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ORIGINAL RESEARCH

# Synthesis, antibacterial evaluation and molecular docking studies of novel series of acridone- 1,2,3-triazole derivatives

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## Abstract

Development of new drugs with antibacterial potency is an important solution to overcome drug-resistance problems. In the goal to develop novel structure with antibacterial potency, we designed and synthesized novel acridone derivatives bearing triazole nucleus. The novel synthesized compounds were tested for their in vitro antibacterial activity against four bacterial human pathogenic strains. The compound 4h displayed significant antibacterial activities against *Staphylcoccus aureus* (MRSA) with MIC = 19.6 μg/mL. The synthesized compounds were subjected for docking study to understand the interaction of our compounds and the dihydropteroate synthase (DHPS) in methicillin-resistant *Staphylcoccus aureus* (MRSA).

**Keywords** Synthesis · Acridone · Triazole · Antibacterial activity · Docking

## Introduction

Antibiotic resistance is currently considered as major public health problem [1]. Therefore, the development of new compounds with antimicrobial activity is important errands of this century. One of the researched therapeutic targets in the field of antibacterial discovery is dihydrofolate reductase (DHFR), an essential enzyme whose role is to regenerate folic acid into its reduced form tetrahydrofolate; this enzyme represents an attractive antibacterial target [2].

Triazole is an important heterocyclic skeleton with large biological activity. 1,2,3-triazoles are important class in the triazoles series [3]; these compounds continue to be attractive for synthesis since they possess diverse pharmacological properties such as antifungal [4], antibacterial [5], anti-malarial [6], antiviral [7], antitumoral [8], and anti-inflammatory [9] activities. Moreover, the synthesis of 1,2,3-triazole via click chemistry by 1,3-dipolar cycloaddition of substituted azides and alkynes in the presence of Cu(I) gives regioselective 1,4-disubstituted 1,2,3-triazole with good yields.

On the other hand, acridone and acridine derivatives are known for many years for their biological activities such as antitumor [10], antiviral [11], anti-inflammatory [12], antimalarial [13], and antimicrobial bioactivity [14] . Numerous acridone compounds that have antibacterial activity have been developed, including some heterocyclic such as triazole, thiazole, and 1,3,4-oxadiazole nucleus [15–17] . Several methods are reported for the preparation of the acridone ring, among them are Ullmann reaction which was considered as the most efficient [18]; it consist the condensation of o-halobenzoic acid with aromatic amine in the presence of Cu followed by intramolecular cyclization using sulfuric acid.

In a view of the significant bio-potential of 1,2,3-triazole and acridone nucleus and in order to develop novel bioactive therapeutic agents, we focused our work on the preparation and evaluation of novel acridone bearing 1,2,3-triazole nucleus as antibacterial agents against four bacteria. The synthesized compounds had led to some important structure with interesting antibacterial activity.

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s11224-020-01512-0) contains supplementary material, which is available to authorized users.

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## Materials and methods

### Materials

All materials were purchased from commercial suppliers. IR spectra were recorded using JASCO FT-IR 4100 spectrophotometer. Mass spectrometric measurements were recorded using SHIMADZU 8040 LC/MS/MS. The $^{1}H$, $^{13}C$ NMR spectra was recorded with Bruker Avance 300 at 25 °C.

### Determination of minimum inhibitory concentration

The synthesized compounds were tested for their antibacterial activity by the disk diffusion method; the active compounds were subjected to the determination of the MIC, using the broth microdilution method. The microorganisms utilized for the test were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas putida*, and *Klebsiella pneumonia*. They were collected from clinical isolates. Bacterial inoculums were prepared by subculturing microorganisms into MHB at 37 °C for 18 h and were diluted to approximately $10^6$ CFU $mL^{-1}$. Initial solution with concentration 0.5 mg/mL of the compounds (**4a-h**) were prepared in DMF; further serial dilutions were made in the microplates, and 100 µl of MHB containing each test microorganism were added to the microplate [19–21], then incubated at 36 °C for 24 h. After incubation, 20 µL of TTC (0.04 mg/mL) were added to each microplate. The color changes of TTC from colorless to red were accepted as microbial growth [22]. A negative control was prepared using the inoculum and DMF without derivative, whereas chloramphenicol was used as positive control.

### Molecular docking studies

Molecular docking of the compounds (**4a-h**) into the *S. aureus* DHFR complex structure (PDB code, 2W9S) [23] was carried out using Molecular Operating Environment (MOE-Dock 2015.10) software [24]. The Discovery Studio (version 4.5) was used for graphical visualization. The structure of 2W9S in docking study was downloaded from Protein Data Bank. The 3D structures of compounds (**4a-h**) were constructed using Chem ultra 12.0 software and were energetically minimized, then the 3D structure of compounds (**4a-h**) was placed in database. For protein preparation, the hydrogen atoms were added; water and impurities were removed. Different types of interactions between protein and the docked compounds (**4a-h**) were analyzed using Discovery Studio.

### Synthesis

#### Synthesis of acridone from 2-(phenylamino)benzoic acid

A mixture of aniline (0.65 g, 7 mmol), *o*-bromobenzoic acid (1 g, 5 mmol), anhydrous potassium carbonate (0.82 g, 6 mmol), 0.1 g of copper powder, and 0.04 g of copper oxide was refluxed for 4 h in 15 mL of amyl alcohol. The amyl alcohol was evaporated then poured into (100 mL) hot water, cooled to room temperature, acidification with HCl lead to ***1*** as a white precipitate which was then recrystallized from ethanol. The N-phenylanthranilic acid (1 g, 4.7 mmol) was taken in 4 mL of concentrated sulfuric acid and heated on water bath for 3 h. Reaction mixture was added to hot water and the resulting precipitates were filtered to get acridone. The sample of acridone 2 was recrystallized from acetic acid. Yield 70%, $R_f$ 0.71, mp > 330 C. IR (KBr) 3275 (N–H), 3084 (C–H aromatic), 1640 (C=O), 1570 (C=C). $^{1}H$ NMR (300 MHz, [$D_6$]DMSO, 25 °C, TMS): δ 11.74 (s, 1H, NH), 8.23 (dd, J = 8.1, 1.2 Hz, 2H, $H_1$-$H_8$), 7.70 (td, J = 8.4, 1.5 Hz, 2H, $H_3$-$H_6$), 7.53 (d, J = 8.1 Hz, 2H, $H_4$-$H_5$), 7.23 (td, J = 8.4, 1.5 Hz, 2H, $H_2$-$H_7$). $^{13}C$ NMR (75 MHz, [$D_6$]DMSO, 25 °C, TMS) δ: 177.29, 141.35, 133.94, 126.46, 121.49, 120.91, 117.80.

### Synthesis of 10-(prop-2-yn-1-yl)acridone (3)

To a mixture of acridone **2** (0.5 g, 2.5 mmol), sodium hydride (45 mg, 3.6 mmol) and TBAB (0.5 g, 2 mmol) in DMF (3 mL), propargyl bromide (0.38 g, 3.6 mmol) was added and the mixture was stirred at 85 °C for 3 h. After which, it was poured into crushed ice and the white yellow formed precipitate was recrystallized from ethanol-DMF.

White solid; yield 74%, $R_f$ 0.84, mp = 178 °C. IR (KBr): 3206, 3008, 2913, 1639, 1600, 1495 $cm^{-1}$. $^{1}H$ NMR (300 MHz, [$D_6$]DMSO, 25 °C, TMS): δ 8.34 (d, 2H, H1-H8), 7.85–7.81 (m, 4H, H3-H6-H4-H5), 7.38–7.32 (m, 2H, H2-H7), 5.34 (s, 2H, CH2), 2.48 (s, 1H, CH); $^{13}C$ NMR (75 MHz, [$D_6$]DMSO, 25 °C, TMS) δ 177.08, 141.70, 134.76, 134.46, 127.18, 127.00, 122.37, 122.20, 117.44, 116.35, 79.12, 76.16, 36.

### General procedure for the synthesis of acridon-1,2,3-triazole derivatives (4a-h)

A mixture of 10-(prop-2-yn-1-yl)acridone (0.1 g, 0.42 mmol), azide (0.63 mmol), copper sulfate (21 mg, 0.081 mmol), and sodium ascorbate (33 mg, 0.16 mmol) in DMF (5 mL) was stirred at room temperature for 12 h. Then the mixture was diluted with water, poured onto ice, and the precipitate was filtered off, washed with cold water, and purified by flash chromatography on silica gel using hexane/diethyl ether (1:4) to afford desire product.

**Ethyl 2-{4-[(9-oxoacridin-10-yl)methyl] -1,2,3-triazol-1-yl}acetate (4a)**

Yellow solid; yield 80%, $R_f$ 0.73 (hexane:diethyl ether = 1:6), mp > 300 °C. IR (KBr): 3132, 2986, 2914, 1745, 1634, 1600, 1493, 1227 $cm^{-1}$. $^1H$ NMR (300 MHz, [$D_6$]DMSO, 25 °C, TMS): δ 8.36 (dd, J = 8.1, 1.5 Hz,2H, H1-H8), 8.10 (s, 1H, triazole), 7,95 (d, J = 8,7 Hz, 2H, H4, H5), 7.80 (td, J = 15.6, 6.9 1.5 Hz, 2H, H3, H6), 7.33 (t, J = 7.5 Hz, 2H, H2,H7), 5.83 (s, 2H, CH2), 5.31(s, 2H, CH2), 4,11 (q, J = 7.2 Hz, 2H, CH2), 1,13 (t, J = 7.2 Hz, 3H,CH3). $^{13}C$ NMR (75 MHz, [$D_6$]DMSO, 25 °C, TMS): 177.07, 167.53, 143.01, 142.23, 134.65, 127.13, 125.25, 122.20, 122.00, 116.74, 61.89, 50.86, 41.94, 14.34. MS (ESI) for $C_{20}H_{18}N_4O_3$ $[M + 1]^+$, calcd 363,13, found 363.14.

**{4-[(9-oxoacridin-10-yl)methyl]-1,2,3-triazol-1-yl}acetic acid (4b)**

Yellow solid; yield 82%, $R_f$ 0.62 (hexane:diethyl ether = 1:4), mp > 300 °C. IR (KBr): 3400, 3120, 2970, 1700, 1635, 1601, 1497, 1236 $cm^{-1}$. $^1H$ NMR (300 MHz, [$D_6$]DMSO, 25 °C, TMS): 10.82 (s, 1H, OH), 8.36 (dd, J = 8.0, 1.1 Hz,2H, H1-H8), 8.11 (s, 1H, triazole), 7,96 (d, J = 8,7 Hz, 2H, H4, H5), 7.80 (td, J = 15.6, 6.9 1.8 Hz, 2H, H3, H6), 7.33 (t, J = 8.1 Hz, 2H, H2,H7), 5.81 (s, 2H, CH2), 5.19(s, 2H, CH2). $^{13}C$ NMR (75 MHz, [$D_6$]DMSO, 25 °C, TMS): 177.08, 168.91, 142.79, 142.23, 134.66, 127.12, 125.21, 122.16, 122.00, 116.78, 51.08, 41.93. MS (ESI) for $C_{18}H_{14}N_4O_3$ $[M + 1]^+$, calcd 335.10, found 335.12.

**10-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one (4c)**

White solid; yield: 82%, $R_f$ 0.69 (hexane:diethyl ether = 1:4), mp > 300 °C. IR (KBr): 3111, 1638, 1614, 1593, 1502, 1255 $cm^{-1}$. $^1H$ NMR (300 MHz, [$D_6$]DMSO, 25 °C, TMS): 8,71(s, 1H, triazole), 8.42 (d, J = 7.2 Hz,2H, H1-H8), 7,98 (d, J = 8,1 Hz, 2H, H4, H5), 7.81–7.62 (m, 4H, H3, H6, H1',H4'), 7.35 (t, J = 7.2 Hz, 5H, H2,H7,H2',H3',H4'), 5.87 (s, 2H, CH2). $^{13}C$ NMR (75 MHz, [$D_6$]DMSO, 25 °C, TMS):177.12, 144.07, 142.57, 138.75, 134.61, 130.85, 127.24, 122.32, 121.91, 120.42, 116.44, 42.49. MS (ESI) for $C_{22}H_{16}N_4O$ $[M + 1]^+$, calcd 353.13, found 353.12.

**10-{[1-(4-methylphenyl)-1,2,3-triazol-4-yl] methyl}acridin-9-one (4d)**

Yellow solid; yield 90%, $R_f$ 0.70 (hexane:diethyl ether = 1:4), mp > 300 °C. IR (KBr): 3110, 3060, 1637, 1617, 1598, 1504, 1265 $cm^{-1}$. $^1H$ NMR (300 MHz, [$D_6$]DMSO, 25 °C, TMS): 8,72(s, 1H, triazole), 8.40 (d, J = 7.2 Hz,2H, H1-H8), 7,98 (d, J = 8,1 Hz, 2H, H4, H5), 7.82 (t, 2H, H3, H6), 7.73 (d, J = 7.2 Hz, 2H, H1',H4'), 7.36 (d, J = 7.2 Hz, 4H, H2,H7,H2',H3'), 5.87 (s, 2H, CH2), 2.36(s, 3H, CH3). $^{13}C$ NMR (75 MHz, [$D_6$]DMSO, 25 °C, TMS):177.07, 144.10, 142.51, 138.85, 134.61, 130.85, 127.14, 122.42, 121.94, 120.52, 116.74, 42.39, 20.96. MS (ESI) for $C_{23}H_{18}N_4O$ $[M + 1]^+$, calcd 367.17, found 367.15.

**Ethyl 2-{4-[(2-methyl-9-oxoacridin-10-yl)methyl] -1,2,3-triazol-1-yl}acetate (4e)**

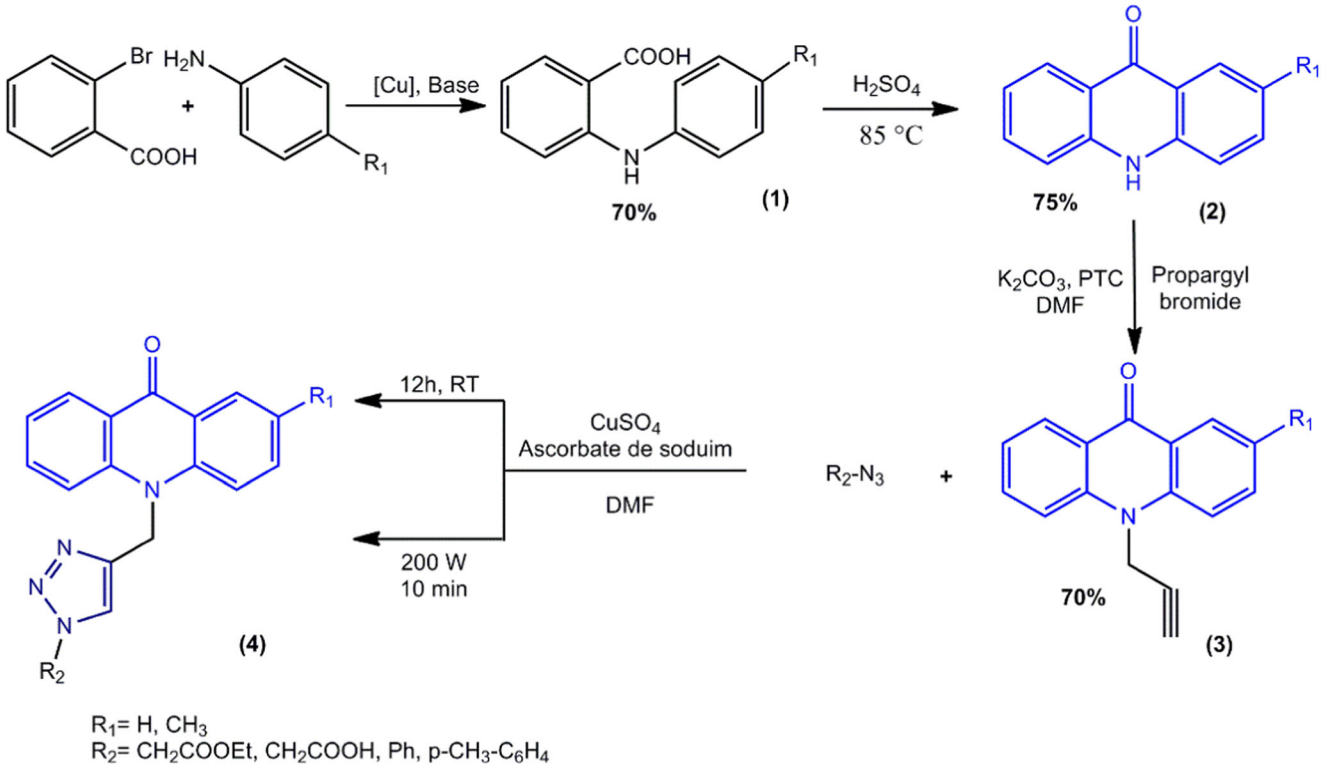
Yellow solid; yield: 84%, $R_f$ 0.77 (hexane:diethyl ether = 1:6), mp > 300 °C. IR (KBr): 3130, 2981, 1746, 1635, 1602, 1496, 1228 $cm^{-1}$. $^1H$ NMR (300 MHz, [$D_6$]DMSO, 25 °C, TMS): 8.34 (d, J = 7.8 Hz,1H, H1), 8.13 (s,1H, H8), 8.05 (s,1H, triazole), 7,93–7.85 (m, 3H, H3,H4, H5), 7.63 (d, J = 8.1 Hz, 1H, H6), 7.30 (t, J = 7.5 Hz, 1H, H2), 5.80 (s, 2H, CH2), 5.29(s, 2H, CH2), 4,11 (q, J = 7.2 Hz, 2H, CH2), 2.41 (s,3H, CH3), 1,13 (t, J = 7.2 Hz, 3H,CH3). $^{13}C$ NMR (75 MHz, [$D_6$]DMSO, 25 °C, TMS): 176.91, 167.47, 143.07, 142.09, 140.36, 135.92, 134.42, 131.21, 127.15, 126.35, 125.16, 122.13, 121.69, 116.73, 116.55, 61.87, 50.87, 41.87, 20.63, 14.32. MS (ESI) for $C_{21}H_{20}N_4O_3$ $[M + 1]^+$, calcd 377.15, found 377.16.

**{4-[(2-methyl-9-oxoacridin-10-yl)methyl] -1,2,3-triazol-1-yl}acetic acid (4f)**

Yellow solid; yield: 81%, $R_f$ 0.65 (hexane:diethyl ether = 1:4), mp > 300 °C. IR (KBr): 3400, 3118, 2976, 1700, 1635, 1601, 1490, 1237 $cm^{-1}$. $^1H$ NMR (300 MHz, [$D_6$]DMSO, 25 °C, TMS): 10.85 (s,1H, OH), 8.34 (d, J = 7.8 Hz,1H, H1), 8.12 (s,1H, H8), 8.06 (s,1H, triazole), 7,94–7.75 (m, 3H, H3,H4, H5), 7.64 (d, J = 8.1 Hz, 1H, H6), 7.30 (t, J = 7.5 Hz, 1H, H2), 5.78 (s, 2H, CH2), 5.13(s, 2H, CH2), 2.40(s,3H, CH3). $^{13}C$ NMR (75 MHz, [$D_6$]DMSO, 25 °C, TMS): 176.89, 167.55, 142.07, 140.34, 135.96, 134.46, 131.20, 127.13, 126.32, 125.19, 122.06, 121.70, 116.80, 116.62, 50.87, 41.84, 20.66. MS (ESI) for $C_{19}H_{16}N_4O_3$ $[M + 1]^+$, calcd 349.13, found 349.13.

**2-methyl-10-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl) acridin-9(10H)-one (4 g)**

White solid; yield: 80%, $R_f$ 0.69 (hexane:diethyl ether = 1:4), mp > 300 °C. IR (KBr): 3110, 3054, 1636, 1614, 1601, 1500 1266 $cm^{-1}$. $^1H$ NMR (300 MHz, [$D_6$]DMSO, 25 °C, TMS): 8,71(s, 1H, triazole), 8.32 (d, J = 7.2 Hz,2H, H1), 8.14 (s, 1H, H8), 7,94–7.78 (m, 2H, H4, H5), 7.71–7.62 (m, 4H, H3, H6, H1',H5'), 7.37 (d, J = 7.2 Hz, 5H, H2,H2',H3', H4'), 5.82 (s, 2H, CH2), 2,38 (s, 3H, CH3). $^{13}C$ NMR (75 MHz, [$D_6$]DMSO, 25 °C, TMS): 177.01, 144.29, 142.13, 140.10, 138.74, 136.12, 134.57,134.12, 131.18, 130.21, 127.22, 126.35, 122.30, 121.13, 120.71, 120.37, 116.92, 116.84,




**Scheme 1** Synthesis of acridone linked to 1,2,3-triazole derivatives 4

42.36, 20.86. MS (ESI) for $C_{23}H_{18}N_4O$ $[M+1]^+$, calcd 367.14, found 367.15.

#### 2-methyl-10-{[1-(4-methylphenyl)-1,2,3-triazol-4-yl] methyl}acridin-9-one (4 h)

Yellow solid; yield: 87%, $R_f$ 0.70 (hexane:diethyl ether = 1:4), mp > 300 °C. IR (KBr): 3112, 3063, 1638, 1616, 1600, 1502 1267 $cm^{-1}$. $^1H$ NMR (300 MHz, $[D_6]$DMSO, 25 °C, TMS): 8,72(s, 1H, triazole), 8.35 (d, J = 7.2 Hz,2H, H1), 8.14 (s, 1H, H8), 7,94–7.78 (m, 2H, H4, H5), 7.71–7.62 (m, 4H, H3, H6, H1',H4'), 7.36 (d, J = 7.2 Hz, 3H, H2,H2',H3'), 5.83 (s, 2H, CH2), 2,41 (s, 3H, CH3), 2.32(s, 3H, CH3). $^{13}C$ NMR (75 MHz, $[D_6]$DMSO, 25 °C, TMS): 177.02, 144.23, 142.23, 140.50, 138.81, 136.00, 134.67,134.52, 131.18, 130.61, 127.12, 126.33, 122.15, 121.83, 121.71, 120.37, 116.82, 116.64, 42.16, 20.99, 20.68. MS (ESI) for $C_{24}H_{20}N_4O$ $[M+1]^+$, calcd 381.17, found 381.16.

**Table 1** Synthesized compounds (4a-h) from alkynes and azides

| Entry | Compounds | $R_1$ | $R_2$ | Yield (%) | |
|---|---|---|---|---|---|
| | | | | Conventional | MW |
| 1 | 4a | H | $CH_2COOEt$ | 74 | 80 |
| 2 | 4b | – | $CH_2COOH$ | 70 | 82 |
| 3 | 4c | – | Ph | 70 | 82 |
| 4 | 4d | – | p-CH3-C6H4 | 75 | 90 |
| 5 | 4e | $CH_3$ | $CH_2COOEt$ | 76 | 84 |
| 6 | 4f | – | $CH_2COOH$ | 69 | 81 |
| 7 | 4g | – | Ph | 75 | 80 |
| 8 | 4h | – | p-CH3-C6H4 | 73 | 87 |

## Results and discussion

### Synthesis

The synthesis of novel acridone bearing 1,2,3-triazole compounds was performed as described in (Scheme 1). Initially acridone **2** was synthesized by Ullmann condensation reaction of o-bromobenzoic acid with various aniline derivatives in the presence of potassium carbonate and copper in isoamyl alcohol at reflux to produce 2-arylamino benzoic acids **1** [25], which was cyclized with sulfuric acid in water bath at 85 °C for 4 h to give compounds **2**. The propargylation of acridone was attempted by refluxing excess of propargyl bromide with acridone derivatives in DMF using sodium hydride.

We synthesized various azides by utilizing literature protocols [9, 26]. Alkyl azides were prepared by refluxing alkyl halides with $NaN_3$ in DMF at 80–90 °C; 4-Azidotoluene and azidobenzene were prepared from p-toluidine and aniline using diazotization followed by adding $NaN_3$.

1,3-diploar cycloaddition was performed with 10-(prop-2-yn-1-yl)acridone derivatives (1 equiv) (**3**) and aromatic,

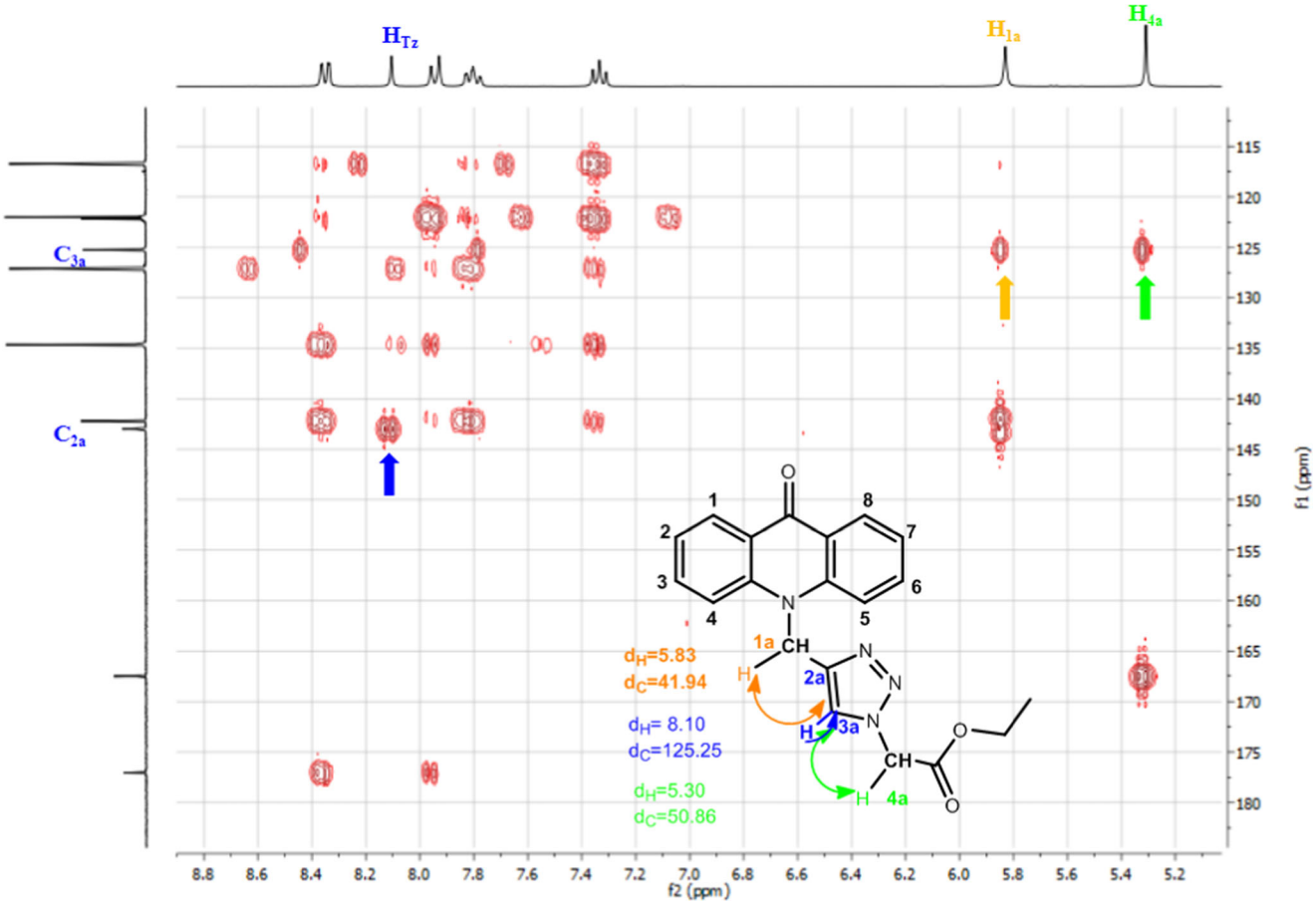



**Fig. 1** The NMR 2D HMBC of compound 4a

aliphatic azides (1.5 equiv) in the presence of copper sulfate (0.1 equiv), and sodium ascorbate (0.2 equiv). The reaction was conducted at room temperature in DMF for 12 h. Then the mixture was poured into ice water; the precipitate was filtered off and purified by flash chromatography using hexane/diethyl ether (1:4); the novel 1,4-disubstituted 1,2,3-triazoles (**4a-h**) were obtained with good yields (Scheme 1). In the goal to obtain these 1,2,3-triazoles with excellent yields and shorter reaction times under mild reaction conditions, we focused our attention to microwave-assisted synthesis. The model reaction was examined in various solvents with controlled MW powers (100–300 W). The best result was obtained in DMF as solvent at 200 W. The desired products of **4a–h** were obtained in yields ranging from 85 to 98%. The reaction times under

**Table 2** Antibacterial data for the synthesized compounds (4a-h)

| *compounds* | *S. aureus* | *E.coli* | *K. pneumoniae* | *P. putida* |
|---|---|---|---|---|
| *2a* | *122.8* | *133.4* | *137.9* | *156.3* |
| *2b* | *118.4* | *124.2* | *130.4* | *145.5* |
| *4a* | *90.91* | *122.81* | *122.81* | *122.81* |
| *4b* | *56.60* | *122.81* | *137.93* | *122.81* |
| *4c* | *46.60* | *112.36* | *138.32* | *126.12* |
| *4d* | *38.46* | *90.91* | *107.14* | *107.14* |
| *4e* | *56.60* | *74.07* | *74.07* | *115.04* |
| *4f* | *56.60* | *74.07* | *56.60* | *107.14* |
| *4 g* | *41.32* | *66.36* | *81.32* | *119.36* |
| *4 h* | *19.61* | *56.60* | *90.91* | *122.81* |
| *Chloramphenicol* | 11.65 | 22.41 | 15.38 | 37.03 |
| *DMF* | – | – | – | – |

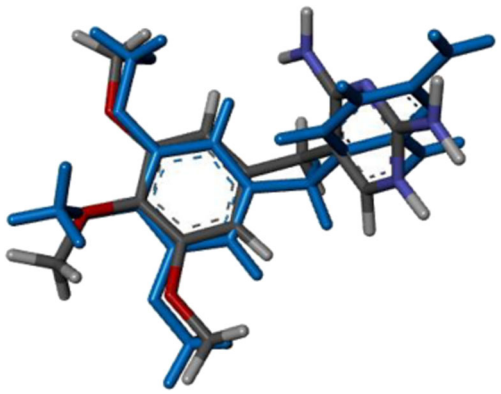


**Fig. 2** Redocking pose and RMSD value of 0.9 Å (blue = docked, gray = original)

**Table 3** Docking score, intramolecular hydrogen bonds (C=O···Ala7), and hydrophobic interactions of the synthesized compounds

| *Compounds* | *Docking score* | *C=O—ALA7 (d in Å)* | *Hydrophobic interactions* |
|---|---|---|---|
| *4a* | *6.74* | – | *Ile 50, Ile 31, Ile 14, Leu 20, Leu 54, and Leu 28* |
| *4b* | *6.42* | – | *Ile 31, Ile 14, and Leu 20* |
| *4c* | *6.80* | *C=O---ALA7 (2.49 Å)* | *Ile 50, Ile 31, Ile 14, and Leu 20* |
| *4d* | *6.98* | *C=O---ALA7 (2.50 Å)* | *Ile 50, Ile 31, Ile 14, and Leu 20* |
| *4e* | *6.75* | – | *Ile 50, Ile 31, Ile 5, and Phe 92* |
| *4f* | *6.58* | – | *Ile 50, Ile 31, Ile 5, Leu 20, and Phe 92* |
| *4 g* | *6.98* | *C=O---ALA7 (2.55 Å)* | *Ile 50, Ile 14, and Leu 20* |
| *4 h* | *7.04* | *C=O---ALA7 (2.55 Å)* | *Ile 5 and Phe 29* |

microwave irradiation are more acceptable than those of conventional methods (Table 1).

The structure of the 1,2,3-triazoles were demonstrated by their spectral data. The IR spectra of compounds **(4a-h)** showed characteristic absorption bands in the region of 1746–1700 $cm^{-1}$ (C=O) due to the presence of ester and carboxylic acid groups, and characteristic bands of 1,2,3-triazole ring was detected in the range 1500–1300 $cm^{-1}$ which corresponds to N=N & C=C bonds. However, the desperation of the vibration bonds of alkyne group in the region of 2110 $cm^{-1}$ and 3210 $cm^{-1}$ confirmed the formation of compounds.

The $^{1}H$ NMR spectra of compounds **4a–h** showed a singlet at $\delta_H$ 8.10–8.72 ppm attributable to the proton $H_3$, of the triazole moiety and signals in the aromatic region $\delta_H$ 8.40–7.31 ppm relative to the aromatic protons. In addition, the compounds 4a-d showed signals at $\delta_H$ 5.83–5.13 ppm corresponded to –CH2– groups attached to triazole ring, and the compounds **4e-f** present singlet peak that appeared at $\delta_H$ 2.3 ppm corresponded to $CH_3$. These structures were further supported by $^{13}C$ and DEPT NMR spectra, which showed all the expected carbon signals corresponding to acridone–triazole derivatives, essentially the aromatic carbons resonating at $\delta_C$ 125.2–143.0 ppm corresponding to triazole ring and signals of CH2 carbons resonated at $\delta_C$ 50.8–41-9 ppm.

The regioselectivity of the cycloaddition reaction under microwave irradiation was confirmed by using 1 H and 13C long-range NMR experiments (HMBC). The selective formation of 1,4-disubstituted 1,2,3-triazoles was confirmed by the 3-bond HMBC correlations of the methylene protons $H_{1a}$ (δ 5.83 ppm) and $H_{4a}$ (δ 5.31 ppm) with the carbon of triazole $C_{3a}$ (δ 125 ppm). Also, we observe that the signal of the $H_{Tz}$ (δ 8.10 ppm) correlate with the $C_{3a}$ (δ 125 ppm) (Fig. 1).

## Antibacterial activity

The antibacterial activity of the compounds **(4a-h)** was tested in vitro against one gram-positive bacteria *Staphyloccocus aureus* and three gram-negative bacteria *Pseudomonas putida,*

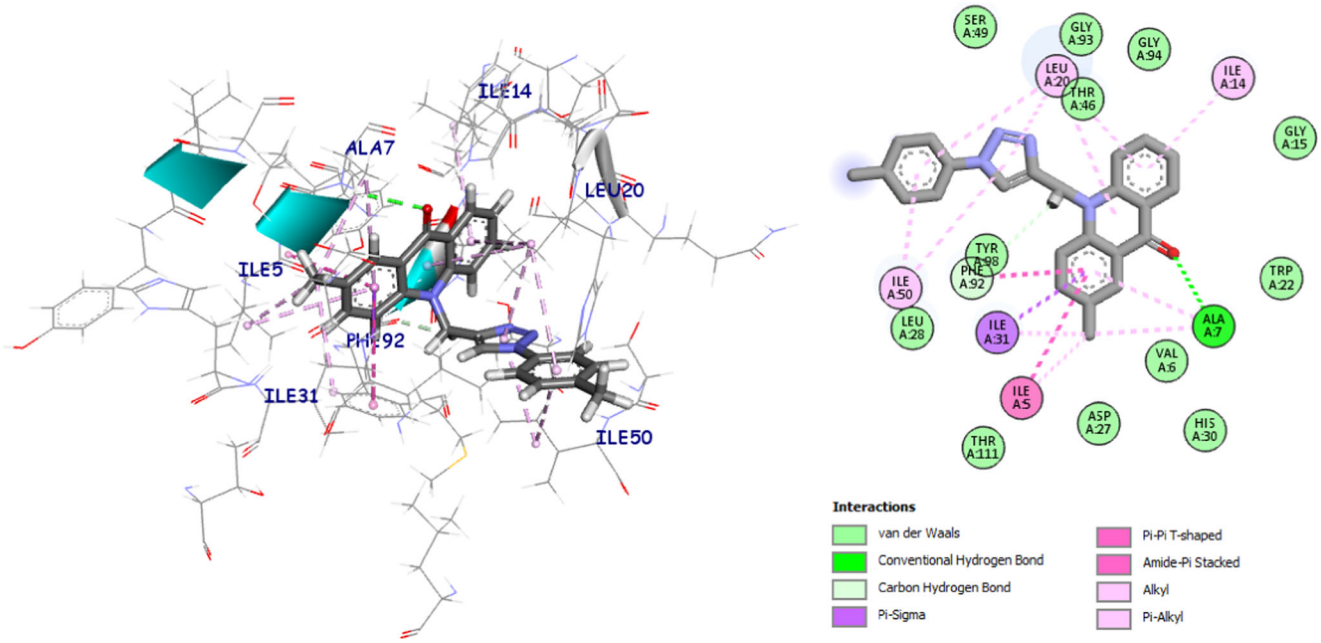



**Fig. 3** The binding conformations and ligand interactions of the compound 4h at the active site of *S. aureus* DHFR

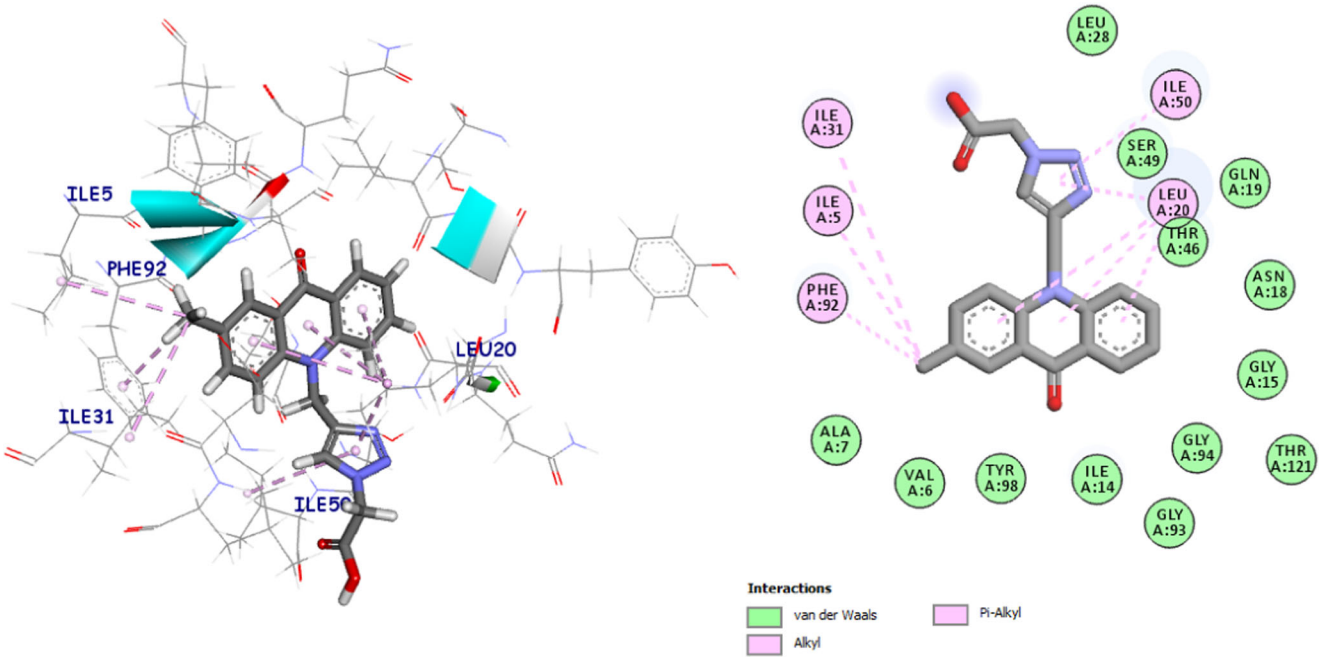



**Fig. 4** The binding conformations and ligand interactions of the compound 4b at the active site of *S. aureus* DHFR

*Klebsiella pneumonia*, and *Escherichia coli*. Primary screening test of the antibacterial effect of the compounds elicited as inhibition zones of the growth of the tested bacteria. Therefore, the MIC was determined for synthesized compounds.

According to the antibacterial activity results (Table 2), all compounds **(4a-h)** were found to have good antibacterial activity against *Staphylococcus aureus*, the compound **4h** was found to be the best active derivatives with MIC = 19.6 μg/mL. The tested compounds **4e-h** show moderate activity against *Escherichia coli* and *Klebsiella pneumonia* with the MIC values between 56.6–74.0 μg/mL. The low antibacterial activity was observed against *Pseudomonas putida* with MIC value 107.1 μg/mL. The results indicate that the substitution of the acridone ring at position 2 with methyl increase the antibacterial activity. In the methyl-substituted series, the antibacterial activity of the compound 4e showed higher activity against gram-negative bacteria. Moreover, there is a development in

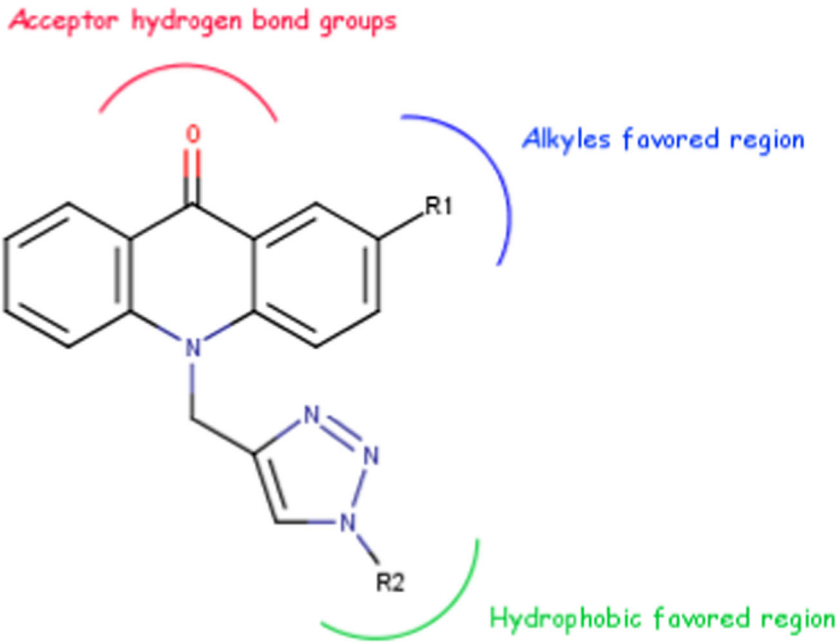



**Fig. 5** Structure-activity relationship (SAR) of the acridone-1,2,3-triazole skeleton

antibacterial activity after the substitution of acridone ring with triazole ring for all bacteria. The results of the antibacterial activity revealed that the compounds **(4a-h)** have good antibacterial activity against gram-positive bacteria compared with gram-negative bacteria.

### Molecular docking studies

The antibacterial potency of the compounds **(4a-h)** was subjected for docking study to understand the interaction of our compounds and methicillin-resistant *Staphylcoccus aureus* (MRSA). The dihydrofolate reductase (DHFR) is an important enzyme whose role is to regenerate folic acid into reduced form of tetrahydrofolate [27]. The dihydrofolate reductase (DHFR) is an interesting target for antibacterial agents in several studies [2, 28, 29]. Crystal structure of *S. aureus* DHFR (PDB ID: 2W9S) with trimethoprim as co-crystallized ligands and NADPH with 1.80 Å resolution was retrieved from Protein Data Bank. In order to evaluate and validate the binding prediction of docking protocol, the co-crystallized ligand TOP was redocked into the active site of *S. aureus* DHFR protein (2W9S). The evaluation of the precision of the docking protocol was based on the RMSD parameter. The RMSD was calculated on the basis of the difference between redocking and original poses using the MOE software. The results show the redocked ligand superimposed on the co-crystallized one (Fig. 2) with a low RMSD value of 0.9 Å.

The analysis of the binding site revealed that the synthesized ligands is stabilized by several favorable interactions including polar, hydrogen bond, hydrophobic, and Van der Waals interactions (Table 3); the binding site pocket contains the following amino acids: Ala 7, Ile 31, Ile 14, Leu 20, Ile 50, Ile 5, Phe 92, Ser 49, Leu 28, Tyr 98, Thr 111, Gly 93, Thr 46, Gly 94, Gly 15, Trp 22, Val 6, His 30, and Asp 27.

According to molecular docking analysis, **4h** showed a docking score value of 7.04, reflecting that **4h** could bind with the *S. aureus* DHFR receptor well. Table 3 indicates that the key residues *Ala 7*, *Ile 31*, *Ile 14*, *Leu 20*, *Ile 50*, *Ile 5*, and *Phe 92* are involved in the recognition of compound **4h** in the active site of *S. aureus* DHFR. 2D interactions of compounds **4c**, **4d**, **4g**, and **4h** presented in Fig. 3 showed that the oxygen of acridone moiety formed hydrogen bond with Ala 7 (distance of 2.5 Å). Additionally, it revealed hydrophobic interactions with Ile 14, Phe 29, Ile 5, Leu 20, and Ile 50. While the compounds **4a**, **4b**, **4e**, and **4f** (Fig. 4) showed less affinity compared with compound **4h** which can be explained by the absence of hydrogen bond of the oxygen of acridone moiety with Ala 7 in these compounds. Furthermore, hydrophobic interactions were observed with Ile 50, Ile 31, Ile 5, and Phe 92.

Various research have demonstrated the presence of the hydrogen bond interaction between inhibitors and Ala 7, which reveals the importance of this residue as a hotspot for the inhibition of the *S. aureus* DHFR receptor [2, 28, 29]. These docking results indicate that the compound **4h** played a significant role in binding with the active site of *S. aureus* DHFR receptor, and this may explain its high antibacterial activity. Furthermore, the study shows that all compounds with high potency for antibacterial activity present a hydrogen bond interaction with Ala7 and hydrophobic interactions into *S. aureus* DHFR active site.

The results of the antibacterial activity and docking studies revealed that the substitution of the acridone ring with methyl group and the acridone-1,2,3-triazole skeleton by aromatic moiety increases the antibacterial activity against *S. aureus; this could be explained* by the hydrophobic interactions in *S. aureus* DHFR active site (Fig. 5).

## Conclusion

The present work reports synthesis of novel heterocyclic compounds combining the 1,2,3-triazole and acridone rings. Efficient microwave-assisted synthesis of novel acridone bearing 1,2,3-triazole compounds has been carried out effectively with short time reaction and in good yields. The compounds **(4a-h)** were investigated for their in vitro antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas putida*, and *Staphyloccocus aureus*, indicating that the introduction of 1,2,3-triazole ring on the acridone ring increases the antibacterial activity. The docking results showed that compounds with high antibacterial potency showed hydrogen bond and hydrophobic interactions with *S. aureus* DHFR active site.

## References


1. López-Pueyo MJ, Barcenilla-Gaite F, Amaya-Villar R, Garnacho-Montero J (2011) Antibiotic multiresistance in critical care units. Med Intensiva (Engl Ed) 35:41–53. https://doi.org/10.1016/S2173-5727(11)70008-X
2. Ahmad MJ, Hassan SF, Nisa RU et al (2016) Synthesis, in vitro potential and computational studies on 2-amino-1, 4-dihydropyrimidines as multitarget antibacterial ligands. Med Chem Res 25:1877–1894. https://doi.org/10.1007/s00044-016-1613-z
3. Belay Y, Coetzee L-C, Williams DBG, Muller A (2019) Synthesis of novel 1,2,3-triazole based polycarboxylic acid functionalised ligands for MOF systems. Tetrahedron Lett 60:501–503. https://doi.org/10.1016/J.TETLET.2019.01.014
4. Yan W, Wang X, Li K et al (2019) Design, synthesis, and antifungal activity of carboxamide derivatives possessing 1,2,3-triazole as potential succinate dehydrogenase inhibitors. Pestic Biochem Physiol. https://doi.org/10.1016/j.pestbp.2019.02.017
5. Zhang B (2019) Comprehensive review on the anti-bacterial activity of 1,2,3-triazole hybrids. Eur J Med Chem 168:357–372. https://doi.org/10.1016/J.EJMECH.2019.02.055

6. Devender N, Gunjan S, Chhabra S et al (2016) Identification of β-amino alcohol grafted 1,4,5 trisubstituted 1,2,3-triazoles as potent antimalarial agents. Eur J Med Chem 109:187–198. https://doi.org/10.1016/J.EJMECH.2015.12.038
7. Bonandi E, Christodoulou MS, Fumagalli G et al (2017) The 1,2,3-triazole ring as a bioisostere in medicinal chemistry. Drug Discov Today 22:1572–1581. https://doi.org/10.1016/J.DRUDIS.2017.05.014
8. Pokhodylo N, Shyyka O, Matiychuk V (2014) Synthesis and anticancer activity evaluation of new 1,2,3-triazole-4-carboxamide derivatives. Med Chem Res 23:2426–2438. https://doi.org/10.1007/s00044-013-0841-8
9. Angajala KK, Vianala S, Macha R et al (2016) Synthesis, anti-inflammatory, bactericidal activities and docking studies of novel 1,2,3-triazoles derived from ibuprofen using click chemistry. Springerplus 5:423. https://doi.org/10.1186/s40064-016-2052-5
10. Sharma V, Sharma PC, Kumar V (2015) A mini review on pyridoacridines: prospective lead compounds in medicinal chemistry. J Adv Res 6:63–71. https://doi.org/10.1016/J.JARE.2014.11.002
11. Sepúlveda CS, García CC, Fascio ML et al (2012) Inhibition of Junin virus RNA synthesis by an antiviral acridone derivative. Antivir Res 93:16–22. https://doi.org/10.1016/J.ANTIVIRAL.2011.10.007
12. Zhao J, Larock RC (2006) Synthesis of xanthones, thioxanthones, and acridones by the coupling of arynes and substituted benzoates. https://doi.org/10.1021/JO0620718
13. Cholewiński G, Dzierzbicka K, Kołodziejczyk AM (2011) Natural and synthetic acridines/acridones as antitumor agents: their biological activities and methods of synthesis. Pharmacol Rep 63:305–336. https://doi.org/10.1016/S1734-1140(11)70499-6
14. Parveen M, Aslam A, Nami SAA et al (2016) Potent acetylcholinesterase inhibitors: synthesis, biological assay and docking study of nitro acridone derivatives. J Photochem Photobiol B Biol 161:304–311. https://doi.org/10.1016/J.JPHOTOBIOL.2016.05.028
15. Kudryavtseva TN, Lamanov AY, Klimova LG, Nazarov GV (2018) Synthesis and antibacterial activity of 9-Oxo-9,10-dihydroacridinecarboxylic acids esters bearing a triazole fragment. Russ J Gen Chem 88:676–681. https://doi.org/10.1134/S1070363218040102
16. Markovich YD, Kudryavtseva TN, Bogatyrev KV et al (2014) Synthesis of 2-(4-methyl-1,3-thiazol-5-yl)ethyl esters of acridone carboxylic acids and evaluation of their antibacterial activity. Russ Chem Bull 63:1153–1158. https://doi.org/10.1007/s11172-014-0564-0
17. Salimon J, Salih N, Yousif E et al (2010) Synthesis and pharmacological evaluation of 9(10H)-acridone bearing 1,3,4-oxadiazole derivatives as antimicrobial agents. Arab J Chem 3:205–210. https://doi.org/10.1016/J.ARABJC.2010.06.001
18. (2010) Ullmann Acridine Synthesis. Comprehensive Organic Name Reactions and Reagents. John Wiley & Sons, Inc., Hoboken, pp 2838–2841
19. Olson O, Balasubramaniam CR, Shrum T, et al (2005) Novel antimicrobial activity of nanocrystalline silver dressings. In: International Conference on MEMS,NANO and Smart Systems. IEEE, pp 129–134
20. Sarkar S, Jana AD, Samanta SK, Mostafa G (2007) Facile synthesis of silver nano particles with highly efficient anti-microbial property. Polyhedron 26:4419–4426. https://doi.org/10.1016/J.POLY.2007.05.056
21. Holetz FB, Pessini GL, Sanches NR et al (2002) Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz 97:1027–1031. https://doi.org/10.1590/S0074-02762002000700017
22. Navarro V, Villarreal ML, Rojas G, Lozoya X (1996) Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. J Ethnopharmacol 53:143–147
23. Heaslet H, Harris M, Fahnoe K et al (2009) Structural comparison of chromosomal and exogenous dihydrofolate reductase from Staphylococcus aureus in complex with the potent inhibitor trimethoprim. Proteins 76:706–717. https://doi.org/10.1002/prot.22383
24. Chemical Computing Group ULC 1010 Molecular Operating Environment (MOE), 2015.10
25. Martín A, Mesa M, Docampo ML et al (2006) Fast synthesis of substituted N-Phenylanthranilic acids using Ullmann condensation under microwave irradiation in dry media. Synth Commun 36:271–277. https://doi.org/10.1080/00397910500374955
26. Wilkening I, del Signore G, Hackenberger CPR (2011) Synthesis of phosphonamidate peptides by Staudinger reactions of silylated phosphinic acids and esters. Chem Commun 47:349–351. https://doi.org/10.1039/C0CC02472D
27. Skierka K, Wilamowski P, Wielechowska M et al (2019) Human dihydrofolate reductase is a substrate of protein kinase CK2α. Biochem Biophys Res Commun. https://doi.org/10.1016/J.BBRC.2019.03.186
28. Rashid U, Ahmad W, Hassan SF et al (2016) Design, synthesis, antibacterial activity and docking study of some new trimethoprim derivatives. Bioorg Med Chem Lett 26:5749–5753. https://doi.org/10.1016/J.BMCL.2016.10.051
29. Rehman W, Haq S, Muhammad B et al (2014) Organotin (IV) based complexes as promiscuous antibacterials: synthesis, in vitro, in silico pharmacokinetic and docking studies. J Organomet Chem 767:91–100. https://doi.org/10.1016/J.JORGANCHEM.2014.05.027