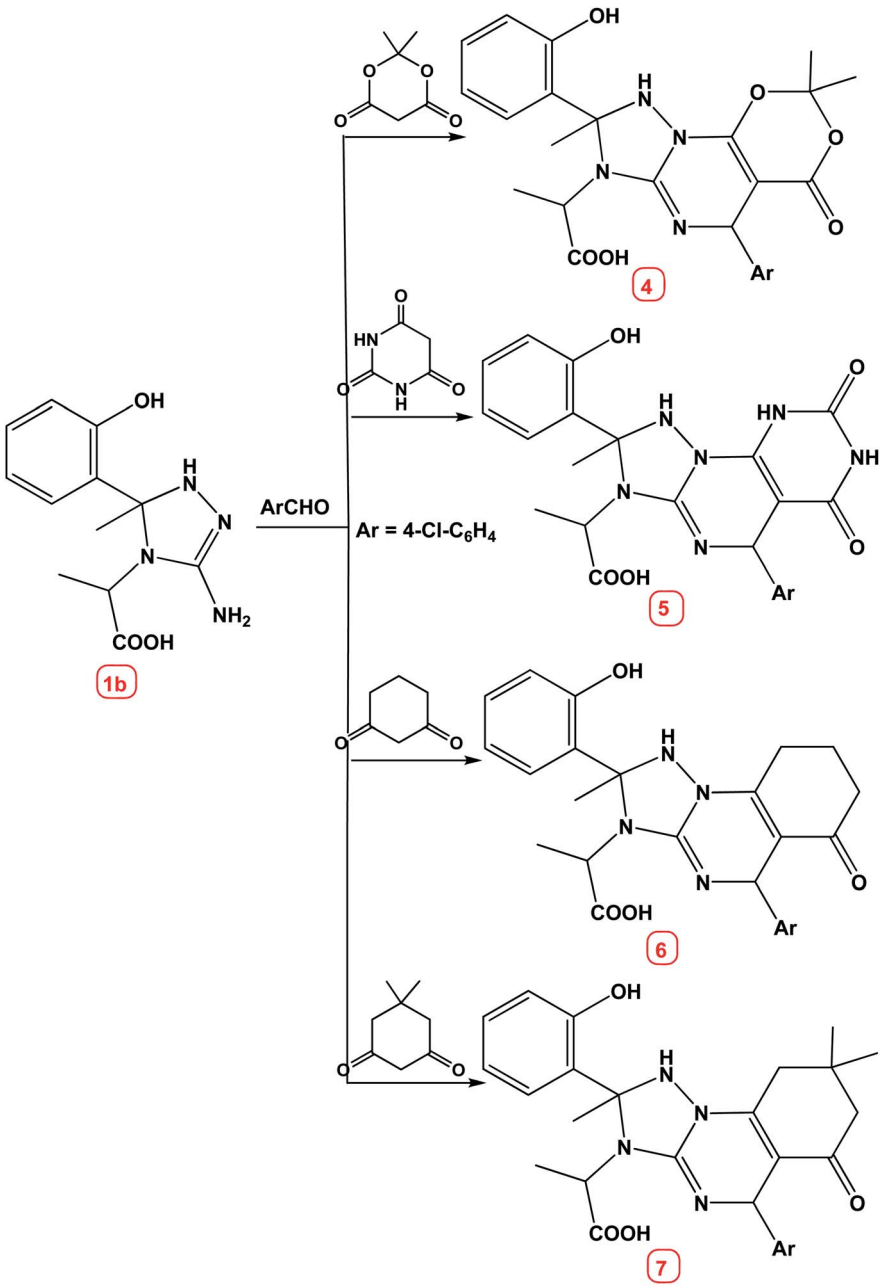
Scheme 1 Synthesis of [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives.

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-*a*]pyrimidine derivatives **4–7** respectively, Scheme 2.

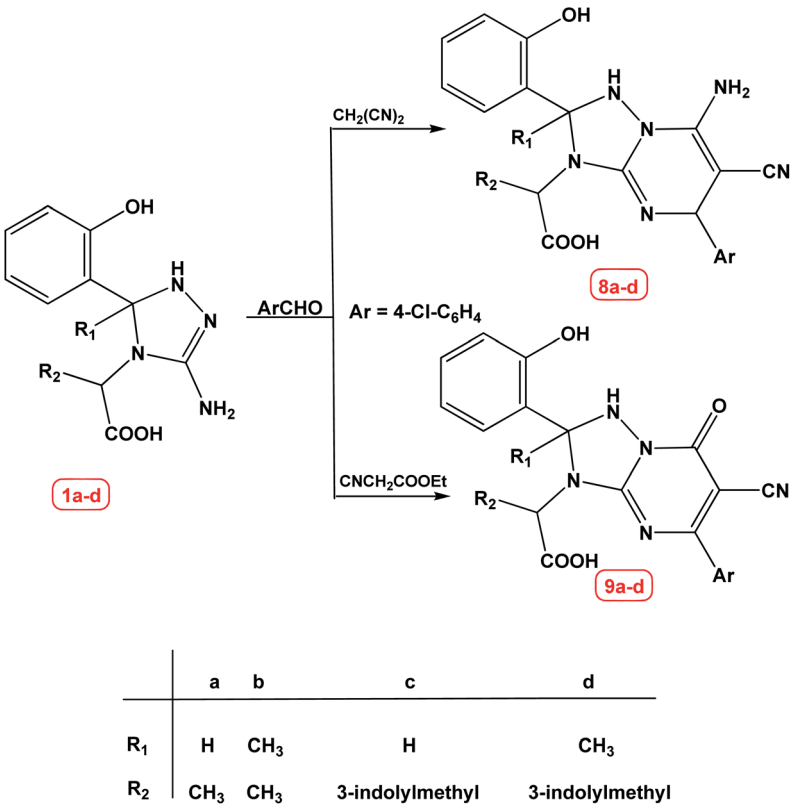
On continuation of our work, 2-(3-amino-5-(2-hydroxyphenyl)-1*H*-1,2,4-triazol-4(5*H*)-yl)propanoic acid derivatives **1a–d** were allowed to react with 4-chlorobenzaldehyde and malononitrile or ethyl cyanoacetate under the same experimental conditions, where the corresponding [1,2,4]triazolo[1,5-*a*]pyrimidine 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-*a*]pyrimidin-3(7*H*)-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-*a*]pyrimidine derivatives.

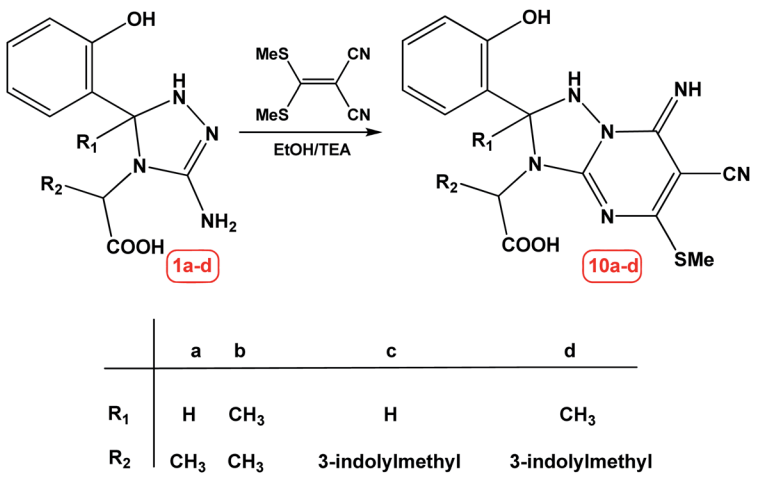


Scheme 3 Synthesis of [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives.

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) and *S. pyogenes* (ATCC 19615)] and Gram-negative bacteria [*P. phaseolicola* (GSPB 2828) 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



Scheme 4 Synthesis of [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives.

Table 1 *In vitro* antibacterial activities of the synthesized compounds against standard bacteria[a]

	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*	
Comp. no.	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}	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	23	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			
Chloramphenicol	NT		NT		25		30			
Clotrimazole	NT		NT		NT		NT		36	44

[a] 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,[36,37] *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 concentration (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.[38,39] Results revealed that test compounds have MIC index 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.[22]

***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.[37,40] The 50% cytotoxic concentration (CC_{50}) 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 antimicrobial activity against *K. pneumoniae* and *MRSA1* compared to mammalian cells, thus showing a good toxicity profile, Fig. 1.

3. Methods

Chemistry

All melting points were determined on a Koffler melting point apparatus and are uncorrected. ^{1}H-NMR and ^{13}C 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, ν_{max} in cm^{-1}). Mass spectra were recorded on a GC-MSQP 1000EX Schimadzu 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)-1*H*-1,2,4-triazol-4(5*H*)-yl)propanoic acid derivative **1a–d** (0.001

Table 2 *In vitro* antibacterial activities and minimum inhibitory concentration of the synthesized compounds against MDR clinical isolates

	K. pneumoniae		*MRSA1*	
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	8	—
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**

		K. pneumoniae (μg mL^{-1})		*MRSA1* (μg mL^{-1})	
Comp. no.	CC_{50}[a]	MIC[a]	MBC	MIC[a]	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 CC_{50} values of the most active compounds against normal VERO cells and their selectivity index

		K. pneumoniae (μg mL^{-1})		*MRSA1* (μg mL^{-1})	
Comp. no.	CC_{50}[a]	MIC[a]	SI[b]	MIC[a]	SI[b]
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] CC_{50} 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] SI is the selectivity index regarding antimicrobial activity against *K. pneumoniae* and *MRSA1*; SI = CC_{50}/MIC.

mol), 4-chlorobenzaldehyde (0.14 g, 0.001 mol) and acetylacetone or ethyl acetoacetate (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 for 4–6 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-(1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(5*H*)-yl)propanoic acid derivatives **2a–d** and **3a–d** respectively.

2-(6-Acetyl-5-(4-chlorophenyl)-2-(2-hydroxyphenyl)-7-methyl-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(5*H*)-yl)propanoic acid 2a. Yield (74%), pale yellow needles, mp: 126–128 °C, anal. data: ($C_{23}H_{23}ClN_4O_4$, 454.90), calc.: C, 60.67; H, 5.05; N, 12.31; Cl, 7.80. Found: C, 60.33; H, 4.88; N, 12.09; Cl, 7.54. IR (ν_{max}, cm^{-1}): 3452 (br, 2OH), 3188 (NH), 3040 (CH_{arom}), 2956 (CH_{aliph}), 1690 (C=O), 1622 (COO_{asy}), 1582 (COO_{sy}), 810 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 1.48 (d, 3H, J = 7.20 Hz, CH_3), 2.10 (s, 3H, $COCH_3$), 2.32 (s, 3H, CH_3), 3.72 (q, 1H, J = 7.14 Hz, CH–COOH), 5.24 (s, 1H, $CH_{triazole}$), 5.36 (s, 1H, $CH_{pyrimidine}$), 6.88–7.58 (m, 8H, 2ArH), 8.02 (s, 1H, NH, exchangeable by D_2O), 9.06 (s, 1H, OH exchangeable by D_2O), 12.25 (br, 1H, OH, exchangeable by D_2O). ^{13}CMR (DMSO-d_6), δ ppm: 17.06, 20.12, 22.02, 41.82, 57.80, 118.12, 119.21, 122.23, 123.46, 124.55, 126.57, 127.39, 128.60, 134.56, 136.08, 138.12, 143.20, 152.11, 178.16, 192.47. MS (*m*/*z*, I%): 453 ($M^+ - 1$, 0.25%).

2-(6-Acetyl-5-(4-chlorophenyl)-2-(2-hydroxyphenyl)-2,7-dimethyl-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(5*H*)-yl) propanoic acid 2b. Yield (74%), yellow crystals, mp: 187–189 °C, anal. data: ($C_{24}H_{25}ClN_4O_4$, 468.93), calc.: C, 61.53; H, 5.34; N, 11.96; Cl, 7.57. Found: C, 61.28; H, 5.12; N, 11.70; Cl, 5.41. IR (ν_{max}, cm^{-1}): 3468 (br, 2OH), 3175 (NH), 3055 (CH_{arom}), 2940 (CH_{aliph}), 1696 (CO), 1620 (COO_{asy}), 1580 (COO_{sy}), 815 (C–Cl).

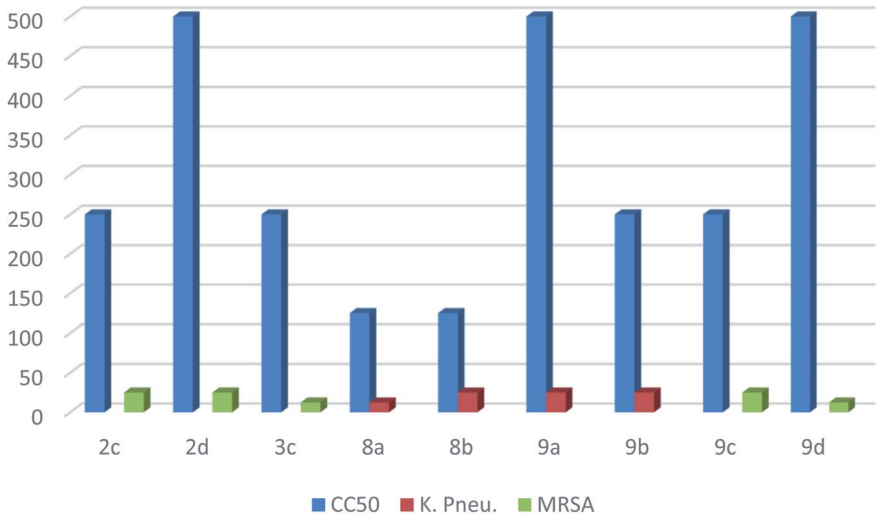


Fig. 1 CC_{50} of the test compound (blue), their MIC against *K. pneumoniae* (red) and MRSA (green).

^{1}H-NMR (DMSO-d_6), δ ppm: 1.48 (d, 3H, $J = 7.20$ Hz, CH_3), 2.12 (s, 3H, $CO\underline{CH_3}$), 2.22 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 3.76 (q, 1H, $J = 7.14$ Hz, $\underline{CH}$–COOH), 5.35 (s, 1H, $CH_{pyrimidine}$), 6.90–7.59 (m, 8H, 2ArH), 8.01 (s, 1H, NH, exchangeable by D_2O), 9.05 (s, 1H, OH exchangeable by D_2O), 12.23 (br, 1H, OH, exchangeable by D_2O). ^{13}CMR (DMSO-d_6), δ ppm: 17.22, 20.50, 22.37, 28.43, 41.88, 54.10, 57.67, 117.21, 118.65, 122.11, 123.42, 124.67, 127.23, 128.25, 133.16, 134.29, 136.27, 138.19, 141.16, 151.20, 178.14, 191.89. MS (*m*/*z*, I%): 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(5*H*)-yl)-3-(1*H*-indol-3-yl)propanoic acid 2c. Yield (70%), yellow brown crystals, mp: 212–214 °C, anal. data: ($C_{31}H_{28}ClN_5O_4$, 570.04), calc.: C, 65.26; H, 4.91; N, 12.88, Cl, 6.22. Found: C, 65.07; H, 4.76; N, 12.68; Cl, 6.08. IR (ν_{max}, cm^{-1}): 3460 (br, 2OH), 3212, 3175 (2NH), 3062 (CH_{arom}), 2938 (CH_{aliph}), 1690 (CO), 1621 (COO_{asy}), 1583 (COO_{sy}), 811 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 2.10 (s, 3H, $CO\underline{CH_3}$), 2.31 (s, 3H, CH_3), 3.12 (d, 2H, $J = 7.6$ Hz, CH_2), 3.76 (q, 1H, $J = 7.14$ Hz, $\underline{CH}$–COOH), 5.23 (s, 1H, $CH_{triazole}$), 5.38 (s, 1H, $CH_{pyrimidine}$), 6.76 (s, 1H, CH_{indole}), 6.90–7.66 (m, 12H, 3ArH), 8.05 (s, 1H, NH, exchangeable by D_2O), 9.05 (s, 1H, OH exchangeable by D_2O), 10.24 (s, 1H, NH_{indole}), 12.23 (br, 1H, OH, exchangeable by D_2O). ^{13}CMR (DMSO-d_6), 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, 123.42, 124.67, 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*, I%): 570 ($M^+ - 1$, 0.2%).

2-(6-Acetyl-5-(4-chlorophenyl)-2-(2-hydroxyphenyl)-2,7-dimethyl-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(5*H*)-yl)-3-(1*H*-indol-3-yl)propanoic acid 2d. Yield (72%), yellow needles, mp: 230–232 °C, anal. data: ($C_{32}H_{30}ClN_5O_4$, 584.06), calc.: C, 65.26; H, 4.91; N, 12.88; Cl, 6.08. Found: C, 65.07; H, 4.76; N, 12.68; Cl, 5.92. IR (ν_{max}, cm^{-1}): 3466 (br, 2OH), 3218, 3186 (2NH), 3055 (CH_{arom}), 2942 (CH_{aliph}), 1695 (CO), 1623 (COO_{asy}), 1580 (COO_{sy}), 817 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 2.11 (s, 3H, $CO\underline{CH_3}$), 2.22 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.12 (d, 2H, $J =$ 7.6 Hz, CH_2), 3.76 (q, 1H, $J = 7.14$ Hz, $\underline{CH}$–COOH), 5.36 (s, 1H, $CH_{pyrimidine}$), 6.75 (s, 1H, CH_{indole}), 6.87–7.65 (m, 12H, 3ArH), 8.05 (s, 1H, NH, exchangeable by D_2O), 9.10 (s, 1H, OH exchangeable by D_2O), 10.22 (s, 1H, NH_{indole}, exchangeable by D_2O), 12.20 (br, 1H, OH, exchangeable by D_2O). ^{13}CMR (DMSO-d_6), δ ppm: 20.31, 22.01, 34.52, 41.06, 44.22, 53.58, 57.06, 110.57, 117.26, 118.43, 121.80, 122.36, 123.12, 123.85, 124.6, 127.11, 127.94, 128.48, 133.25, 134.37, 135.41, 136.50, 138.45, 138.94, 143.10, 151.22, 178.66, 192.73. MS (*m*/*z*, I%): 583 ($M^+ - 1$, 0.35%).

2-(5-(4-Ahlorophenyl)-6-(ethoxycarbonyl)-2-(2-hydroxyphenyl)-7-methyl-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(5*H*)-yl)propanoic acid 3a. Yield (80%), pale yellow crystals, mp: 180–182 °C, anal. data: ($C_{24}H_{25}ClN_4O_5$, 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 (ν_{max}, cm^{-1}): 3458 (br, 2OH), 3176 (NH), 3053 (CH_{arom}), 2948 (CH_{aliph}), 1698 (CO), 1620 (COO_{asy}), 1583 (COO_{sy}), 810 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 1.14 (t, 3H, $J =$ 7.2 Hz, CH_3), 1.48 (d, 3H, $J = 7.20$ Hz, CH_3), 2.30 (s, 3H, CH_3), 3.76 (q, 1H, $J = 7.14$ Hz, $\underline{CH}$–COOH), 4.02 (q, 2H, $J = 7.2$ Hz, CH_2), 5.24 (s, 1H, $CH_{triazole}$), 5.36 (s, 1H, $CH_{pyrimidine}$), 6.94–7.52 (m, 8H, 2ArH), 8.02 (s, 1H, NH, exchangeable by D_2O), 9.12 (s, 1H, OH exchangeable by D_2O), 12.24 (br, 1H, OH, exchangeable by D_2O). ^{13}CMR (DMSO-d_6), δ ppm: 14.27, 18.25, 28.55, 44.10, 53.78, 57.88, 117.36, 118.47, 121.50, 122.30, 123.55, 124.19, 127.28, 128.72, 134.32, 136.55, 138.83, 143.01, 151.26, 178.06, 193.05.

2-(5-(4-Chlorophenyl)-6-(ethoxycarbonyl)-2-(2-hydroxyphenyl)-2,7-dimethyl-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(5*H*)-yl)propanoic acid 3b. Yield (78%), yellow crystals, mp: 196–198 °C, anal. data: ($C_{25}H_{27}ClN_4O_5$, 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 (ν_{max}, cm^{-1}): 3450 (br, 2OH), 3180 (NH), 3058 (CH_{arom}), 2940 (CH_{aliph}), 1692 (CO), 1622 (COO_{asy}), 1580 (COO_{sy}), 811 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 1.14 (t, 3H, $J =$ 7.2 Hz, CH_3), 1.48 (d, 3H, $J = 7.20$ Hz, CH_3), 2.21 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.72 (q, 1H, $J = 7.14$ Hz, $\underline{CH}$–COOH), 4.01 (q, 2H, $J = 7.2$ Hz, CH_2), 5.38 (s, 1H, $CH_{pyrimidine}$), 6.90–7.50 (m, 8H,

2ArH), 8.01 (s, 1H, NH, exchangeable by D_2O), 9.10 (s, 1H, OH exchangeable by D_2O), 12.20 (br, 1H, OH, exchangeable by D_2O). ^{13}CMR (DMSO-d_6), 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-(2-hydroxyphenyl)-7-methyl-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(5*H*)-yl)-3-(1*H*-indol-3-yl)propanoic acid 3c. Yield (75%), brownish powder, mp: 232–234 °C, anal. data: ($C_{32}H_{30}ClN_5O_5$, 600.06), calc.: C, 63.99; H, 4.99; N, 11.66; Cl, 5.91. Found: C, 63.75; H, 4.78; N, 11.40; Cl, 5.72. IR (ν_{max}, cm^{-1}): 3458 (br, 2OH), 3232, 3185 (2NH), 3066 (CH_{arom}), 2954 (CH_{aliph}), 1690 (CO), 1621 (COO_{asy}), 1583 (COO_{sy}), 817 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 1.12 (t, 3H, J = 7.2 Hz, CH_3), 2.22 (s, 3H, CH_3), 3.12 (s, 2H, CH_2), 3.71 (q, 1H, J = 7.14 Hz, CH–COOH), 4.03 (q, 2H, J = 7.2 Hz, CH_2), 5.23 (s, 1H, $CH_{triazole}$), 5.38 (s, 1H, $CH_{pyrimidine}$), 6.90–7.64 (m, 12H, 2ArH), 8.05 (s, 1H, NH, exchangeable by D_2O), 9.10 (s, 1H, OH exchangeable by D_2O), 10.18 (s, 1H, CH_{indole}, exchangeable by D_2O), 12.23 (br, 1H, OH, exchangeable by D_2O). ^{13}CMR (DMSO-d_6), δ ppm: 14.21, 28.46, 36.48, 41.81, 53.12, 57.66, 107.86, 117.40, 118.62, 121.67, 122.15, 122.24, 123.02, 123.40, 124.61, 125.41, 127.25, 128.20, 133.32, 134.25, 135.70, 136.21, 138.22, 141.18, 144.30, 151.12, 178.25, 191.80.

2-(5-(4-Chlorophenyl)-6-(ethoxycarbonyl)-2-(2-hydroxyphenyl)-2,7-dimethyl-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(5*H*)-yl)-3-(1*H*-indol-3-yl)propanoic acid 3d. Yield (75%), brown sheets, mp: 251–253 °C, anal. data: ($C_{33}H_{32}ClN_5O_5$, 614.09), calc.: C, 64.48; H, 5.21; N, 11.39; Cl, 5.78. Found: C, 64.22; H, 4.98; N, 11.20; Cl, 5.62. IR (ν_{max}, cm^{-1}): 3451 (br, 2OH), 3230, 3182 (2NH), 3061 (CH_{arom}), 2950 (CH_{aliph}), 1698 (CO), 1622 (COO_{asy}), 1581 (COO_{sy}), 811 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 1.10 (t, 3H, J = 7.2 Hz, CH_3), 2.22 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 3.11 (s, 2H, CH_2), 3.73 (q, 1H, J = 7.14 Hz, CH–COOH), 4.02 (q, 2H, J = 7.2 Hz, CH_2), 5.38 (s, 1H, $CH_{pyrimidine}$), 6.93–7.65 (m, 12H, 2ArH), 8.03 (s, 1H, NH, exchangeable by D_2O), 9.12 (s, 1H, OH exchangeable by D_2O), 10.23 (s, 1H, CH_{indole}, exchangeable by D_2O), 12.25 (br, 1H, OH, exchangeable by D_2O). ^{13}CMR (DMSO-d_6), δ ppm: 14.21, 22.58, 28.46, 36.48, 41.81, 53.12, 57.66, 107.86, 117.40, 118.62, 121.64, 122.15, 122.24, 123.02, 123.40, 124.61, 125.41, 127.25, 128.20, 133.32, 134.25, 135.70, 136.21, 138.22, 141.18, 144.30, 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(2*H*,5*H*,6*H*)-yl)propanoic acid derivatives 4–7

General procedures. A mixture of 2-(3-amino-5-(2-hydroxyphenyl)-1*H*-1,2,4-triazol-4(5*H*)-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) *vis.* 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 was 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-(2-hydroxyphenyl)-2,8,8-trimethyl-6-oxo-1H-[1,3]dioxino[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. calcd for ($C_{25}H_{25}ClN_4O_6$, 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 (2OH), 3199 (NH), 3067 ($CH_{aromatic}$), 2978 ($CH_{aliphatic}$), 1675 ($C{=}O_{meldurium}$), 1616 (COO_{asy}), 1590 (COO_{sy}), 816 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 2.22 (d, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.63 (s, 3H, C–CH_3), 2.65 (s, 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 D_2O), 11.50 (s, 1H, NH, exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ ppm: 14.51 (CH_3), 15.57 (CH_3), 18.87 (CH_3), 24.61 (CH_3), 46.23 (CH–COOH), 50.11 ($CH_{pyrimidine}$), 56.54 ($C_{triazole}$), 104.90, 117.38, 119.08, 120.60, 129.36, 131.32, 133.63, 135.17, 141.38, 142.89, 149.31 (m, 2ArC), 153.61 (C=N), 157.74 (C–Cl), 167.95 (C–OH), 178.57 (C=O), 181.09 (C=O).

*2-(5-(4-Chlorophenyl)-2-(2-hydroxyphenyl)-2-methyl-6,8-dioxo-1,2,6,7,8,9-hexahydropyrimido[5,4-e][1,2,4]triazolo[1,5-a]pyrimidin-3(5H)-yl)propanoic acid **5**.* Yield (62%), yellow crystals, mp 218–220 °C, anal. calcd for ($C_{23}H_{21}ClN_6O_5$, 496.91): C, 55.59; H, 4.26; Cl, 7.13; N, 16.91. Found: C, 55.70; H, 4.10; Cl, 7.33; N, 16.75. IR (ν_{max}, cm^{-1}): 3396 (2OH), 3294 (NH), 3238 ($NH_{barbaturic}$), 3206 ($NH_{barbaturic}$), 3024 ($CH_{aromatic}$), 2996 (C–$H_{aliphatic}$), 1707 ($C{=}O_{barbaturic}$), 1643 ($C{=}O_{barbaturic}$), 1621 (COO_{asy}), 1591 (COO_{sy}), 753 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 2.23 (d, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.02 (m, 1H, CH–COOH), 5.87 (s, 1H, $CH_{pyrimidine}$), 6.86 (s, 1H, NH, exchangeable by D_2O), 6.98–7.77 (m, 8H, 2ArH), 7.35 (s, 1H, OH, exchangeable by D_2O), 10.76 (s, 1H, $NH_{barbaturic}$, exchangeable by D_2O), 12.10 (s, 1H, $NH_{barbaturic}$, exchangeable by D_2O), 12.32 (s, 1H, OH exchangeable by D_2O).

*2-(5-(4-Chlorophenyl)-2-(2-hydroxyphenyl)-2-methyl-6-oxo-1,2,6,7,8,9-hexahydro-[1,2,4]triazolo[1,5-a]quinazolin-3(5H)-yl) propanoic acid **6**.* Yield (54%), yellow crystals, mp 273–275 °C, anal. calcd for ($C_{25}H_{25}ClN_4O_4$, 480.94): C, 62.43; H, 5.24; Cl, 7.37; N, 11.65. Found: C, 62.11; H, 5.44; Cl, 7.25; N, 16.87. IR (ν_{max}, cm^{-1}): 3414 (2OH), 3148 (NH), 3048 ($CH_{aromatic}$), 2926 ($CH_{aliphatic}$), 1663 ($C{=}O_{cyclohexanedione}$), 1605 (COO_{asy}), 1592 (COO_{sy}), 750 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 1.95 (m, 2H, CH_2(a)), 2.28 (t, 2H, CH_2(b)), 2.34 (s, 3H, CH_3), 2.53 (d, 3H, CH_3), 2.64 (t, 2H, CH_2(c)), 3.12 (m, 1H, CH–COOH), 5.58 (s, 1H, $CH_{pyrimidine}$), 6.97–7.93 (m, 8H, 2ArH), 11.92 (s, 1H, OH exchangeable by D_2O), 12.48 (s, 1H, NH, exchangeable by D_2O), 13.17 (s, 1H, OH exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ ppm: 15.38 (CH_3), 20.31 (CH_2), 21.26 (CH_3), 26.94 (CH_2), 31.12 (CH–COOH), 36.86 (CH_2), 48.61 ($CH_{pyrimidine}$), 62.46 ($C_{triazole}$), 115.61, 117.69, 118.02, 119.92, 128.31, 129.52, 130.34, 133.12, 136.65, 144.00, 149.91 (m, 2ArC), 160.33 (C=N), 165.36 (C–Cl), 168.33 (C–OH), 171.32 (C=O), 196.64 (C=O).

*2-(5-(4-Chlorophenyl)-2-(2-hydroxyphenyl)-2,8,8-trimethyl-6-oxo-1,2,6,7,8,9-hexahydro-[1,2,4]triazolo[1,5-a]quinazolin-3(5H)-yl)propanoic acid **7**.* Yield (58%), pale yellow crystals, mp 257–

259 °C, anal. calcd for ($C_{27}H_{29}ClN_4O_4$, 509): C, 63.71; H, 5.74; Cl, 6.97; N, 11.01. Found: C, 63.55; H, 5.91; Cl, 6.95; N, 11.11. IR (ν_{max}, cm^{-1}): 3316 (2OH), 3177 (NH), 3038 ($CH_{aromatic}$), 2956 ($CH_{aliphatic}$), 1705 ($C{=}O_{dimedone}$), 1627 (COO_{asy}), 1598 (COO_{sy}), 751 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 0.91–1.07 (m, 6H, $2CH_3$), 2.11 (s, 2H, CH_2), 2.38 (d, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.66 (s, 2H, CH_2), 3.47 (m, 1H, CH–COOH), 5.61 (s, 1H, $CH_{pyrimidine}$), 6.95–7.93 (m, 8H, 2ArH), 11.93 (s, 1H, OH exchangeable by D_2O), 12.86 (s, 2H, NH, OH exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ ppm: 15.36 (CH_3), 18.92 (CH_3), 27.03 (CH_2), 28.19 (CH_3), 29.44 (CH_3), 32.24 (CH_2), 47.55 (CH–COOH), 50.75 ($CH_{pyrimidine}$), 53.69 (C–$(CH_3)_2$), 56.53 ($C_{triazole}$), 117.68, 119.48, 119.93, 128.24, 130.04, 131.79, 133.12, 136.66, 149.97 (m, 2ArC), 159.98 (C=N), 163.54 (C–Cl), 168.30 (C–OH), 181.36 (C=O), 196.47 (C=O).

Synthesis of 2-(5-(4-chlorophenyl)-6-cyano-2-(2-hydroxyphenyl)-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)propanoic acid derivatives 8a–d and 9a–d

An equimolar mixture of 2-(3-amino-5-(2-hydroxyphenyl)-1*H*-1,2,4-triazol-4(5*H*)-yl)propanoic acid derivative **1a–d** (0.001 mol), 4-chlorobenzaldehyde (0.14 g, 0.001 mol) and malononitrile or ethyl cyanoacetate (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 for 3–6 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-(5-(4-chlorophenyl)-6-cyano-2-(2-hydroxyphenyl)-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)propanoic acid derivatives **8a–d** and **9a–d** respectively.

2-(5-(4-Chlorophenyl)-6-cyano-2-(2-hydroxyphenyl)-7-imino-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)propanoic acid 8a. Yield (54%), pale brown crystals, mp 170–172 °C, anal. calcd for ($C_{21}H_{17}ClN_6O_3$, 436.85): C, 57.74; H, 3.92; Cl, 8.12; N, 19.24. Found: C, 57.20; H, 4.42; Cl, 8.03; N, 19.34. IR (ν_{max}, cm^{-1}): 3442 (OH), 3318 ($NH_{pyrimidine}$), 3175 (NH), 3032 ($CH_{aromatic}$), 2990 ($CH_{aliphatic}$), 2222 (C≡N), 1617 (COO_{asy}), 1539 (COO_{sy}), 752 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 1.59 (d, 3H, CH_3), 3.48 (q, 1H, CH–COOH), 4.44 (s, 1H, $CH_{triazole}$), 5.36 (s, 1H, $CH_{pyrimidine}$), 6.75 (s, 2H, NH_2, exchangeable by D_2O), 6.89–7.92 (m, 8H, 2ArH), 8.05 (s, 1H, OH, exchangeable by D_2O), 8.40 (s, 1H, NH, exchangeable by D_2O), 10.02 (s, 1H, OH, exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ ppm: 19.91 (CH_3), 44.55 (CH–COOH), 53.53 ($CH_{triazole}$), 80.99 (C–CN), 111.44 (C≡N), 116.86, 119.00, 120.84, 121.87, 127.20, 129.14, 131.46, 131.94, 133.98, (m, ArC), 140.81 (C=N), 157.92 (C–Cl), 167.14 (C–OH), 177.96 (C=NH), 178.20 (C=O).

2-(5-(4-Chlorophenyl)-6-cyano-2-(2-hydroxyphenyl)-7-imino-2-methyl-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl) propanoic acid 8b. Yield (46%), orange crystals, mp 102–104 °C, anal. calcd for ($C_{22}H_{19}ClN_6O_3$, 450.88): C, 58.60; H, 4.25; Cl, 7.86; N, 18.64. Found: C, 58.20; H, 4.72; Cl, 7.73; N, 18.61. IR (ν_{max}, cm^{-1}): 3409 (2OH), 3290 ($NH_{pyrimidine}$), 3148 (NH), 3032 ($CH_{aromatic}$), 2956 ($CH_{aliphatic}$), 2227 (C≡N), 1618 (COO_{asy}), 1589 (COO_{sy}), 766 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 2.54 (d, 3H, CH_3), 2.65 (s, 3H, CH_3), 3.35 (q, 1H, CH–COOH), 6.86 (s, 2H, NH_2, exchangeable by D_2O), 6.95–8.24 (m, 8H, 2ArH), 8.08 (s, 1H, OH, exchangeable by D_2O), 11.49 (s, 1H, NH, exchangeable by D_2O), 11.98 (s, 1H, OH, exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ ppm: 20.61 (CH_3), 28.03 (CH_3), 46.48 (CH–COOH), 63.12 ($C_{triazole}$), 78.17 (C–CN), 118.02 (C≡N), 119.63, 127.76, 129.16, 129.41, 130.48, 131.19, 131.89, 133.67, 134.69, (m, ArC), 136.74 (C=N), 141.30 (C–Cl), 159.45 (C–OH), 161.28 (C=NH), 178.5 (C=O). MS (*m*/*z*, I%): 452 (M + 2, 0.15%); 450 (0.05), 405 (0.27), 375 (0.22), 363 (0.53), 347 (0.42), 317 (0.89), 259 (4.13), 189 (6.36), 113 (24.84), 87 (19.42), 59 (100).

2-(5-(4-Chlorophenyl)-6-cyano-2-(2-hydroxyphenyl)-7-imino-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)-3-(1*H*-indol-3-yl)propanoic acid 8c. Yield (67%), yellow crystals, mp 118–120 °C, anal. calcd for ($C_{29}H_{22}ClN_7O_3$, 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 (ν_{max}, cm^{-1}): 3430 (OH), 3400 (OH), 3312 ($NH_{pyrimidine}$), 3230 (NH), 3162 (NH_{indole}), 3065 ($CH_{aromatic}$), 2958 ($CH_{aliphatic}$), 2201 (C≡N), 1621 (COO_{asy}), 1578 (COO_{sy}), 765 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 3.12 (t, 1H, CH–COOH), 3.35, 3.58 (dd, 2H, CH_2), 5.25 (s, 1H, $CH_{triazole}$), 6.72 (s, 2H, NH_2, exchangeable by D_2O), 6.80–8.12 (m, 13H, 3ArH), 8.40 (s, 1H, OH, exchangeable by D_2O), 10.22 (s, 1H, NH, exchangeable by D_2O), 10.48 (s, 1H, $NH_{pyrimidine}$, exchangeable by D_2O), 12.80 (s, 1H, OH, exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ ppm: 34.81 (CH_2), 53.87 (CH–COOH), 66.75 ($CH_{triazole}$), 77.55 (C–CN), 107.77 (C≡N), 117.30 (CH_{indole}), 119.32, 119.50, 119.84, 121.60, 123.60, 123.96, 124.84, 128.31, 128.95, 129.12, 129.43, 130.05, 131.38, 131.50, 133.71 (ArC), 141.20 (C=N), 153.76 (C–NH), 157.18 (C–Cl), 165.89 (C–OH), 168.50 (C=NH), 175.45 (C=O).

2-(5-(4-Chlorophenyl)-6-cyano-2-(2-hydroxyphenyl)-7-imino-2-methyl-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)-3-(1*H*-indol-3-yl)propanoic acid 8d. Yield (67%), yellow crystals, mp 130 °C, anal. calcd for ($C_{30}H_{24}ClN_7O_3$, 566.01): C, 63.66; H, 4.27; Cl, 6.26; N, 17.32. Found: C, 63.01; H, 4.42; Cl, 6.59; N, 17.41. IR (ν_{max}, cm^{-1}): 3431 (OH), 3408 (OH), 3310 ($NH_{pyrimidine}$), 3238 (NH), 3166 (NH_{indole}), 3080 ($CH_{aromatic}$), 2975 ($CH_{aliphatic}$), 2189 (C≡N), 1616 (COO_{asy}), 1579 (COO_{sy}), 765 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 2.33 (s, 3H, CH_3), 3.10 (t, 1H, CH–COOH), 3.35, 3.58 (dd, 2H, CH_2), 6.76 (s, 2H, NH_2, exchangeable by D_2O), 6.84–8.15 (m, 13H, 3ArH), 8.41 (s, 1H, OH, exchangeable by D_2O), 10.20 (s, 1H, NH, exchangeable by D_2O), 10.54 (s, 1H, $NH_{pyrimidine}$, exchangeable by D_2O), 12.86 (s, 1H, OH, exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ ppm: 20.68 (CH_3), 34.80 (CH_2), 53.84 (CH–COOH), 66.76 ($CH_{triazole}$), 77.52 (C–CN), 114.70 (C≡N), 117.32 (CH_{indole}), 119.28, 119.54, 119.81, 121.63, 123.63, 123.96, 124.86, 128.30, 128.96, 129.10, 129.23, 130.03, 131.32, 131.55, 133.73 (m, ArC), 141.20 (C=N), 153.74 (C–NH), 157.11 (C–Cl), 165.14 (C–OH), 168.32 (C=NH), 175.65 (C=O). MS (*m*/*z*, I%): 568 (M + 2, 0.13), 566 (0.51), 553 (0.44), 405 (0.38), 370 (0.48), 326 (0.43), 298 (0.25), 204.20 (1.86), 194 (0.61), 158.20 (5.11), 130.15 (100), 103.10 (9.99), 77.05 (13.13).

2-(5-(4-Chlorophenyl)-6-cyano-2-(2-hydroxyphenyl)-7-oxo-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)propanoic acid 9a. Yield (52%), pale yellow crystals, mp 158–160 °C, anal. calcd for ($C_{21}H_{16}ClN_5O_4$, 437.93): C, 57.55; H, 3.65; Cl, 8.10; N, 15.98. Found: C, 57.28; H, 3.42; Cl, 8.00; N, 18.64. IR (ν_{max}, cm^{-1}): 3442 (OH), 3170 (NH), 3040 ($CH_{aromatic}$), 2956 ($CH_{aliphatic}$), 2201 (C≡N), 1676 (CO), 1622 (COO_{asy}), 1548 (COO_{sy}), 750 (C–Cl). ^{1}H-

NMR (DMSO-d_6), δ 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_2O), 10.02 (s, 1H, OH, exchangeable by D_2O), 12.22 (s, 1H, OH, exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ ppm: 19.91 (CH_3), 44.55 (CH–COOH), 53.53 ($CH_{triazole}$), 107.44 (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=N), 157.92 (C–Cl), 167.14 (C–OH), 177.96 (C=NH), 178.20 (C=O).

2-(5-(4-Chlorophenyl)-6-cyano-2-(2-hydroxyphenyl)-2-methyl-7-oxo-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)propanoic acid 9b. Yield (64%), yellow crystals, mp 170–172 °C, anal. calcd for ($C_{22}H_{18}ClN_5O_4$, 451.86): C, 58.42; H, 3.98; Cl, 7.85; N, 15.49. Found: C, 58.25; H, 3.76; Cl, 7.68; N, 15.30. IR (ν_{max}, cm^{-1}): 3450 (OH), 3182 (NH), 3056 ($CH_{aromatic}$), 2949 ($CH_{aliphatic}$), 2218 (C≡N), 1675 (CO), 1620 (COO_{asy}), 1553 (COO_{sy}), 757 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ 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_2O), 9.24 (s, 1H, OH, exchangeable by D_2O), 12.18 (s, 1H, OH, exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ ppm: 19.87 (CH_3), 18.12, 44.23 (CH–COOH), 80.65 ($C_{triazole}$), 107.44 (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=N), 153.96 (C=NH), 157.92 (C–Cl), 167.14 (C–OH), 178.15 (C=O).

2-(5-(4-Chlorophenyl)-6-cyano-2-(2-hydroxyphenyl)-7-oxo-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)-3-(1*H*-indol-3-yl)propanoic acid 9c. Yield (75%), yellow needles, mp 188–190 °C, anal. calcd for ($C_{29}H_{21}ClN_6O_4$, 552.97): C, 62.93; H, 3.79; Cl, 6.41; N, 15.97. Found: C, 62.71; H, 3.52; Cl, 6.41; N, 15.01. IR (ν_{max}, cm^{-1}): 3430 (OH), 3405 (OH), 3230 (NH), 3176 (NH_{indole}), 3060 ($CH_{aromatic}$), 2952 ($CH_{aliphatic}$), 2206 (C≡N), 1623 (COO_{asy}), 1578 (COO_{sy}), 765 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ 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_2O), 6.80–8.12 (m, 13H, 3ArH), 8.40 (s, 1H, OH, exchangeable by D_2O), 10.25 (s, 1H, NH, exchangeable by D_2O), 12.23 (s, 1H, OH, exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ ppm: 34.80, 53.83, 66.75, 107.77, 117.30, 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, 133.71, 141.20, 153.76, 157.16, 165.81, 168.33, 178.41.

2-(5-(4-Chlorophenyl)-6-cyano-2-(2-hydroxyphenyl)-2-methyl-7-oxo-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)-3-(1*H*-indol-3-yl)propanoic acid 9d. Yield (72%), brown needles, mp 202–205 °C, anal. calcd for ($C_{30}H_{23}ClN_6O_4$, 566.99): C, 63.49; H, 4.05; Cl, 6.26; N, 14.81. Found: C, 63.33; H, 3.92; Cl, 6.01; N, 14.55. IR (ν_{max}, cm^{-1}): 3436 (2OH), 3236 (NH), 3180 (NH_{indole}), 3059 ($CH_{aromatic}$), 2948 ($CH_{aliphatic}$), 2212 (C≡N), 1625 (COO_{asy}), 1575 (COO_{sy}), 771 (C–Cl). ^{1}H-NMR (DMSO-d_6), δ ppm: 2.23 (s, 3H, CH_3), 3.13 (t, 1H, CH–COOH), 3.58 (d, 2H, CH_2), 6.75 (s, 1H, NH, exchangeable by D_2O), 6.83–8.12 (m, 13H, 3ArH), 8.38 (s, 1H, OH, exchangeable by D_2O), 10.23 (s, 1H, NH, exchangeable by D_2O), 12.25 (s, 1H, OH, exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ 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, 133.72, 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)-1*H*-1,2,4-triazol-4(5*H*)-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-(2-hydroxyphenyl)-7-imino-5-(methylthio)-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)propanoic acid derivatives **10a–d** respectively.

2-(6-Cyano-2-(2-hydroxyphenyl)-7-imino-5-(methylthio)-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)propanoic acid 10a. Yield (55%), yellow crystals, mp 186 °C, anal. calcd for ($C_{16}H_{16}N_6O_3S$, 372.40): C, 51.55; H, 4.29; N, 22.55; S, 8.59. Found: C, 51.28; H, 4.08; N, 22.32; S, 8.40%. IR (ν_{max}, cm^{-1}): 3412 (OH), 3265 ($NH_{pyrimidine}$), 3142 (NH), 3054 ($CH_{aromatic}$), 2945 ($CH_{aliphatic}$), 2112 (C≡N), 1625 (COO_{asy}), 1581 (COO_{sy}). ^{1}H-NMR (DMSO-d_6), δ ppm: 2.23 (d, 3H, CH_3), 2.58 (s, 3H, S–CH_3), 3.45 (q, 1H, CH–COOH), 5.25 (s, 1H, $CH_{triazole}$), 6.78 (s, 1H, NH exchangeable by D_2O), 7.34–7.56 (m, 4H, ArH), 7.96 (s, 1H, NH, exchangeable by D_2O), 8.04 (s, 1H, OH, exchangeable by D_2O), 12.22 (s, 1H, OH, exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ ppm: 15.35, 18.90, 46.76, 57.70, 66.95, 117.45, 119.11, 119.43, 128.25, 128.76, 130.13, 130.99, 131.48, 152.55, 157.02, 168.43, 181.05.

2-(6-Cyano-2-(2-hydroxyphenyl)-7-imino-2-methyl-5-(methylthio)-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)propanoic acid 10b. Yield (53%), orange crystals, mp 186 °C, anal. calcd for ($C_{17}H_{18}N_6O_3S$, 386.43): C, 52.84; H, 4.70; N, 21.75; S, 8.30. Found: C, 52.38; H, 4.90; N, 21.81; S, 8.42%. IR (ν_{max}, cm^{-1}): 3403 (OH), 3284 ($NH_{pyrimidine}$), 3137 (NH), 3048 ($CH_{aromatic}$), 2953 ($CH_{aliphatic}$), 2108 (C≡N), 1605 (COO_{asy}), 1587 (COO_{sy}). ^{1}H-NMR (DMSO-d_6), δ ppm: 2.23 (d, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.53 (s, 3H, S–CH_3), 3.47 (m, 1H, CH–COOH), 6.84 (s, 1H, NH exchangeable by D_2O), 7.32–7.54 (m, 4H, ArH), 7.88 (s, 1H, NH, exchangeable by D_2O), 8.06 (s, 1H, OH, exchangeable by D_2O), 12.23 (s, 1H, OH, exchangeable by D_2O). ^{13}C NMR (DMSO-d_6), δ ppm: 15.38, 18.96, 24.53, 46.80, 57.72, 66.99, 117.40, 119.08, 119.47, 128.33, 128.95, 130.04, 130.98, 131.48, 152.93, 157.72, 168.29, 180.98.

2-(6-Cyano-2-(2-hydroxyphenyl)-7-imino-5-(methylthio)-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)-3-(1*H*-indol-3-yl)propanoic acid 10c. Yield (65%), brown needles, mp 226–228 °C, anal. calcd for ($C_{24}H_{21}N_7O_3S$, 487.53): C, 59.07; H, 4.30; N, 20.10; S, 6.65. Found: C, 58.86; H, 4.08; N, 19.87; S, 6.43. IR (ν_{max}, cm^{-1}): 3415 (2OH), 3284 ($NH_{pyrimidine}$), 3189, 3137 (2NH), 3056 ($CH_{aromatic}$), 2953 ($CH_{aliphatic}$), 2112 (C≡N), 1625 (COO_{asy}), 1587 (COO_{sy}). ^{1}H-NMR (DMSO-d_6), δ ppm: 2.53 (s, 3H, S–CH_3), 3.47 (m, 1H, CH–COOH), 3.57 (d, 3H, CH_2), 5.25 (s, 1H, $CH_{triazole}$), 6.85 (s, 1H, NH exchangeable by D_2O), 7.32–7.54 (m, 8H, ArH), 8.04 (s, 1H, NH, exchangeable by D_2O), 9.23 (s, 1H, OH, exchangeable by D_2O), 12.21 (s, 1H, OH, exchangeable by D_2O).

2-(6-Cyano-2-(2-hydroxyphenyl)-7-imino-2-methyl-5-(methylthio)-1,2-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-3(7*H*)-yl)-3-(1*H*-indol-3-yl)propanoic acid 10d. Yield (65%), orange crystals, mp 235–237 °C, anal. calcd for ($C_{25}H_{23}N_7O_3S$, 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 (2OH), 3280 ($NH_{pyrimidine}$), 3188, 3141 (2NH), 3059 ($CH_{aromatic}$), 2956 ($CH_{aliphatic}$), 2111 (C≡N), 1623 (COO_{asy}), 1588 (COO_{sy}). ^{1}H-NMR (DMSO-d_6), δ ppm: 2.58 (s, 3H, S–CH_3), 3.48 (m, 1H, CH–COOH), 3.56 (d, 3H, CH_2), 6.81 (s, 1H, NH exchangeable by D_2O), 7.30–7.62 (m, 8H, ArH), 8.05 (s, 1H, NH, exchangeable by D_2O), 9.20 (s, 1H, OH, exchangeable by D_2O), 12.24 (s, 1H, OH, exchangeable by D_2O).

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 phaseolicola* and *Pseudomonas fluorescens*) for yeast-like fungi *C. albicans*. For all bacteria (nutrient medium), consisting of (g L^{-1} distilled water): peptone, 5 and meat extract, 3. pH was adjusted to 7.0. For solid media, 2% agar was added. All media were sterilized at 121 °C for 20 min.

The antimicrobial screening was performed according to the procedure of "Agar Diffusion Method".[35,37] One mg of each of the newly synthesized compounds was dissolved in dimethyl sulphoxide (DMSO, 1 mL) then made up to 10 mL with sterile water to give a concentration of 100 μg mL^{-1}. A solution of the tested compounds was placed separately in the agar medium. The inhibition zones were measured after 24 h incubation.

Minimal inhibitory concentration (MIC) of active compounds against MDR bacteria. A mixture of 80 μl of sterile Müeller–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 1.5×10^6 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.[36,37]

Determination of minimum bactericidal concentration (MBC) and MIC index of compounds 3c, 8a and 9d. The most active compound against MRSA (**3c** & **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 Müeller–Hinton agar plates and incubated at 35 ± 2 °C for 18 h[38] 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.[39]

***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% confluency 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.

$$\text{Cell viability (\%)} = \text{mean optical density/control optical density} \times 100\%$$

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 **2c**, **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 **3c**, **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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References

1 B. Beović, *Int. J. Food Microbiol.*, 2006, **112**, 280–287.
2 D. T. W. Chu, J. J. Plattner and L. Katz, *J. Med. Chem.*, 1996, **39**, 3853–3874.
3 R. Finch and P. A. Hunter, *J. Antimicrob. Chemother.*, 2006, **58**(Suppl 1), i3–i22.
4 T. D. Gootz, *Crit. Rev. Immunol.*, 2010, **30**, 79–93.
5 D. Niccolai, L. Tarsi and R. J. Thomas, *Chem. Commun.*, 1997, 2333–2342, DOI: 10.1039/a704497f.
6 K. M. Overbye and J. F. Barrett, *Drug Discovery Today*, 2005, **10**, 45–52.
7 N. Suree, M. E. Jung and R. T. Clubb, *Mini-Rev. Med. Chem.*, 2007, **7**, 991–1000.
8 C. Walsh, *Nature*, 2000, **406**, 775–781.
9 M. M. Cowan, *Clin. Microbiol. Rev.*, 1999, **12**, 564–582.
10 V. Cattoir and B. Felden, *J. Infect. Dis.*, 2019, **220**, 350–360.
11 E. D. Brown and G. D. Wright, *Nature*, 2016, **529**, 336–343.
12 A. Travis, O. Chernova, V. Chernov and R. Aminov, *Expert Opin. Drug Discovery*, 2018, **13**, 983–985.
13 F. Gao, T. Wang, J. Xiao and G. Huang, *Eur. J. Med. Chem.*, 2019, **173**, 274–281.
14 S. A. Shahzad, M. Yar, Z. A. Khan, L. Shahzadi, S. A. R. Naqvi, A. Mahmood, S. Ullah, A. J. Shaikh, T. A. Sherazi, A. T. Bale, J. Kukułowicz and M. Bajda, *Bioorg. Chem.*, 2019, **85**, 209–220.
15 H. A. M. El-Sherief, B. G. M. Youssif, S. N. Abbas Bukhari, A. H. Abdelazeem, M. Abdel-Aziz and H. M. Abdel-Rahman, *Eur. J. Med. Chem.*, 2018, **156**, 774–789.
16 İ. Küçükgüzel, E. Tatar, Ş. G. Küçükgüzel, S. Rollas and E. De Clercq, *Eur. J. Med. Chem.*, 2008, **43**, 381–392.
17 S. Zhang, Z. Xu, C. Gao, Q.-C. Ren, L. Chang, Z.-S. Lv and L.-S. Feng, *Eur. J. Med. Chem.*, 2017, **138**, 501–513.
18 Z. Xu, S. Zhang, C. Gao, J. Fan, F. Zhao, Z.-S. Lv and L.-S. Feng, *Chin. Chem. Lett.*, 2017, **28**, 159–167.
19 J. Xu, Y. Cao, J. Zhang, S. Yu, Y. Zou, X. Chai, Q. Wu, D. Zhang, Y. Jiang and Q. Sun, *Eur. J. Med. Chem.*, 2011, **46**, 3142–3148.
20 A. M. El-Saghier, M. A. Mohamed, O. A. Abd-Allah, A. M. Kadry, T. M. Ibrahim and A. A. Bekhit, *Med. Chem. Res.*, 2019, **28**, 169–181.
21 S. Eswaran, A. V. Adhikari and N. S. Shetty, *Eur. J. Med. Chem.*, 2009, **44**, 4637–4647.
22 H. Wang, M. Lee, Z. Peng, B. Blázquez, E. Lastochkin, M. Kumarasiri, R. Bouley, M. Chang and S. Mobashery, *J. Med. Chem.*, 2015, **58**, 4194–4203.
23 M. A. T. Blaskovich, *J. Med. Chem.*, 2016, **59**, 10807–10836.
24 A. Cosquer, M. Ficamos, M. Jebbar, J.-C. Corbel, G. Choquet, C. Fontenelle, P. Uriac and T. Bernard, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 2061–2065.
25 S. Mishra, U. Narain, R. Mishra and K. Misra, *Bioorg. Med. Chem.*, 2005, **13**, 1477–1486.
26 E. J. Corey, L. Kürti and B. Czakó, *Molecules and Medicine*, Wiley & Sons, 2008, p. 272, ISBN: 978-0-470-26096-8.
27 M. K. Khera, I. A. Cliffe, T. Mathur and O. Prakash, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 2887–2889.
28 T. Krishnaraj and S. Muthusubramanian, *J. Heterocycl. Chem.*, 2014, **51**, E68–E70.
29 R. Kumar, R. R. Nair, S. S. Dhiman, J. Sharma and O. Prakash, *Eur. J. Med. Chem.*, 2009, **44**, 2260–2264.
30 O. Prakash, V. Bhardwaj, R. Kumar, P. Tyagi and K. R. Aneja, *Eur. J. Med. Chem.*, 2004, **39**, 1073–1077.
31 R. H. Abd El-Aleam, R. F. George, G. S. Hassan and H. M. Abdel-Rahman, *Bioorg. Chem.*, 2020, **94**, 103411.
32 A. A. Bekhit, A. M. Farghaly, R. M. Shafik, M. M. A. Elsemary, A. E.-D. A. Bekhit, A. A. Guemei, M. S. El-Shoukrofy and T. M. Ibrahim, *Bioorg. Chem.*, 2018, **77**, 38–46.
33 L. Zaharani, N. G. Khaligh, T. Mihankhah, Z. Shahnavaz and M. R. Johan, *Synth. Commun.*, 2020, **50**, 1633–1640.
34 L. P. Garrod and P. M. Waterworth, *J. Clin. Pathol.*, 1971, **24**, 779–789.
35 A. O. Abdelhamid, I. E. El Sayed, M. Z. Hussein and M. M. Mangoud, *Molecules*, 2016, 21.
36 N. Nina, C. Quispe, F. Jiménez-Aspee, C. Theoduloz, G. E. Feresín, B. Lima, E. Leiva and G. Schmeda-Hirschmann, *Molecules*, 2015, **20**, 18144–18167.
37 D. R. Ramadan, A. A. Elbardan, A. A. Bekhit, A. El-Faham and S. N. Khattab, *New J. Chem.*, 2018, **42**, 10676–10688.
38 G. Chamandi, Z. Olama and H. Holail, *Int. J. Curr. Microbiol. Appl. Sci.*, 2015, **4**, 328–342.
39 A. R. L. L. A. Tukappa, *Am. J. Drug Discovery Dev.*, 2013, **3**, 72–83.
40 T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55–63.