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3-Aryl-4-acyloxyethoxyfuran-2(5*H*)-ones as inhibitors of tyrosyl-tRNA synthetase: Synthesis, molecular docking and antibacterial evaluation

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ABSTRACT

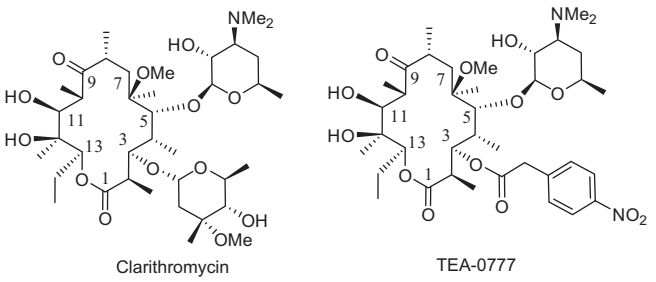
Thirty-eight 3-aryl-4-acyloxyethoxyfuran-2(5*H*)-ones were designed, prepared and tested for antibacterial activities. Some of them showed significant antibacterial activity against Gram-positive organism, Gram-negative organism and fungus. Out of these compounds, 4-(2-(3-chlorophenylformyloxy)ethoxy)-3-(4-chlorophenyl)furan-2(5*H*)-one (**d40**) showed the widest spectrum of activity with MIC_{50} of 2.0 μg/mL against *Staphylococcus aureus*, 4.3 μg/mL against *Escherichia coli*, 1.5 μg/mL against *Pseudomonas aeruginosa* and 1.2 μg/mL against *Candida albicans*. Our data disclosed that MIC_{50} values against whole cell bacteria are positive correlation with MIC_{50} values against tyrosyl-tRNA synthetase. Meanwhile, molecular docking of **d40** into *S. aureus* tyrosyl-tRNA synthetase active site was also performed, and the inhibitor tightly fitting the active site might be an important reason why it has high antimicrobial activity.

1. Introduction

The widely use of antimicrobial drugs as human and veterinary medicine for the prevention and treatment of infectious diseases has brought significant health benefits.[1,2] However, the bacteria which resist to conventional antibiotics have enabled major killers.[3,4] For example, methicillin-resistant *Staphylococcus aureus* leads to nearly 100,000 infections and 20,000 deaths in the United States annually,[5] and drug-resistant *Mycobacterium tuberculosis* significantly undermine the major advances achieved in treatment.[6,7] With the resistance increasing, antibacterial research for finding new antibacterial agents becomes more and more emergency.[8–10]

The antibacterial mechanism of actions mainly includes alterations of membrane permeability and interruptions of protein or nucleic acid synthesis. Aminoacyl-tRNA synthetase (aaRS) is an enzyme which ligates specific amino acid to its cognate tRNA molecule(s) and plays an important role in the production of proteins.[11,12] Therefore, more and more researchers are focusing on the development of aaRS inhibitors as new antibacterial agents. AaRSs as the leading targets of antimicrobial agents have been proven by Mupirocin (an inhibitor of isoleucyl-tRNA synthetase) and AN-2690 (an inhibitor of leucyl-tRNA synthetase).[13,14]

In our group, some 3-aryl-4-aminofuran-2(5*H*)-ones were reported as potent inhibitors of tyrosyl-tRNA synthetase (TyrRS)[15,16] and several of them exhibit excellent growth inhibition activity against bacteria. However, all of them show no activity against fungi. TEA-0777, a derivative of clarithromycin (Scheme 1) by esterification at C-3, has the potential as the next generation of macrolide antibiotics, which shows not only good activity against erythromycin-susceptible Gram-positive pathogens but also inducibly macrolides–lincosamides–streptogramin B (MLS_B)-resistant *S. aureus* and efflux-resistant *Streptococcus pneumoniae*.[17–19] Perhaps



Scheme 1. Structure of clarithromycin and TEA-0777.

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because of introducing ester group, TEA-0777 becomes a potent agent with a broad spectrum. Based on this finding, we therefore introduced ester group to furan-2(5*H*)-ones, trying to endow them with a broader spectrum. Thirty-eight esters of furan-2(5*H*)-one were synthesized and evaluated for antimicrobial activity. The results displayed that some of the compounds show good antibacterial and antifungal activity.

2. Materials and methods

2.1. Chemistry

All chemicals (reagent grade) used were purchased from Aldrich (U.S.A.) and Sinopharm Chemical Reagent Co., Ltd (China). Separation of the compounds by column chromatography was carried out with silica gel 60 (200–300 mesh ASTM, E. Merck). The quantity of silica gel used was 30–70 times the weight charged on the column. Then, the eluates were monitored using TLC. Melting points (uncorrected) were determined on a XT4 MP apparatus (Taike Corp., Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer, and ^{1}H NMR spectra were recorded on Bruker AV-300 spectrometer at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts were reported in ppm (δ). Elemental analyses were performed on a CHN–O-Rapid instrument and were within ±0.4% of the theoretical values.

2.1.1. General procedure for preparation of compounds a1–a2

An appropriately substituted phenylacetic acid (40 mmol) was dissolved in 0.1 M NaOH with pH in the range of 9.0–10.0. Then, the solvent was removed under reduced pressure to give the corresponding sodium phenylacetate. To a solution of the resulted sodium phenylacetate (40 mmol) in DMSO (45 mL) was added 1.1 equiv of ethyl bromoacetate, which was stirred at 25 °C for 3–4 h. The mixture was then poured into ice-water (50 mL), and extracted twice with 200 mL of AcOEt. The organic layer was dried over $MgSO_4$ followed by removal of the solvent under reduced pressure. The residue was then purified by column chromatography on silica gel to give **a1**–**a2** (Scheme 2) in yields of 90–95%.

2.1.2. General procedure for preparation of compounds b1–b2

A dropwise solution of compound (**a1** or **a2**) (10 mmol) in dry THF (10 mL) was added to a suspension of NaH (24 mmol) in dry THF (20 mL) in an ice cold bath and the stirring was maintained at room temperature for several hours (monitored by TLC). Water (30 mL) was added and the solution was extracted twice with ethyl ether. The aqueous phase was cooled to 0 °C and then acidified with concentrated hydrochloric acid to give a solid precipitate. Filtration and washing with water furnished compound (**b1** or **b2**) (Scheme 2), which was used without further purification.

2.1.3. General procedure for preparation of compounds c1–c2

Compound (**b1** or **b2**) (5 mmol) was dissolved in 35 mL of dry acetone, followed by addition of 1,2-dibromoethane (4.3 mL, 50 mmol) and triethylamine (2.2 mL, 15 mmol). The resulted mixture was refluxed for 3–5 h. After the solvent was removed, the residue was partitioned between AcOEt and water. The organic layer was then dried over $MgSO_4$ and concentrated under reduced pressure. Flash chromatography (AcOEt/petroleum ether, from 3:2 to 2:3) furnished compound (**c1** or **c2**) (Scheme 2) in good yield.

2.1.4. General procedure for preparation of 3-aryl-4-acyloxyethoxyfuran-2(5*H*)-ones (d3–d40)

Appropriately substituted sodium carboxylate (1.0 mmol) was added in a solution of compound (**c1** or **c2**) (0.5 mmol) in dry DMSO (15 mL). The mixture was then stirred for 8–12 h at room temperature (monitored by TLC). After 30 mL of water was added, the precipitate was collected, which was purified by column chromatography on silica gel, eluting with AcOEt/petroleum ether.

2.1.5. 4-(2-Butyryloxyethoxy)-3-phenylfuran-2(5*H*)-one (d3)

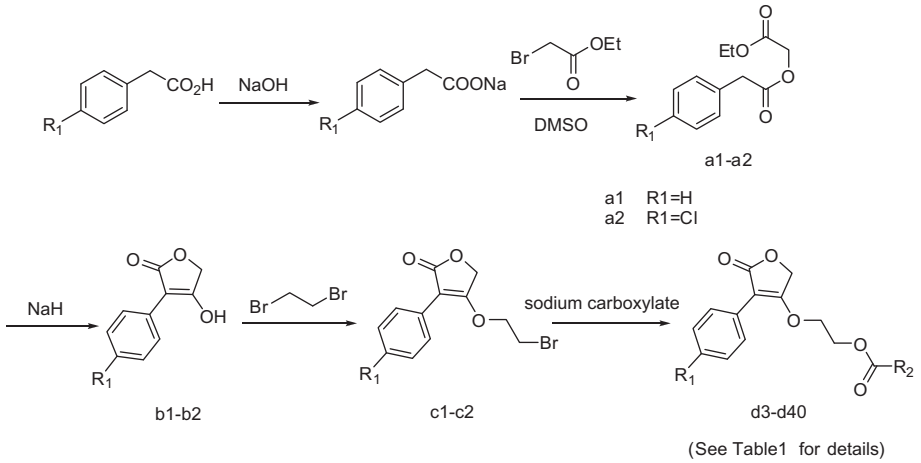
Yellow oily liquid, 68.0%; ^{1}H NMR ($CDCl_3$): 0.92–0.97(m, 3H, CH_3); 1.60–1.71 (m, 2H, CH_2); 2.32 (t, J = 7.3 Hz, 2H, CH_2); 4.29 (t, J = 4.3 Hz, 2H, CH_2); 4.39 (t, J = 4.0 Hz, 2H, CH_2); 4.85 (s, 2H, CH_2); 7.26–7.33 (m, 2H, ArH); 7.39 (t, J = 7.4 Hz, 1H, ArH); 7.81 (d, J = 7.1 Hz, 2H, ArH); EIMS m/z 290 (M^+). Anal. Calcd for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25; O, 27.56; Found: C, 66.28; H, 6.16; O, 27.56.

2.1.6. 4-(2-Hexanoyloxyethoxy)-3-phenylfuran-2(5*H*)-one (d4)

Red-brown oily liquid, 71.2%; ^{1}H NMR ($CDCl_3$): 0.88 (t, J = 6.9 Hz, 3H, CH_3); 1.27–1.31 (m, 4H, CH_2); 1.57–1.65 (m, 2H, CH_2); 2.34 (m, 2H, CH_2); 4.29–4.32 (m, 2H, CH_2); 4.37–4.41 (m, 2H, CH_2); 4.85 (s, 2H, CH_2); 7.26–7.33 (m, 1H, ArH); 7.37–7.42 (m, 2H, ArH); 7.82 (dd, J = 7.1 Hz, J = 8.6 Hz, 2H, ArH); EIMS m/z 318 (M^+). Anal. Calcd for $C_{18}H_{22}O_5$: C, 67.91; H, 6.97; O, 25.13; Found: C, 67.80; H, 6.95; O, 25.25.

2.1.7. 4-(2-Octanoyloxyethoxy)-3-phenylfuran-2(5*H*)-one (d5)

Pale yellow oily liquid, 67.5%; ^{1}H NMR ($CDCl_3$): 0.85–0.91 (m, 3H, CH_3); 1.15–1.28 (m, 10H, CH_2); 1.65–1.70 (m, 2H, CH_2); 2.34



Scheme 2. Synthetic route of 3-aryl-4-acyloxyethoxyfuran-2(5*H*)-ones.

(t, J = 7.5 Hz, 2H, CH_2); 4.39 (t, J = 4.8 Hz, 2H, CH_2); 4.85 (s, 2H, CH_2); 7.36–7.41 (m, 3H, ArH), 7.81 (d, J = 7.1 Hz, 2H, ArH); EIMS m/z 346 (M^+). Anal. Calcd for $C_{20}H_{26}O_5$: C, 69.34; H, 7.56; O, 23.09; Found: C, 69.21; H, 7.66; O, 23.13.

2.1.8. 4-(2-Phenylacetyloxylethoxy)-3-phenylfuran-2(5*H*)-one (d6)

White powder, 64.3%, mp 134–136 °C; ^{1}H NMR ($CDCl_3$): 3.66 (s, 2H, CH_2); 4.24 (t, J = 4.4 Hz, 2H, CH_2); 4.41 (t, J = 4.5 Hz, 2H, CH_2); 4.66 (s, 2H, CH_2); 7.23 (m, 2H, ArH); 7.26–7.38 (m, 4H, ArH); 7.42 (t, J = 7.0 Hz, 2H, ArH); 7.77 (d, J = 7.1 Hz, 2H, ArH); EIMS m/z 338 (M^+). Anal. Calcd for $C_{20}H_{18}O_5$: C, 70.99; H, 5.36; O, 23.64; Found: C, 70.87; H, 5.53; O, 23.60.

2.1.9. 4-(2-(3-Pyridylformyloxy)ethoxy)-3-phenylfuran-2(5*H*)-one (d7)

Orange powder, 52.7%, 111–113 °C; ^{1}H NMR ($CDCl_3$): 4.63 (t, J = 4.6 Hz, 2H, CH_2); 4.69 (t, J = 4.5 Hz, 2H, CH_2); 4.87 (s, 2H, CH_2); 7.30–7.39 (m, 3H, ArH); 7.46 (t, J = 7.9 Hz, 1H, ArH); 7.78 (d, J = 7.2 Hz, 2H, ArH); 8.32 (d, J = 7.9 Hz, 1H, ArH); 8.82 (d, J = 3.5 Hz, 1H, ArH); 9.25 (s, 1H, ArH); EIMS m/z 325 (M^+). Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31; O, 24.59; Found: C, 66.38; H, 4.69; N, 4.30; O, 24.63.

2.1.10. 4-(2-(Naphthalen-2-ylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d8)

Colorless crystal, 79.2%, mp 194–196 °C; ^{1}H NMR ($CDCl_3$): 4.49 (t, J = 4.5 Hz, 2H, CH_2); 4.74 (t, J = 4.5 Hz, 2H, CH_2); 4.89 (s, 2H, CH_2); 7.30–7.40 (m, 3H, ArH); 7.47–7.57 (m, 3H, ArH); 7.85–7.92 (m, 3H, ArH); 8.06 (d, J = 8.2 Hz, 1H, ArH); 8.16 (d, J = 7.1 Hz, 1H, ArH); 8.84 (d, J = 9.5 Hz, 1H, ArH); EIMS m/z 374 (M^+). Anal. Calcd for $C_{23}H_{18}O_5$: C, 73.79; H, 4.85; O, 21.37; Found: C, 73.81; H, 4.80; O, 21.39.

2.1.11. 4-(2-(6-Hydroxynaphthalen-2-ylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d9)

Gray white powder, 77.1%, mp 202–204 °C; ^{1}H NMR ($CDCl_3$): 4.70 (t, J = 4.7 Hz, 4H, CH_2); 5.20 (s, 2H, CH_2); 7.16–7.24 (m, 3H, ArH); 7.30 (t, J = 7.4 Hz, 2H, ArH); 7.79 (d, J = 8.8 Hz, 1H, ArH); 7.87–7.95 (m, 4H, ArH); 8.51 (s, 1H, ArH); 10.22 (s, 1H, ArH); EIMS m/z 390 (M^+). Anal. Calcd for $C_{23}H_{18}O_6$: C, 70.76; H, 4.65; O, 24.59; Found: C, 70.85; H, 4.72; O, 24.43.

2.1.12. 4-(2-(2-Methylphenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d10)

White powder, 68.1%, mp 121–123 °C; ^{1}H NMR ($CDCl_3$): 2.58 (s, 3H, CH_3); 4.43 (t, J = 4.6 Hz, 2H, CH_2); 4.63 (t, J = 4.6 Hz, 2H, CH_2); 4.87 (s, 2H, CH_2); 7.21–7.24 (m, 1H, ArH); 7.28–7.43 (m, 5H, ArH); 7.82 (d, J = 7.1 Hz, 2H, ArH); 7.89 (d, J = 7.5 Hz, 1H, ArH); EIMS m/z 338 (M^+). Anal. Calcd for $C_{20}H_{18}O_5$: C, 70.99; H, 5.36; O, 23.64; Found: C, 70.84; H, 5.31; O, 23.85.

2.1.13. 4-(2-(2-Chlorophenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d11)

White powder, 86.5%, mp 117–119 °C; ^{1}H NMR ($CDCl_3$): 4.43 (t, J = 4.4 Hz, 2H, CH_2); 4.65 (t, J = 4.2 Hz, 2H, CH_2); 4.87 (s, 2H, CH_2); 7.30–7.41 (m, 4H, ArH); 7.45–7.47 (m, 2H, ArH); 7.81 (t, J = 7.4 Hz, 3H, ArH); EIMS m/z 358 (M^+). Anal. Calcd for $C_{19}H_{15}ClO_5$: C, 63.61; H, 4.21; Cl, 9.88; O, 22.30; Found: C, 63.64; H, 4.27; Cl, 9.83; O, 22.26.

2.1.14. 4-(2-(2-Nitrophenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d12)

White powder, 71.9%, mp 84–85 °C; ^{1}H NMR ($CDCl_3$): 4.36 (t, J = 4.5, 2H, CH_2); 4.65 (t, J = 4.4, 2H, CH_2); 4.87 (s, 2H, CH_2); 7.26–7.41 (m, 3H, ArH); 7.64–7.76 (m, 3H, ArH); 7.77 (d, J = 7.0 Hz, 2H, ArH); 7.93–7.96 (m, 1H, ArH); EIMS m/z 369 (M^+). Anal. Calcd for $C_{19}H_{15}NO_7$: C, 61.79; H, 4.09; N, 3.79; O, 30.32; Found: C, 61.81; H, 4.14; N, 3.75; O, 30.30.

2.1.15. 4-(2-(2-hydroxyphenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d13)

White powder, 37.8%, mp 132–134 °C; ^{1}H NMR ($CDCl_3$): 4.43 (s, 2H, CH_2); 4.66 (d, J = 4.2 Hz, 2H, CH_2); 4.87 (s, 2H, CH_2); 6.90 (t, J = 7.6 Hz, 1H, ArH); 7.01 (d, J = 8.4 Hz, 1H, ArH); 7.30–7.40 (m, 3H, ArH); 7.50 (t, J = 7.8 Hz, 1H, ArH); 7.81 (t, J = 6.7 Hz, 3H, ArH); 10.50 (s, 1H, OH); EIMS m/z 340 (M^+). Anal. Calcd for $C_{19}H_{16}O_6$: C, 67.05; H, 4.74; O, 28.21; Found: C, 67.17; H, 4.68; O, 28.15.

2.1.16. 4-(2-(2-Methoxyphenylformyloxy)ethoxy)-3-phenylfuran-2(5*H*)-one (d14)

White powder, 63.8%, mp 90–91 °C; ^{1}H NMR ($CDCl_3$): 3.85 (s, 3H, CH_3); 4.43 (t, J = 5.3 Hz, 2H, CH_2); 4.61 (t, J = 5.3 Hz, 2H, CH_2); 4.90 (s, 2H, CH_2); 6.96–7.01 (m, 2H, ArH); 7.28–7.32 (m, 1H, ArH); 7.35–7.41 (m, 2H, ArH); 7.47–7.53 (m, 1H, ArH); 7.77 (d, J = 7.9 Hz, 1H, ArH); 7.86 (d, J = 8.6 Hz, 2H, ArH); EIMS m/z 354 (M^+). Anal. Calcd for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12; O, 27.09; Found: C, 67.81; H, 5.15; O, 27.04.

2.1.17. 4-(2-(3-Nitrophenylformyloxy)ethoxy)-3-phenylfuran-2(5*H*)-one (d15)

Colorless crystal, 61.2%, mp 151–152 °C; ^{1}H NMR ($CDCl_3$): 4.55–4.60 (m, 4H, CH_2); 5.17 (s, 2H, CH_2); 6.85 (d, J = 8.8 Hz, 2H, ArH); 7.24–7.34 (m, 3H, ArH); 7.84–7.87 (m, 4H, ArH); EIMS m/z 369 (M^+). Anal. Calcd for $C_{19}H_{15}NO_7$: C, 61.79; H, 4.09; N, 3.79; O, 30.32; Found: C, 61.88; H, 4.15; N, 3.61; O, 30.36.

2.1.18. 4-(2-(3-Methylphenylformyloxy)ethoxy)-3-phenylfuran-2(5*H*)-one (d16)

White solid, 52.9%, mp 134–136 °C; ^{1}H NMR ($CDCl_3$): 2.40 (s, 3H, CH_3); 4.43 (t, J = 4.6 Hz, 2H, CH_2), 4.63 (t, J = 4.6 Hz, 2H, CH_2), 4.88 (s, 2H, CH_2), 7.29–7.39 (m, 5H, ArH), 7.82–7.84 (m, 4H, ArH); EIMS m/z 338 (M+). Anal. Calcd for $C_{20}H_{18}O_5$: C, 70.99; H, 5.36; O, 23.64; Found: C, 70.85; H, 5.41; O, 23.74.

2.1.19. 4-(2-(3-Trifluoromethylphenylformyoxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d17)

White powder, 71.2%, mp 110–112 °C; ^{1}H NMR ($CDCl_3$): 4.44 (t, J = 4.4 Hz, 2H, CH_2); 4.67 (t, J = 4.4 Hz, 2H, CH_2); 4.87 (s, 2H, CH_2); 7.30–7.39 (m, 3H, ArH); 7.61 (t, J = 7.8 Hz, 1H, ArH); 7.79–7.87 (m, 3H, ArH); 8.21 (d, J = 7.9 Hz, 1H, ArH); 8.30 (s, 1H, ArH); EIMS m/z 392 (M^+). Anal. Calcd for $C_{20}H_{15}F_3O_5$: C, 61.23; H, 3.85; F, 14.53; O, 20.39; Found: C, 61.32; H, 3.80; F, 14.54; O, 20.34.

2.1.20. 4-(2-(4-Nitrophenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d18)

Chartreuse powder, 56.0%, mp 147–149 °C; ^{1}H NMR (DMSO-d_6): 4.65 (d, J = 4.4 Hz, 2H, CH_2); 4.76 (t, J = 4.0 Hz, 2H, CH_2); 5.18 (s, 2H, CH_2); 7.25–7.30 (m, 3H, ArH); 7.86 (t, J = 4.3 Hz, 2H, ArH); 8.23 (d, J = 8.8 Hz, 2H, ArH); 8.38 (d, J = 8.9 Hz, 2H, ArH); EIMS m/z 369 (M^+). Anal. Calcd for $C_{19}H_{15}NO_7$: C, 61.79; H, 4.09; N, 3.79; O, 30.32; Found: C, 61.83; H, 4.15; N, 3.56; O, 30.46.

2.1.21. 4-(2-(4-Methylphenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d19)

White powder, 71.3%, mp 122–124 °C; ^{1}H NMR ($CDCl_3$): 2.42 (s, 3H, CH_3); 4.42 (t, J = 4.8 Hz, 2H, CH_2); 4.63 (t, J = 4.5 Hz, 2H, CH_2); 4.87 (s, 2H, CH_2); 7.24–7.32 (m, 3H, ArH); 7.37 (t, J = 7.3 Hz, 2H, ArH); 7.83 (d, J = 8.3 Hz, 2H, ArH); 7.91 (d, J = 8.2 Hz, 2H, ArH); EIMS m/z 338 (M^+). Anal. Calcd for $C_{20}H_{18}O_5$: C, 70.99; H, 5.36; O, 23.64; Found: C, 70.82; H, 5.38; O, 23.80.

2.1.22. 4-(2-(4-fluorophenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d20)

White powder, 36.8%, mp 104–107 °C; ^{1}H NMR ($CDCl_3$): 4.42 (m, 2H, CH_2); 4.65 (m, 2H, CH_2); 4.87 (s, 2H, CH_2); 7.30–7.48 (m, 5H, ArH); 7.70 (d, J = 8.8 Hz, 1H, ArH); 7.80 (d, J = 7.0 Hz, 3H, ArH); EIMS m/z 342 (M^+). Anal. Calcd for $C_{19}H_{15}FO_5$: C, 66.66; H, 4.42; F, 5.55; O, 23.37; Found: C, 66.61; H, 4.42; F, 5.54; O, 23.43.

2.1.23. 4-(2-(4-Chlorophenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d21)

White powder, 76.1%, mp 155–157 °C; ^{1}H NMR ($CDCl_3$): 4.42 (d, J = 4.4 Hz, 2H, CH_2); 4.64 (s, 2H, CH_2); 4.87 (s, 2H, CH_2); 7.30–7.39 (m, 3H, ArH); 7.43 (d, J = 8.4 Hz, 2H, ArH); 7.81 (d, J = 7.3 Hz, 2H, ArH); 7.95 (d, J = 8.4 Hz, 2H, ArH); EIMS m/z 358 (M^+). Anal. Calcd for $C_{19}H_{15}ClO_5$: C, 63.61; H, 4.21; Cl, 9.88; O, 22.30; Found: C, 63.65; H, 4.23; Cl, 9.86; O, 22.26.

2.1.24. 4-(2-(4-Hydroxyphenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d22)

White powder, 49.5%, mp 160–162 °C; ^{1}H NMR ($CDCl_3$): 4.42 (d, J = 4.4 Hz, 2H, CH_2); 4.59 (d, J = 4.4 Hz, 2H, CH_2); 4.87 (s, 2H, CH_2); 6.97 (d, J = 6.8 Hz, 2H, ArH); 7.32–7.41 (m, 3H, ArH); 7.44 (d, J = 8.3 Hz, 2H, ArH); 7.86 (d, J = 7.4 Hz, 2H, ArH); 10.45 (s, 1H, OH); EIMS m/z 340 (M^+). Anal. Calcd for $C_{19}H_{16}O_6$: C, 67.05; H, 4.74; O, 28.21; Found: C, 67.21; H, 4.83; O, 27.96.

2.1.25. 4-(2-(2,4-Dichlorophenylformyloxy)ethoxy)-3-phenylfuran-2(5*H*)-one (d23)

White powder, 61.2%, mp 150–151 °C; ^{1}H NMR ($CDCl_3$): 4.41 (t, J = 4.5 Hz, 2H, CH_2); 4.65 (t, J = 4.5 Hz, 2H, CH_2); 4.68 (s, 2H, CH_2); 7.29–7.33 (m, 4H, ArH); 7.49 (s, 1H, ArH); 7.75–7.82 (m, 3H, ArH); EIMS m/z 392 (M^+). Anal. Calcd for $C_{19}H_{14}Cl_2O_5$: C, 58.03; H, 3.59; Cl, 18.03; O, 20.34; Found: C, 58.13; H, 3.76; Cl, 18.10; O, 20.01.

2.1.26. 4-(2-(2,5-Dichlorophenylformyloxy)ethoxy)-3-phenylfuran-2(5*H*)-one (d24)

White powder, 68.6%, mp 118–120 °C; ^{1}H NMR ($CDCl_3$): 4.45 (t, J = 4.3 Hz, 2H, CH_2); 4.60 (t, J = 4.3 Hz, 2H, CH_2); 4.81 (s, 2H, CH_2); 7.20–7.42 (m, 6H, ArH); 7.70–7.79 (m, 2H, ArH); EIMS m/z 392 (M^+). Anal. Calcd for $C_{19}H_{14}Cl_2O_5$: C, 58.03; H, 3.59; Cl, 18.03; O, 20.34; Found: C, 58.10; H, 3.74; Cl, 18.11; O, 20.05.

2.1.27. 4-(2-(3,4-Dichlorophenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d25)

Gray white powder, 57.0%, mp 127–128 °C; ^{1}H NMR ($CDCl_3$): 4.34–4.42 (m, 2H, CH_2); 4.64 (t, J = 4.2 Hz, 2H, CH_2); 4.87 (s, 2H, CH_2); 7.30–7.40 (m, 3H, ArH); 7.54 (d, J = 8.4 Hz, 1H, ArH); 7.78–7.86 (m, 3H, ArH); 8.10 (s, 1H, ArH); EIMS m/z 392 (M^+). Anal. Calcd for $C_{19}H_{14}Cl_2O_5$: C, 58.03; H, 3.59; Cl, 18.03; O, 20.34; Found: C, 58.13; H, 3.57; Cl, 18.08; O, 20.22.

2.1.28. 4-(2-(2,4-Dihydroxyphenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d26)

White powder, 48.5%, mp 200–203 °C; ^{1}H NMR (DMSO-d_6): 4.66 (t, J = 4.7 Hz, 4H, CH_2); 5.17 (s, 2H, CH_2); 6.31–6.39 (m, 2H, ArH); 7.24–7.34 (m, 3H, ArH); 7.68 (d, J = 8.6 Hz, 1H, ArH); 7.85 (d, J = 7.1 Hz, 2H, ArH); 10.50 (s, 1H, OH); 10.62 (s, 1H, OH); EIMS m/z 356 (M^+). Anal. Calcd for $C_{19}H_{16}O_7$: C, 64.04; H, 4.53; O, 31.43; Found: C, 64.08; H, 4.59; O, 31.33.

2.1.29. 4-(2-(3,4-Dihydroxyphenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d27)

White powder, 50.4%, mp 175–177 °C; ^{1}H NMR (DMSO-d_6): 4.58 (s, 4H, CH_2); 5.16 (s, 2H, CH_2); 6.82 (d, J = 8.4 Hz, 1H, ArH); 7.23–7.41 (m, 5H, ArH); 7.86 (d, J = 7.7 Hz, 2H, ArH); 9.37 (s, 1H, OH); 9.86 (s, 1H, OH); EIMS m/z 356 (M^+). Anal. Calcd for $C_{19}H_{16}O_7$: C, 64.04; H, 4.53; O, 31.43; Found: C, 64.21; H, 4.48; O, 31.31.

2.1.30. 4-(2-(3,5-Dihydroxyphenylformyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d28)

White powder, 60.8%, mp 182–185 °C; ^{1}H NMR (DMSO-d_6): 4.60 (d, J = 5.3 Hz, 4H, CH_2); 5.17 (s, 2H, CH_2); 6.43–6.52 (m, 1H, ArH); 6.83–6.92 (m, 2H, ArH); 7.21–7.64 (m, 3H, ArH); 7.85 (d, J = 7.1 Hz, 2H, ArH); 9.66 (s, 2H, OH); EIMS m/z 370 (M^+). Anal. Calcd for $C_{19}H_{16}O_7$: C, 64.04; H, 4.53; O, 31.43; Found: C, 64.14; H, 4.58; O, 31.48.

2.1.31. 4-(2-(3-Chlorophenylacetyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d29)

White powder, 57.9%, mp 141–142 °C; ^{1}H NMR ($CDCl_3$): 3.63 (s, 2H, CH_2); 4.26 (t, J = 4.5 Hz, 2H, CH_2); 4.42 (t, J = 4.5 Hz, 2H, CH_2); 4.73 (s, 2H, CH_2); 7.11 (d, J = 7.1 Hz, 1H, ArH); 7.21–7.26 (m, 2H, ArH); 7.31–7.41 (m, 3H, ArH); 7.72 (dd, J = 7.1 Hz, J = 8.6 Hz, 2H, ArH); EIMS m/z 372 (M^+). Anal. Calcd for $C_{20}H_{17}ClO_5$: C, 64.44; H, 4.60; Cl, 9.51; O, 21.46; Found: C, 64.30; H, 4.81; Cl, 9.56; O, 21.33.

2.1.32. 4-(2-(4-Fluorophenylacetyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d30)

White powder, 75.2%, mp 119–120 °C; ^{1}H NMR ($CDCl_3$): 3.62 (s, 2H, CH_2); 4.26 (t, J = 4.7 Hz, 2H, CH_2); 4.41 (t, J = 4.5 Hz, 2H, CH_2); 4.75 (s, 2H, CH_2); 6.97 (t, J = 8.7 Hz, 2H, ArH); 7.19 (t, J = 8.6 Hz, 2H, ArH); 7.31–7.42 (m, 3H, ArH); 7.79 (d, J = 8.4 Hz, 2H, ArH); EIMS m/z 356 (M^+). Anal. Calcd for $C_{20}H_{17}FO_5$: C, 67.41; H, 4.81; F, 5.33; O, 22.45; Found: C, 67.35; H, 4.75; F, 5.43; O, 22.47.

2.1.33. 4-(2-(4-Bromophenylacetyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d31)

Yellow oily liquid, 65.6%, mp 101–103 °C; ^{1}H NMR ($CDCl_3$): 3.60 (s, 2H, CH_2); 4.26 (t, J = 4.4 Hz, 2H, CH_2); 4.41 (t, J = 4.5 Hz, 2H, CH_2); 4.76 (s, 2H, CH_2); 7.09 (d, J = 8.4 Hz, 2H, ArH); 7.29–7.42 (m, 5H, ArH); 7.79 (dd, J = 7.0 Hz, J = 8.4 Hz, 2H, ArH); EIMS m/z 416 (M^+). Anal. Calcd for $C_{20}H_{17}BrO_5$: C, 57.57; H, 4.11; Br, 19.15; O, 19.17; Found: C, 57.64; H, 4.18; Br, 19.16; O, 19.02.

2.1.34. 4-(2-(4-Hydroxyphenylacetyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d32)

White powder, 49.7%, mp 149–151 °C; ^{1}H NMR (DMSO-d_6): 3.45 (s, 2H, CH_2); 4.28–4.33 (m, 4H, CH_2); 4.77 (s, 2H, CH_2); 6.65 (t, J = 8.4 Hz, 2H, ArH); 6.94 (d, J = 8.4 Hz, 2H, ArH); 7.19–7.30 (m, 3H, ArH); 7.72 (d, J = 7.3 Hz, 2H, ArH); EIMS m/z 354 (M^+). Anal. Calcd for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12; O, 27.09; Found: C, 67.92; H, 5.05; O, 27.03.

2.1.35. 4-(2-(3,4-Dimethoxyphenylacetyloxyl)ethoxy)-3-phenylfuran-2(5*H*)-one (d33)

Red-brown oily liquid, 77.0%; ^{1}H NMR ($CDCl_3$): 3.59 (s, 2H, CH_2); 3.85 (s, 6H,OCH_3); 4.26 (t, J = 4.5 Hz, 2H, CH_2); 4.41 (t, J = 4.5 Hz, 2H, CH_2); 4.71 (s, 2H, CH_2); 6.70–6.76 (m, 3H, ArH); 7.30–7.41 (m, 3H, ArH); 7.80 (t, J = 7.1 Hz, 2H, ArH); EIMS m/z 398 (M^+). Anal. Calcd for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57; O, 28.11; Found: C, 66.40; H, 5.51; O, 28.09.

2.1.36. 4-(2-(3-Chlorophenylacetyloxy)ethoxy)-3-(4-chlorophenyl)furan-2(5*H*)-one (d34)

White powder, 57.4%, mp 99–100 °C; ^{1}H NMR (DMSO-d_6): 3.62 (s, 2H, CH_2); 4.27–4.30 (m, 2H, CH_2); 4.40 (t, J = 4.2 Hz, 2H, CH_2); 4.73 (s, 2H, CH_2); 7.09 (d, J = 7.3 Hz, 1H, ArH); 7.18 (d, J = 8.5 Hz, 1H, ArH); 7.23–7.25 (m, 2H, ArH); 7.32–7.36 (m, 2H, ArH); 7.75–7.79 (m, 2H, ArH); EIMS m/z 406 (M^+). Anal. Calcd for $C_{20}H_{16}Cl_2O_5$: C, 58.99; H, 3.96; Cl, 17.41; O, 19.64; Found: C, 58.95; H, 3.93; Cl, 17.40; O, 19.72.

2.1.37. 4-(2-(3-Bromophenylacetyloxy)ethoxy)-3-(4-chlorophenyl)furan-2(5*H*)-one (d35)

White powder, 64.4%, mp 79–81 °C; ^{1}H NMR (DMSO-d_6): 3.62 (s, 2H, CH_2); 4.27–4.30 (m, 2H, CH_2); 4.44–4.47 (m, 2H, CH_2); 4.73 (s, 2H, CH_2); 7.13–7.15 (m, 2H, ArH); 7.32–7.37 (m, 2H, ArH); 7.38–7.41 (m, 2H, ArH); 7.76–7.80 (m, 2H, ArH); EIMS *m*/*z* 449 (M^+). Anal. Calcd for $C_{20}H_{16}BrClO_5$: C, 53.18; H, 3.57; Br, 17.69; Cl, 7.85; O, 17.71; Found: C, 53.25; H, 3.60; Br, 17.75; Cl, 7.45; O, 17.95.

2.1.38. 4-(2-(4-Hydroxyphenylacetyloxy)ethoxy)-3-(4-chlorophenyl)furan-2(5*H*)-one (d36)

White powder, 56.6%, mp 110–112 °C; ^{1}H NMR (DMSO-d_6): 3.57 (s, 2H, CH_2); 4.25 (t, *J* = 4.40 Hz, 2H, CH_2); 4.42 (t, *J* = 4.3 Hz, 2H, CH_2); 4.55 (s, 2H, CH_2); 5.65 (s, 1H, OH); 6.73 (d, *J* = 8.6 Hz, 2H, ArH); 7.05 (d, *J* = 8.4 Hz, 2H, ArH); 7.35 (d, *J* = 8.6 Hz, 2H, ArH); 7.78 (d, *J* = 8.6 Hz, 2H, ArH); EIMS *m*/*z* 388 (M^+). Anal. Calcd for $C_{20}H_{17}ClO_6$: C, 61.78; H, 4.41; Cl, 9.12; O, 24.69; Found: C, 61.70; H, 4.45; Cl, 9.11; O, 24.74.

2.1.39. 4-(2-(4-Fluorophenylacetyloxy)ethoxy)-3-(4-chlorophenyl)furan-2(5*H*)-one (d37)

White powder, 62.6%, mp 110–112 °C; ^{1}H NMR (DMSO-d_6): 3.62 (s, 2H, CH_2); 4.30 (t, *J* = 4.4 Hz, 2H, CH_2); 4.45 (t, *J* = 4.5 Hz, 2H, CH_2); 4.75 (s, 2H, CH_2); 6.95–7.00 (m, 2H, ArH); 7.19 (d, *J* = 8.6 Hz, 2H, ArH); 7.34 (d, *J* = 8.6 Hz, 2H, ArH); 7.80 (d, *J* = 8.8 Hz, 2H, ArH); EIMS *m*/*z* 390 (M^+). Anal. Calcd for $C_{20}H_{16}ClFO_5$: C, 61.47; H, 4.13; Cl, 9.07; F, 4.86; O, 20.47; Found: C, 61.29; H, 4.10; Cl, 9.12; F, 4.75; O, 20.74.

2.1.40. 4-(2-(4-Chlorophenylacetyloxy)ethoxy)-3-(4-chlorophenyl)furan-2(5*H*)-one (d38)

White powder, 39.2%, mp 138–139 °C; ^{1}H NMR (DMSO-d_6): 3.65 (s, 2H, CH_2); 4.29 (t, *J* = 4.5 Hz, 2H, CH_2); 4.45 (t, *J* = 4.5 Hz, 2H, CH_2); 4.76 (s, 2H, CH_2); 7.15 (d, *J* = 8.6 Hz, 2H); 7.25–7.36 (m, 4H, ArH); 7.80 (d, *J* = 8.8 Hz, 2H, ArH); EIMS *m*/*z* 406 (M^+). Anal. Calcd for $C_{20}H_{16}Cl_2O_5$: C, 58.99; H, 3.96; Cl, 17.41; O, 19.64; Found: C, 58.90; H, 3.93; Cl, 17.47; O, 19.70.

2.1.41. 4-(2-(4-Bromophenylacetyloxy)ethoxy)-3-(4-chlorophenyl)furan-2(5*H*)-one (d39)

White powder, 60.0%, mp 137–139 °C; ^{1}H NMR (DMSO-d_6): 3.60 (s, 2H, CH_2); 4.29 (t, *J* = 4.2 Hz, 2H, CH_2); 4.44 (t, *J* = 4.2 Hz, 2H, CH_2); 4.77 (s, 2H, CH_2); 7.09 (d, *J* = 8.4 Hz, 2H, ArH); 7.35 (t, *J* = 2.0 Hz, 2H, ArH); 7.41 (d, *J* = 8.4 Hz, 2H, ArH); 7.80 (d, *J* = 8.7 Hz, 2H, ArH); EIMS *m*/*z* 449 (M^+). Anal. Calcd for $C_{20}H_{16}BrClO_5$: C, 53.18; H, 3.57; Br, 17.69; Cl, 7.85; O, 17.71; Found: C, 53.20; H, 3.51; Br, 17.81; Cl, 7.65; O, 17.83.

2.1.42. 4-(2-(3-Chlorophenylformyloxy)ethoxy)-3-(4-chlorophenyl)furan-2(5*H*)-one (d40)

White powder, 53.0%, mp 108–110 °C; ^{1}H NMR (DMSO-d_6): 4.55 (t, *J* = 4.4 Hz, 2H, CH_2); 4.75 (t, *J* = 4.0 Hz, 2H, CH_2); 4.76 (s, 2H, CH_2); 7.11–7.19 (m, 2H, ArH); 7.29 (t, *J* = 2.0 Hz, 2H, ArH); 7.37–7.42 (m, 2H, ArH); 7.75–7.81 (m, 2H, ArH); EIMS *m*/*z* 393 (M^+). Anal. Calcd for $C_{19}H_{14}Cl_2O_5$: C, 58.03; H, 3.59; Cl, 18.03; O, 20.34; Found: C, 57.95; H, 3.62; Cl, 17.99; O, 20.44.

2.2. Preparation of the TyrRS and enzyme assay

S. aureus TyrRS was over-expressed in *Escherichia coli* and purified to near homogeneity (~98% as judged by SDS–PAGE) using standard purification procedures.[20] TyrRS activity was measured by aminoacylation using modifications to previously described methods.[21] The assays were performed at 37 °C in a mixture containing (final concentrations) 100 mM Tris/Cl pH 7.9, 50 mM KCl, 16 mM $MgCl_2$, 5 mM ATP, 3 mM DTT, 4 mg/ml *E. coli* MRE600 tRNA (Roche) and 10 μM L-tyrosine (0.3 μM L-[ring-3,5-^{3}H] tyrosine (PerkinElmer, Specific activity: 1.48–2.22 TBq/mmol), 10 μM carrier). TyrRS (0.2 nM) was pre-incubated with a range of inhibitor concentrations for 10 min at room temperature followed by the addition of pre-warmed mixture at 37 °C. After specific intervals, the reaction was terminated by adding aliquots of the reaction mix into ice-cold 7% trichloroacetic acid and harvesting onto 0.45 mm hydrophilic Durapore filters (Millipore Multiscreen 96-well plates) and counted by liquid scintillation. The rate of reaction in the experiments was linear with respect to protein and time with less than 50% total tRNA acylation. IC_{50}s correspond to the concentration at which half of the enzyme activity is inhibited by the compound. The results are presented in Table 1.

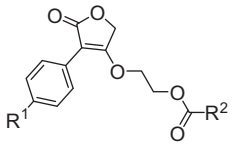
2.3. Antimicrobial activity

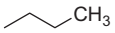
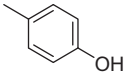
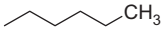
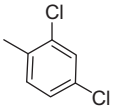
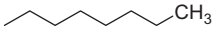
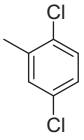
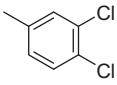
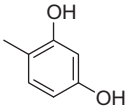
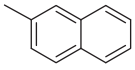
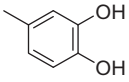
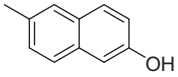
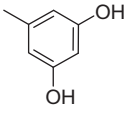
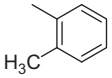
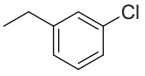
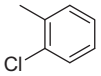
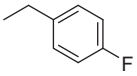
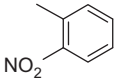
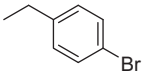
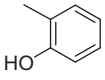
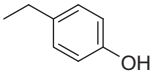
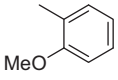
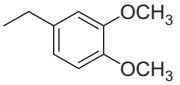
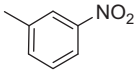
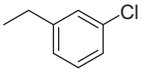
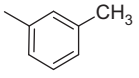
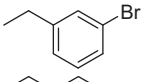
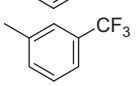
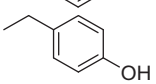
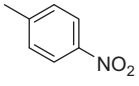
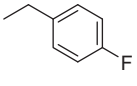
The antibacterial activities of the synthesized compounds were tested against Gram-positive bacterial strain (*S. aureus* ATCC 25923, kanamycin as positive control) and two Gram-negative bacterial strains (*E. coli* ATCC 35218, kanamycin as positive control and *P. aeruginosa* ATCC 27853, norfloxacin as positive control) using LB medium. The antifungal activities of the compounds were tested against *C. albican* ATCC 10231 (ketoconazol as positive control) using LB medium. The MTT proliferation assay was used to measure the MIC_{50}s of the test compounds by determining the absorbance of the cells in culture.[22] A stock solution of the synthesized compound (1000 μg/ml) in DMSO was prepared with different concentrations using sterilized liquid medium (50% (v/v) of DMSO in PBS). A specified quantity of the medium containing the test compound was added into 96-well plates, which was replaced by the sterilized liquid medium as blank control. Suspension of the microorganism was prepared to contain approximate 10^5 cfu/mL and applied to 96-well plates with serially diluted compounds (or blank control) to be tested and incubated at 37 °C. In the case of fungi, plates were incubated at 28 °C. Fifty microliter of PBS containing 3 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of 10% sodium dodecyl sulfate containing 5% isopropanol and 10 mol/mL HCl was added to extract the dye. After 8–10 h of incubation at room temperature, the control OD value (at 570 nm) should be controlled under 1.0–2.0 to achieve reliable results. The observed MIC_{50}s were presented in Table 2.

2.4. Protocol of docking study

The automated docking studies were carried out using AutoDock version 4.2. First, AutoGrid component of the program precalculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. A grid box of 60 × 60 × 60 Å size (*x*, *y* and *z*) with a spacing of 0.375 Å and grid maps were created representing the catalytic active target site region where the native ligand was embedded. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitor within the macromolecules. The genetic algorithm with local search (GA-LS) was chosen to search for the best conformers. The parameters were set using the software ADT (AutoDockTools package, version 1.5.4) on PC which is associated with AutoDock 4.2. Default settings were used with an initial population of 50 randomly placed individuals, a maximum number of 7.5×10^6 energy evaluations, and a maximum number of 2.7×10^4 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen. Results differing by less than 0.5 Å in positional root-mean-square deviation (RMSD) were clustered together and

Table 1
In vitro inhibitory activity data of the synthesized compounds against *S. aureus* TyrRS



Compound			IC_{50} (μM)	Compound			IC_{50} (μM)
Entry	R^1	R^2		Entry	R^1	R^2	
d3	H		84.6 ± 2.7	**d22**	H		11.2 ± 0.3
d4	H		47.8 ± 3.2	**d23**	H		16.5 ± 1.5
d5	H		41.0 ± 2.6	**d24**	H		5.2 ± 0.3
d6	H		12.8 ± 1.1	**d25**	H		33.4 ± 3.1
d7	H		26.0 ± 0.9	**d26**	H		50.2 ± 3.7
d8	H		17.7 ± 0.7	**d27**	H		75.3 ± 4.7
d9	H		16.8 ± 0.7	**d28**	H		6.7 ± 0.5
d10	H		>100	**d29**	H		>100
d11	H		56.8 ± 1.2	**d30**	H		93.6 ± 5.5
d12	H		18.2 ± 0.6	**d31**	H		48.1 ± 0.9
d13	H		41.4 ± 0.5	**d32**	H		>100
d14	H		64.7 ± 6.5	**d33**	H		83.8 ± 1.4
d15	H		>100	**d34**	Cl		1.1 ± 0.1
d16	H		8.1 ± 0.5	**d35**	Cl		3.5 ± 0.2
d17	H		4.3 ± 0.3	**d36**	Cl		7.9 ± 0.3
d18	H		5.2 ± 0.2	**d37**	Cl		5.7 ± 0.4

(*continued on next page*)

Table 1 (*continued*)

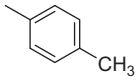
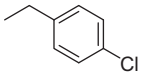
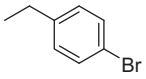
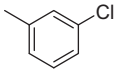
Compound			IC_{50} (μM)	Compound			IC_{50} (μM)
Entry	R^1	R^2		Entry	R^1	R^2	
d19	H		17.8 ± 0.9	**d38**	Cl		9.3 ± 0.7
d20	H		7.5 ± 0.2	**d39**	Cl		16.7 ± 1.5
d21	H		8.8 ± 0.7	**d40**	Cl		0.9 ± 0.05

Table 2
Inhibitory activity (IC_{50}) of the synthetic compounds against microbes

Compound	IC_{50} (μg/ml)			
	A	B	C	D
d3	>100	41.3	98.1	88.7
d4	>100	43.0	60.8	21.4
d5	48.8	>100	56.8	>100
d6	>100	17.8	13.4	>100
d7	18.6	22.8	44.8	>100
d8	>100	21.9	18.1	>100
d9	>100	16.1	15.8	>100
d10	19.3	>100	90.2	>100
d11	17.9	72.9	52.7	>100
d12	17.5	24.3	35.5	>100
d13	15.7	24.9	47.8	>100
d14	>100	>100	73.6	>100
d15	9.4	>100	>100	>100
d16	>100	15.8	16.7	>100
d17	23.3	17.5	2.4	>100
d18	5.0	24.5	11.4	2.7
d19	>100	18.3	37.7	23.4
d20	>100	8.2	13.8	18.1
d21	>100	17.2	14.5	78.4
d22	19.5	23.3	21.3	23.0
d23	12.8	18.2	21.3	22.9
d24	13.9	24.8	2.9	>100
d25	2.9	19.9	22.7	95.4
d26	>100	15.3	36.7	>100
d27	>100	19.9	>100	>100
d28	>100	2.2	14.4	>100
d29	>100	>100	83.1	>100
d30	>100	16.5	>100	>100
d31	78.7	15.4	22.0	>100
d32	53.6	22.5	68.1	>100
d33	>100	>100	>100	92.3
d34	7.6	5.2	2.5	2.5
d35	16.6	13.6	4.6	4.2
d36	>100	27.4	15.5	>100
d37	>100	14.9	13.2	15.6
d38	45.6	13.3	17.7	>100
d39	14.3	13.0	20.6	13.1
d40	4.3	2.0	1.5	1.2
Kanamycin	3.5	1.5	—	—
Norfloxacin	—	—	2.1	—
Ketoconazole	—	—	—	3.8

(A) *E. coli* ATCC 35218; (B) *S. aureus* ATCC 25923; (C) *P. aeruginosa* ATCC 27853; (D) *C. albicans* ATCC 10231.

the results of the most favorable free energy of binding were selected as the resultant complex structures.

3. Results and discussion

3.1. Chemistry

Thirty-eight 3-aryl-4-acyloxyethoxyfuran-2(5*H*)-ones were synthesized by the routes outlined in Scheme 2. Esters (**a1**–**a2**) were prepared by esterification of the corresponding sodium phenylacetate with 2-bromoacetate, which greatly improved the yield comparing with the previously reported method,[15] and the intermediates (**b1**–**b2** and **c1**–**c2**) were prepared according to previously method.[14,23] Subsequently, esterification of compounds (**c1**–**c2**) with appropriately substituted sodium carboxylate in DMSO gave 3-aryl-4-acyloxyethoxyfuran-2(5*H*)-ones (**d3**–**d40**), which were first reported and fully characterized by elementary analyses, EIMS and ^{1}H NMR.

3.2. Inhibitory activities of 3-aryl-4-acyloxyethoxyfuran-2(5*H*)-ones against TyrRS from *S. aureus*

All synthesized compounds (**d3**–**d40**) were tested for inhibitory activity against TyrRS from *S. aureus*. The IC_{50}s of these compounds are presented in Table 1. Compounds **d3**–**d33** were prepared to study the structure–activity relationship profiles of the acyloxy moiety (R^2). The compounds (**d3**–**d5**) with R^2 being an aliphatic group which show weak activity against TyrRS and the activities increase as carbon chain increase. Compound containing a 3-piperidyl (**d7**) or 3-naphthy (**d8**) group is slightly less active than compound with phenyl group (**d6**). In comparison with **d6**, introduction of any substituent at the 2-position of aryl ring (**d10**–**d14**) leads to a small reduction in potency. Compound containing a 3-trifluoromethyl group (**d17**) is more active than compounds with methyl group (**d16**) or nitro group (**d15**). Compounds with an electron-donating group at 4-position of aryl ring are less active than those with an electron-withdrawing group. As for compounds with two substituents in the aryl ring, compounds with no substituent at 4-position (**d24** and **d28**) show 2 to 12-fold more potent than others, indicating that these substituents may cause a steric clash with the protein. Substituting a benzyl group for the phenyl group (**d30** vs **d20**, **d32** vs **d22**, **d34** vs **d40**) obviously reduced the inhibitory activity.

Introduction of a chlorine atom at 4-position in the 3-phenyl group led to a significant increase in potency with respect to the corresponding unsubstituted compound (**d29** vs **d34**, **d30** vs **d37**, **d31** vs **d39**). Out of these compounds, compound **d40** was proved as the most potent, having IC_{50} of 0.9 ± 0.05 μM.

3.3. Antibacterial activity

All compounds were tested against representative Gram-positive organism (*S. aureus* ATCC 25923), two Gram-negative organisms (*E. coli* ATCC 35218; *P. aeruginosa* ATCC 27853) and a fungus (*C. albican* ATCC 10231), and the results are presented in Table 2. The results revealed that the compounds with strong inhibition against TyrRS also exhibit good antibacterial activities, especially against *P. aeruginosa* ATCC 27853. By compared to other compounds, **d34** and **d40** display good activity against Gram-positive organism, Gram-negative organism and fungus, and **d40** is the

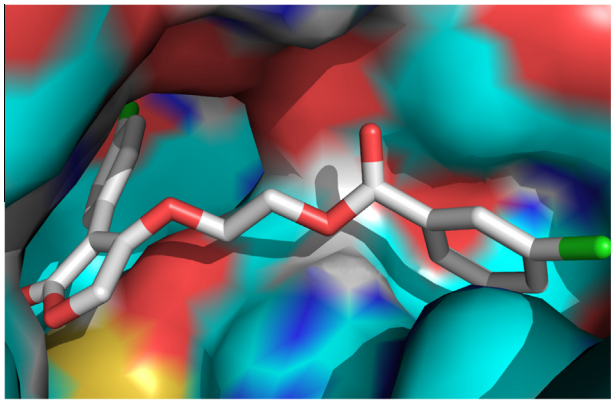


Figure 1. Binding mode of compound **d40** with TyrRS. The enzyme is shown as surface; while **d40** docked structures are shown as sticks. This figure was made using PyMol.

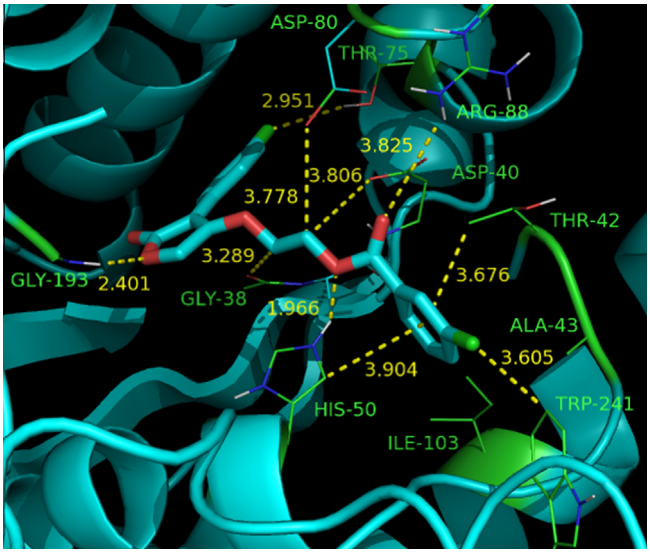


Figure 2. Binding mode of compound **d40** with TyrRS from *S. aureus*. For clarity, only interacting residues were labeled. Hydrogen bonding interactions are shown in dash. This figure was made using PyMol.

most potent compound, which is close to or slightly over that of marketed antibiotics. Out of the synthetic compounds, compound **d28** shows good activity against Gram-positive organism and compound **d17** shows good activity against Gram-negative organism especially against *P. aeruginosa* with IC_{50} value of 2.4 μg/mL.

3.4. Molecular docking

To explain the structure–activity relationships observed in 3-aryl-4-acyloxyethoxy-furan-2(5*H*)-ones, the compounds **d29**, **d34** and **d40** were selected to dock into the binding site of TyrRS which were performed on the binding model based on the TyrRS complex structure (1jij.pdb).[20] The results showed that the most active compound **d40** is held to the active pocket tightly by several hydrogen-bonding interactions and hydrophobic contacts. In the binding model, 3-chlorobenzene-ring moiety of **d40** is oriented towards the entrance cavity (Fig. 1), which hydrophobically interacts with Ile103, Trp241 and Ala43 residues. The benzene ring of 3-chlorobenzene-ring moiety group as hydrogen bond acceptor ishydrogen-bonded with CH_3 group of Thr42 and CH_2 group of His50 (3.676 and 3.904 Å). The chlorine atom in 3-chlorobenzene-ring

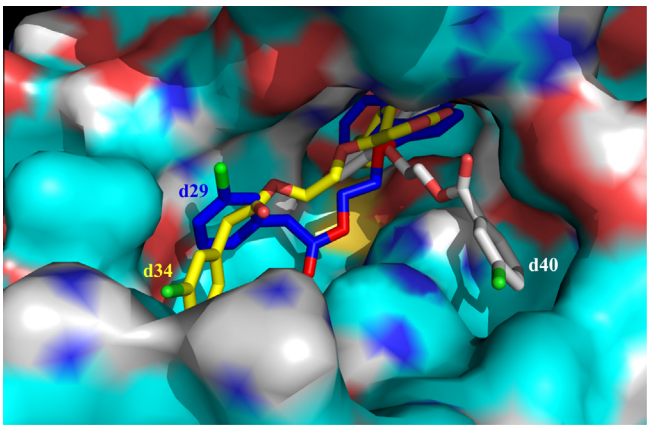


Figure 3. Binding mode of compound **d29**, **d34**, **d40** with TyrRS. The enzyme is shown as surface; while **d29**, **d34**, **d40** docked structures are shown as sticks. This figure was made using PyMol.

Table 3
Binding energy and Δ*G* of the synthetic compounds

Compound	**d29**	**d34**	**d40**
Δ*G*	−4.06	−5.06	−5.80
Binding energy	4.06	5.06	5.80

moiety as acceptor receives a weak hydrogen-bonding interaction from the CH_2 group of Trp241 residue at a distance of 3.605 Å. The unsaturated oxygen in 3-chlorophenylacetate moiety interacts with the backbone amino groups of Arg88 (3.825 Å), while the saturated oxygen in 3-chlorophenylacetate moiety forms another hydrogen-bond with NH groups of His50 residue having the H···O bond length of 1.966 Å. The two CH_2 groups in carbon chain as donors, forming three hydrogen bonds, the CH_2 group which closes to 3-chlorophenylacetate moiety interacts with Asp80 and Asp40 residues through hydrogen bonds at distances of 3.778 and 3.806 Å. The other CH_2 group interacts with Gly38 at adistance of 3.289 Å. The furanone-ring moiety interacts with Gly193 residue through a hydrogen bond which is observed between the NH group of residue Gly193 and saturated oxygen with H···O bond length of 2.401 Å (Fig. 2). The 4-chlorobenzene-ring moiety which is used as an acceptor, forming O–H···Cl hydrogen bonding interaction with Thr75 residue is located at the bottom of the active site cavity (Fig. 1).

Our modeling results revealed that the chlorine atom in 4-chlorobenzene-ring and the position of 3-chlorobenzene-ring had an important influence on the interactions of the protein–ligand complex and were crucial to the potency of TyrRS inhibitory activity (Fig. 3). The compound **d34**, substituting a benzyl group for the phenyl group of **d40**, located at a different position of the active site due to space steric hindrance effects. This may be causes that the enzyme inhibitory activity of **d34** is weaker than **d40**. Removal of the chlorine atom of 4-chlorobenzene-ring (**d34** vs **d29**) leads to a decrease in binding energy (Table 3), which is evidenced by a significant decrease in enzyme inhibitory activity.

4. Conclusions

In this study, thirty-eight 3-aryl-4-acyloxyethoxyfuran-2(5*H*)-ones were synthesized and characterized by elementary analyses, EIMS and ^{1}H NMR, and their biological activities were also evaluated as antimicrobial agents. Compound **d40**, 4-(2-(3-chlorophenylformyloxy)ethoxy)-3-(4-chlorophenyl)furan-2(5*H*)-one, displays good inhibitory activity against TyrRS and has a broad

spectrum against Gram-positive bacteria, Gram-negative bacteria and the yeast-like pathogenic fungus *C. albicans*. This indicated that introducing an ester to the 2(5*H*)-one core could efficiently extend the antimicrobial spectrum. Compound **d40** may be used as a lead for further modification for discovering agents with broad antimicrobial spectrum.

Acknowledgments

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