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Research paper

Synthesis and evaluation of adenosine containing 3-arylfuran-2(5*H*)-ones as tyrosyl-tRNA synthetase inhibitors

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ABSTRACT

Tyrosyl-tRNA synthetase (TyrRS) is an aminoacyl-tRNA synthetase family protein that possesses an essential role in bacterial protein synthesis. The synthesis, structure-activity relationship, and evolution of a novel series of adenosine-containing 3-arylfuran-2(5*H*)-ones as TyrRS inhibitors are described. Advanced compound **d3** from this series exhibited excellent affinity for TyrRS with IC_{50} of 0.61 ± 0.04 μM. Bacterial growth inhibition assays demonstrated that **d3** showed submicromolar antibacterial potency against *Escherichia coli* and *Pseudomonas aeruginosa*, and compared to the marketed antibiotics ciprofloxacin.

1. Introduction

Excessive use of antibiotics for the treatment of bacterial infections in both humans and animals have led to the emergence of drug-resistant bacteria [1,2]. Antibiotic resistance is becoming increasingly serious, and causes great damage to the human health [3]. However, a dramatic decline of efforts from pharmaceutical companies in pursuing development of new anti-infective agents in the past decades, and few novel antibiotics with significantly improved activity against resistant strains have been marketed. Modifications of existing classes of antibiotics have proven unsuccessful in providing potent activity against resistant strains that possess multiple resistance mechanisms [4,5]. With the purpose to avoid falling into a post-antibiotic era, efforts in this field has therefore shifted to find antimicrobials with novel mechanisms or chemical entities, thus avoiding many common efflux and low permeability mutations.

Tyrosyl-tRNA synthetase (TyrRS) is an aminoacyl-tRNA synthetase (aaRS) which ligates specific amino acid to its cognate tRNA molecules and possesses an important role in bacterial protein synthesis [6–8]. These enzymes are required for protein synthesis, and inhibition of any enzyme in the cell will lead to protein synthesis halt and cell growth arrest [9,10]. Therefore, TyrRS inhibitors as a new type of antibacterial agents have been receiving significant attention. In our group, 3-arylfuran-2(5*H*)-one-fluoroquinolone hybrids, 3-aryl-4-aminofuran-2(5*H*)-ones and 3-aryl-4-acyloxyethoxyfuran-2(5*H*)-ones have been reported as TyrRS inhibitors, showing excellent inhibition activity against pathogenic bacteria [3,7,9,11–13].

Recently, in a program for antibacterial screening, some adenosine analogues were (Scheme 1) found to have good inhibition activity against Gram-negative bacterial strains [14]. Intrigued by

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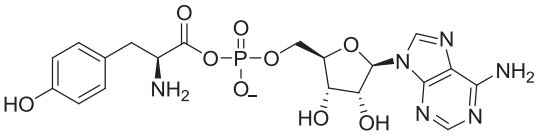
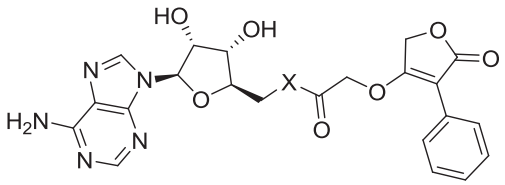


Scheme 1. Structure of tyrosyl adenylate.



Scheme 2. Structure of 3-arylfuran-2(5*H*)-one-adenosine.

this discovery, a series of adenosine containing 3-arylfuran-2(5*H*)-ones was designed and synthesized by emergence of adenosine with 3-arylfuran-2(5*H*)-one (Scheme 2). They were subsequently evaluated for biological activities against a representative Gram-positive organism (*Staphylococcus aureus* ATCC 6538) and two Gram-negative organisms (*Escherichia coli* ATCC 8739; *Pseudomonas aeruginosa* ATCC 9027). The results demonstrated some of the synthesized compounds show very good antibacterial activities.

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 ***b1-b11***

To a suspension of 3-aryl-4-hydroxyfuran-2(5*H*)-one (1.0 mmol) and K_2CO_3 (1.0 mmol) in acetone (20 mL) was added ethyl bromoacetate (1.5 mmol) then stirred at 60–70 °C for 3–5 h (monitored by TLC). The contents were poured into 30 mL aqueous solution to dissolve K_2CO_3, and were acidified to pH 5.0–6.0 with concentrated HCl. The mixture was extracted with EtOAc, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue (**1**) was dissolved in THF (20 mL) then basified to pH 9.0–10.0 with 0.1 M NaOH. The mixture was stirred at the room temperature for 1–2 h (monitored by TLC), acidified with 0.1 M HCl, and extracted thrice with 90 mL of EtOAc. Evaporation to dryness and flash chromatography ($CHCl_3/CH_3OH$, containing 0.3% of acetic acid) gave compounds **b1**-**b11** (Scheme 3).

2.1.2. Preparation of compound ***3***

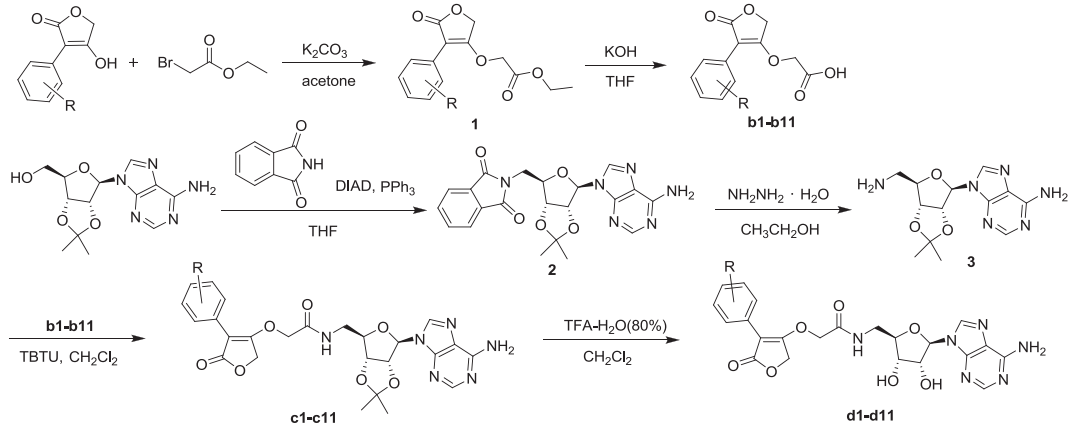
Triphenylphosphine (1.5 mmol), 2′,3′-*O*-isopropylideneadenosine (1.0 mmol) and phthalimide (1.0 mmol) were dissolved in anhydrous THF (20 mL) then cooled to 0 °C. Diisopropyl azodicarboxylate (DIAD 2.0 mmol) was added dropwise, and the obtained mixture was stirred at room temperature for 8 h. Evaporation to dryness and flash chromatography (AcOEt/petroleum ether) afforded compound **2**. To a stirring solution of **2** (1.0 mmol) in EtOH (1.0 mL) was added hydrazine hydrate (1.5 mmol) then the resulted mixture was warmed to 85 °C and stirred for an additional 2 h. Filtration and Evaporation to dryness furnished compounds **3**, which were used without further purification (Scheme 3).

2.1.3. General procedure for preparation of compounds ***c1-c11***

Compound **3** (1.0 mmol) was dissolved in 25 mL of dry CH_2Cl_2, followed by addition of 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 2.0 mmol), compound **b** (1.5 mmol) and triethylamine (3.0 mmol). The mixture was then stirred for 5–7 h at 60 °C (monitored by TLC). After removal of the solvent the residue was partitioned between EtOAc and water. The organic layer was then dried over $MgSO_4$ and concentrated under reduced pressure. Flash chromatography (EtOAc/petroleum ether) furnished compound **c1**-**c11** (Scheme 3) in good yield.

2.1.4. General procedure for preparation of compounds ***d1-d11***

To a solution of compound **c1**-**c11** (0.40 mmol) in CH_2Cl_2 was added 1.5 mL of trifluoroacetic acid (80%) then stirred at room temperature for 5–14 h (monitored by TLC). The reaction was quenched by addition of saturated aqueous Na_2CO_3 (30 mL) and was extracted twice with 100 mL of AcOEt. The organic layer was dried over $MgSO_4$ followed by removal of the solvent under reduced pressure. The residue was purified by column



Scheme 3. Synthetic route of 3-arylfuran-2(5*H*)-one-adenosine.

chromatography on silica gel to give compound **d1**-**d11** (Scheme 3).

*2.1.5. N-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-d] [1,3]- dioxol-4-yl)methyl)-2-(4-(2,4-dichlorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide **(c1)***

White powder, 33.4%, mp 191–192 °C; ^{1}H NMR (DMSO-d_6): 1.32 (s, 3H); 1.59 (s, 3H); 3.35–3.45 (m, 2H); 4.19 (td, J = 5.9 Hz, J = 3.3 Hz, 1H); 4.66 (d, J = 3.6 Hz, 2H); 4.92 (dd, J = 6.3 Hz, J = 3.3 Hz, 1H); 5.07 (s, 2H); 5.39 (dd, J = 6.4 Hz, J = 3.0 Hz, 1H); 6.14 (d, J = 2.9 Hz, 1H); 7.34 (d, J = 8.4 Hz, 1H); 7.38 (bs, 2H); 7.43 (dd, J = 8.3 Hz, J = 2.2 Hz, 1H); 7.66 (d, J = 2.2 Hz, 1H); 8.17 (s, 1H); 8.33 (s, 1H); 8.40 (t, J = 5.8 Hz, 1H); EIMS m/z 590 (M^+). Anal. Calcd for $C_{25}H_{24}Cl_2N_6O_7$: C, 50.77; H, 4.09; Cl, 11.99; N, 14.21; Found: C, 50.74; H, 4.09; Cl, 12.00; N, 14.22.

*2.1.6. N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)- 2-(4-(2,4-dichlorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide **(d1)***

White powder, 40.4%, mp 124–125 °C; ^{1}H NMR (DMSO-d_6): 3.38–3.53 (m, 2H); 3.96 (q, J = 5.0 Hz, 1H); 4.09 (q, J = 4.97 Hz, 1H); 4.70–4.79 (m, 3H); 5.06 (s, 2H); 5.31 (dd, J = 4.9 Hz, J = 1.7 Hz, 1H); 5.48 (dd, J = 6.2 Hz, J = 1.7 Hz, 1H); 5.87 (d, J = 5.9 Hz, 1H); 7.34 (bs, 2H); 7.36 (d, J = 8.3 Hz, 1H); 7.45 (dd, J = 8.3 Hz, J = 2.2 Hz, 1H); 7.67 (d, J = 2.1 Hz, 1H); 8.16 (s, 1H); 8.34 (s, 1H); 8.43 (t, J = 5.8 Hz, 1H); EIMS m/z 550 (M^+). Anal. Calcd for $C_{22}H_{20}Cl_2N_6O_7$: C, 47.93; H, 3.66; Cl, 12.86; N, 15.24; Found: C, 47.88; H, 3.66; Cl, 12.89; N, 15.23.

*2.1.7. N-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-d] [1,3]- dioxol-4-yl)methyl)-2-(4-(2-chlorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide **(c2)***

White powder, 42.4%, mp 140–142 °C; ^{1}H NMR (DMSO-d_6): 1.32 (s, 3H); 1.54 (s, 3H); 3.33–3.45 (m, 2H); 4.18 (td, J = 5.9 Hz, J = 3.3 Hz, 1H); 4.64 (d, J = 2.4 Hz, 2H); 4.92 (dd, J = 6.3 Hz, J = 3.3 Hz, 1H); 5.06 (s, 2H); 5.40 (dd, J = 6.4 Hz, J = 2.9 Hz, 1H); 6.14 (d, J = 2.9 Hz, 1H); 7.26–7.43 (m, 5H); 7.49 (dd, J = 7.4 Hz, J = 1.5 Hz, 1H); 8.17 (s, 1H); 8.33 (s, 1H); 8.37 (t, J = 5.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.72; 27.52; 41.20; 66.66; 69.09; 82.12; 83.36; 84.38; 89.47; 101.75; 114.04; 119.78; 127.40; 128.61; 129.76; 130.49; 132.86; 134.04; 140.57; 149.14; 153.19; 156.68; 166.74; 171.88; 175.01; EIMS m/z 557 (M^+). Anal. Calcd for $C_{25}H_{25}ClN_6O_7$: C, 53.91; H, 4.52; Cl, 6.37; N, 15.09; Found: C, 53.95; H, 4.52; Cl, 6.36; N, 15.08.

*2.1.8. N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)- 2-(4-(2-chlorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide **(d2)***

White powder, 58.3%, mp 177–178 °C; ^{1}H NMR (DMSO-d_6): 3.37–3.54 (m, 2H); 3.95 (dd, J = 5.4 Hz, J = 4.5 Hz, 1H); 4.08 (dd, J = 5.5 Hz, J = 4.6 Hz, 1H); 4.62–4.71 (m, 3H); 5.05 (s, 2H); 5.31 (d, J = 4.9 Hz, 1H); 5.48 (d, J = 6.2 Hz, 1H); 5.86 (d, J = 6.0 Hz, 1H); 7.29–7.43 (m, 5H); 7.50 (dd, J = 6.8 Hz, J = 2.0 Hz, 1H); 8.16 (s, 1H); 8.34 (s, 1H); 8.40 (t, J = 5.8 Hz, 1H); EIMS m/z 516 (M^+). Anal. Calcd for $C_{22}H_{21}ClN_6O_7$: C, 51.12; H, 4.10; Cl, 6.86; N, 16.26; Found: C, 51.16; H, 4.10; Cl, 6.85; N, 16.24.

*2.1.9. N-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-d] [1,3]- dioxol-4-yl)methyl)-2-(4-(3-chlorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide **(c3)***

White powder, 28.9%, mp 174–176 °C; ^{1}H NMR (DMSO-d_6): 1.31 (s, 3H); 1.55 (s, 3H); 3.38–3.52 (m, 2H); 4.23 (td, J = 6.0 Hz, J = 3.4 Hz, 1H); 4.88 (s, 2H); 4.97 (dd, J = 6.3 Hz, J = 3.4 Hz, 1H); 5.06 (s, 2H); 5.44 (dd, J = 6.3 Hz, J = 2.9 Hz, 1H); 6.17 (d, J = 2.8 Hz, 1H); 7.30–7.49 (m, 4H); 7.86 (dt, J = 7.8 Hz, J = 1.3 Hz, 1H); 7.94 (t, J = 1.9 Hz, 1H); 8.20 (s, 1H); 8.35 (s, 1H); 8.55 (t, J = 5.8 Hz, 1H); EIMS m/z 556 (M^+). Anal. Calcd for $C_{25}H_{25}ClN_6O_7$: C, 53.91; H, 4.52; Cl, 6.37; N, 15.09; N, 15.09; Found: C, 53.87; H, 4.52; Cl, 6.38; N, 15.10.

*2.1.10. N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)meth-yl)-2-(4-(3-chlorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide **(d3)***

White powder, 50.5%, mp 151–153 °C; ^{1}H NMR (DMSO-d_6): 3.48–3.52 (m, 2H); 4.01 (q, J = 5.1 Hz, 1H); 4.12 (dd, J = 5.2 Hz, J = 3.6 Hz, 1H); 4.71 (t, J = 5.6 Hz, 1H); 4.91 (d, J = 2.1 Hz, 2H); 5.06 (s, 2H); 5.20–5.70 (bs, 2H); 4.88 (d, J = 6.0 Hz, 2H); 7.25–7.35 (m, 3H); 7.42 (t, J = 7.9 Hz, 1H); 7.86 (dt, J = 7.8 Hz, J = 1.4 Hz, 1H); 7.94 (t, J = 1.9 Hz, 1H); 8.20 (s, 1H); 8.36 (s, 1H); 8.64 (t, J = 5.9 Hz, 1H); EIMS m/z 516 (M^+). Anal. Calcd for $C_{22}H_{21}ClN_6O_7$: C, 51.12; H, 4.10; Cl, 6.86; N, 16.26; Found: C, 51.16; H, 4.10; Cl, 6.85; N, 16.24.

*2.1.11. N-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-d]- [1,3]dioxol-4-yl)methyl)-2-(4-(4-chlorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide **(c4)***

White powder, 40.3%, mp 161–163 °C; ^{1}H NMR (DMSO-d_6): 1.32 (s, 3H); 1.55 (s, 3H); 3.40–3.51 (m, 2H); 4.23 (td, J = 6.0 Hz, J = 3.3 Hz,1H); 4.85 (s, 2H); 4.97 (dd, J = 6.4 Hz, J = 3.4 Hz, 1H); 5.04 (s, 2H); 5.44 (dd, J = 6.4 Hz, J = 2.9 Hz, 1H); 6.17 (d, J = 2.8 Hz, 1H); 7.39 (s, 2H); 7.46 (d, J = 8.7 Hz, 2H); 7.91 (d, J = 8.7 Hz, 2H); 8.20 (s, 1H); 8.35 (s, 1H); 8.53 (t, J = 5.8 Hz, 1H); EIMS m/z 556 (M^+). Anal. Calcd for $C_{25}H_{25}ClN_6O_7$: C, 53.91; H, 4.52; Cl, 6.37; N, 15.09; Found: C, 53.87; H, 4.52; Cl, 6.38; N, 15.11.

*2.1.12. N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)meth-yl)-2-(4-(4-chlorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide **(d4)***

White powder, 61.1%, mp 130–132 °C; ^{1}H NMR (DMSO-d_6): 3.49–3.53 (m, 2H); 4.02 (td, J = 5.4 Hz, J = 3.4 Hz, 1H); 4.10–4.15 (m, 1H); 4.69–4.74 (m, 1H); 4.89 (s, 2H); 5.05 (s, 2H); 5.39 (d, J = 4.9 Hz, 1H); 5.56 (d, J = 6.2 Hz, 1H); 5.89 (d, J = 6.0 Hz, 1H); 7.34 (s, 2H); 7.45 (d, J = 8.7 Hz, 2H); 7.90 (d, J = 8.7 Hz, 2H); 8.19 (s, 1H); 8.36 (s, 1H); 8.66 (t, J = 5.8 Hz, 1H); EIMS m/z 516 (M^+). Anal. Calcd for $C_{22}H_{21}ClN_6O_7$: C, 51.12; H, 4.10; Cl, 6.86; N, 16.26; Found: C, 51.16; H, 4.10; Cl, 6.85; N, 16.24.

*2.1.13. N-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-d]- [1,3]dioxol-4-yl)methyl)-2-(4-(3,4-diethoxyphenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide **(c5)***

White powder, 55.2%, mp 184–185 °C; ^{1}H NMR (DMSO-d_6): 1.24–1.37 (m, 9H); 1.54 (s, 3H); 3.36–3.48 (m, 2H); 3.96 (q, J = 7.0 Hz, 2H); 4.03 (q, J = 7.0 Hz, 2H); 4.18 (q, J = 5.8 Hz, 1H); 4.61 (d, J = 2.5 Hz, 2H); 4.92 (dd, J = 6.3 Hz, J = 3.2 Hz, 1H); 5.00 (s, 2H); 5.40 (dd, J = 5.4 Hz, J = 2.9 Hz, 1H); 6.13 (d, J = 2.9 Hz, 1H); 6.85 (s, 1H); 7.02 (s, 1H); 7.37 (s, 2H); 8.15 (s, 1H); 8.32 (s, 1H); 8.35 (t, J = 5.9 Hz, 1H); EIMS m/z 611 (M^+). Anal. Calcd for $C_{29}H_{34}N_6O_9$: C, 57.04; H, 5.61; N, 13.76; Found: C, 57.15; H, 5.50; N, 13.79.

*2.1.14. N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)meth-yl)-2-(4-(3,4-diethoxyphenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide **(d5)***

White powder, 66.3%, mp 160–162 °C; ^{1}H NMR (DMSO-d_6): 1.24–1.37 (m, 9H); 1.54 (s, 3H); 3.36–3.48 (m, 2H); 3.96 (q, J = 7.0 Hz, 2H); 4.03 (q, J = 7.0 Hz, 2H); 4.18 (q, J = 5.8 Hz, 1H); 4.62 (s, 2H); 4.65–4.72 (m, 1H); 5.00 (s, 2H); 5.29–5.36 (m, 1H); 5.47–5.55 (m, 1H); 5.86 (d, J = 5.9 Hz, 1H); 6.89 (s, 1H); 7.05 (s, 1H); 7.33 (s, 2H); 8.15 (s, 1H); 8.34 (s, 1H); 8.41 (t, J = 6.0 Hz, 1H); EIMS m/z 570 (M^+). Anal. Calcd for $C_{26}H_{30}N_6O_9$: C, 54.73; H, 5.30; N, 14.73; Found: C, 54.68; H, 5.30; N, 14.74.

2.1.15. N-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-d]- [1,3]dioxol-4-yl)methyl)-2-(4-(4-bromophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide ***(c6)***

White powder, 39.8%, mp 170–171 °C; ^{1}H NMR (DMSO-d_6): 1.32 (s, 3H); 1.55 (s, 3H); 3.45 (qt, J = 13.9 Hz, J = 6.2 Hz, 2H); 4.23 (td, J = 6.1 Hz, J = 3.3 Hz, 1H); 4.85 (s, 2H); 4.97 (dd, J = 6.4 Hz, J = 3.4 Hz, 1H); 5.04 (s, 2H); 5.45 (dd, J = 6.4 Hz, J = 2.8 Hz, 1H); 6.17 (d, J = 2.8 Hz, 1H); 7.38 (s, 2H); 7.60 (d, J = 8.7 Hz, 2H); 7.84 (d, J = 8.7 Hz, 2H); 8.20 (s, 1H); 8.35 (s, 1H); 8.53 (t, J = 5.8 Hz, 1H); EIMS m/z 600 (M^+). Anal. Calcd for $C_{25}H_{25}BrN_6O_7$: C, 49.93; H, 4.19; Br, 13.29; N, 13.97; Found: C, 49.89; H, 4.19; Br, 13.30; N, 13.98.

2.1.16. N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)meth-yl)-2-(4-(4-bromophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide ***(d6)***

White powder, 49.8%, mp 197–199 °C; ^{1}H NMR (DMSO-d_6): 3.45–3.55 (m, 2H); 4.01 (q, J = 5.3 Hz, 1H); 4.12 (td, J = 5.0 Hz, J = 3.3 Hz, 1H); 4.71 (q, J = 5.9 Hz, 1H); 4.88 (s, 2H); 5.04 (s, 2H); 5.38 (d, J = 4.9 Hz, 1H); 5.55 (d, J = 6.1 Hz, 1H); 5.88 (d, J = 6.0 Hz, 1H); 7.33 (s, 2H); 7.58 (d, J = 8.7 Hz, 2H); 7.83 (d, J = 8.7 Hz, 2H); 8.19 (s, 1H); 8.36 (s, 1H); 8.65 (t, J = 5.8 Hz, 1H); EIMS m/z 560 (M^+). Anal. Calcd for $C_{22}H_{21}BrN_6O_7$: C, 47.07; H, 3.77; Br, 14.23; N, 14.97; Found: C, 47.13; H, 3.77; Br, 14.22; N, 14.95.

2.1.17. N-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-d]- [1,3]dioxol-4-yl)methyl)-2-(4-(3,4-dichlorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide ***(c7)***

White powder, 38.4%, mp 134–136 °C; ^{1}H NMR (DMSO-d_6): 1.32 (s, 3H); 1.54 (s, 3H); 3.37–3.52 (m, 2H); 4.22 (td, J = 6.0 Hz, J = 3.3 Hz, 1H); 4.89 (s, 2H); 4.97 (dd, J = 6.3 Hz, J = 3.4 Hz, 1H); 5.06 (s, 2H); 5.44 (dd, J = 6.3 Hz, J = 2.9 Hz, 1H); 6.17 (d, J = 2.8 Hz, 1H); 7.37 (s, 2H); 7.67 (d, J = 8.6 Hz, 1H); 7.90 (dd, J = 8.6 Hz, J = 2.1 Hz, 1H); 8.13 (d, J = 2.1 Hz, 1H); 8.20 (s, 1H); 8.34 (s, 1H); 8.56 (t, J = 5.9 Hz, 1H); EIMS m/z 590 (M^+). Anal. Calcd for $C_{25}H_{24}Cl_2N_6O_7$: C, 50.77; H, 4.09; Cl, 11.99; N, 14.21; Found: C, 50.82; H, 4.10; Cl, 11.97; N, 14.19.

2.1.18. N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)meth-yl)-2-(4-(3,4-dichlorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide ***(d7)***

White powder, 54.7%, mp 210–212 °C; ^{1}H NMR (DMSO-d_6): 3.47–3.60 (m, 2H); 3.98–4.06 (m, 1H); 4.10–4.20 (m, 1H); 4.67–4.75 (m, 1H); 4.92 (s, 2H); 5.07 (s, 2H); 5.32–5.40 (m, 1H); 5.48–5.55 (m, 1H); 5.88 (d, J = 6.1 Hz, 1H); 7.33 (s, 2H); 7.65 (d, J = 8.5 Hz, 1H); 7.89 (dd, J = 8.5 Hz, J = 2.1 Hz, 1H); 8.13 (d, J = 2.0 Hz, 1H); 8.19 (s, 1H); 8.35 (s, 1H); 8.62–8.71 (m, 1H); EIMS m/z 550 (M^+). Anal. Calcd for $C_{22}H_{20}Cl_2N_6O_7$: C, 47.93; H, 3.66; Cl, 12.86; N, 15.24; Found: C, 47.88; H, 3.66; Cl, 12.88; N, 15.25.

2.1.19. N-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-d] [1,3]- dioxol-4-yl)methyl)-2-(4-(3,4-dimethoxyphenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide ***(c8)***

White powder, 40.5%, mp 133–136 °C; ^{1}H NMR (DMSO-d_6): 1.32 (s, 3H); 1.54 (s, 3H); 3.36–3.43 (m, 2H); 3.71 (s, 3H); 3.78 (s, 3H); 4.18 (td, J = 5.9 Hz, J = 3.3 Hz, 1H); 4.61 (d, J = 3.0 Hz, 2H); 4.93 (dd, J = 6.3 Hz, J = 3.3 Hz, 1H); 5.01 (s, 2H); 5.40 (dd, J = 6.3 Hz, J = 2.9 Hz, 1H); 6.14 (d, J = 2.9 Hz, 1H); 6.86 (s, 1H); 7.04 (s, 1H); 7.38 (s, 2H); 8.16 (s, 1H); 8.32 (s, 1H); 8.36 (t, J = 5.8 Hz, 1H); EIMS m/z 582 (M^+). Anal. Calcd for $C_{27}H_{30}N_6O_9$: C, 55.67; H, 5.19; N, 14.43; Found: C, 55.70; H, 5.18; N, 14.42.

2.1.20. N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)meth-yl)-2-(4-(3,4-dimethoxyphenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide ***(d8)***

White powder, 62.9%, mp 157–160 °C; ^{1}H NMR (DMSO-d_6): 3.43–3.53 (m, 2H); 3.72 (s, 3H); 3.79 (s, 3H); 3.92–3.98 (m, 1H); 4.06–4.13 (m, 1H); 4.62 (s, 2H); 4.65–4.72 (m, 1H); 5.00 (s, 2H); 5.29–5.36 (m, 1H); 5.47–5.55 (m, 1H); 5.86 (d, J = 5.9 Hz, 1H); 6.89 (s, 1H); 7.05 (s, 1H); 7.33 (s, 2H); 8.15 (s, 1H); 8.34 (s, 1H); 8.41 (t, J = 6.0 Hz, 1H); EIMS m/z 542 (M^+). Anal. Calcd for $C_{24}H_{26}N_6O_9$: C, 53.14; H, 4.83; N, 15.49; Found: C, 53.17; H, 4.83; N, 15.48.

2.1.21. N-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-d]- [1,3]dioxol-4-yl)methyl)-2-(4-(3-fluorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide ***(c9)***

White powder, 34.9%, mp 148–150 °C; ^{1}H NMR (DMSO-d_6): 1.32 (s, 3H); 1.54 (s, 3H); 3.46 (tq, J = 14.5 Hz, J = 6.5 Hz, 2H); 4.19–4.27 (m, 1H); 4.87 (s, 2H); 4.97 (dd, J = 6.3 Hz, J = 3.4 Hz, 1H); 5.05 (s, 2H); 5.44 (dd, J = 6.3 Hz, J = 2.9 Hz, 1H); 6.17 (s, 1H); 7.12 (td, J = 8.6 Hz, J = 2.7 Hz, 1H); 7.38 (s, 2H); 7.44 (dd, J = 14.5 Hz, J = 8.0 Hz, 1H); 7.71 (dd, J = 11.2 Hz, J = 1.7 Hz, 1H); 7.76 (dd, J = 7.9 Hz, J = 1.3 Hz, 1H); 8.20 (s, 1H); 8.34 (s, 1H); 8.55 (t, J = 5.7 Hz, 1H); EIMS m/z 540 (M^+). Anal. Calcd for $C_{25}H_{25}FN_6O_7$: C, 55.55; H, 4.66; F, 3.51; N, 15.55; Found: C, 55.60; H, 4.65; F, 3.51; N, 15.54.

2.1.22. N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)meth-yl)-2-(4-(3-fluorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide ***(d9)***

White powder, 63.2%, mp 161–163 °C; ^{1}H NMR (DMSO-d_6): 3.44–3.56 (m, 2H); 4.01 (q, J = 4.9 Hz, 1H); 4.12 (q, J = 4.6 Hz, 1H); 4.71 (q, J = 5.7 Hz, 1H); 4.90 (s, 2H); 5.05 (s, 2H); 5.34 (d, J = 4.7 Hz, 1H); 5.50 (d, J = 6.2 Hz, 1H); 5.88 (d, J = 5.9 Hz, 1H); 7.11 (td, J = 8.6 Hz, J = 2.8 Hz, 1H); 7.33 (s, 2H); 7.43 (dd, J = 14.6 Hz, J = 8.1 Hz, 1H); 7.71 (dt, J = 11.2 Hz, J = 2.2 Hz, 1H); 7.75 (d, J = 8.0 Hz, 1H); 8.19 (s, 1H); 8.36 (s, 1H); 8.62 (t, J = 5.8 Hz, 1H); EIMS m/z 500 (M^+). Anal. Calcd for $C_{22}H_{21}FN_6O_7$: C, 52.80; H, 4.23; F, 3.80; N, 16.79; Found: C, 52.77; H, 4.25; F, 3.82; N, 16.82.

2.1.23. N-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-d]- [1,3]dioxol-4-yl)methyl)-2-(4-(4-fluorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide ***(c10)***

White powder, 55.6%, mp 166–168 °C; ^{1}H NMR (DMSO-d_6): 1.32 (s, 3H); 1.55 (s, 3H); 3.36–3.56 (m, 2H); 4.23 (td, J = 5.9 Hz, J = 3.3 Hz, 1H); 4.84 (s, 2H); 4.97 (dd, J = 6.3 Hz, J = 3.4 Hz, 1H); 5.04 (s, 2H); 5.44 (dd, J = 6.4 Hz, J = 2.9 Hz, 1H); 6.17 (d, J = 2.8 Hz, 1H); 7.23 (t, J = 9.0 Hz, 2H); 7.38 (s, 2H); 7.91 (dd, J = 8.8 Hz, J = 5.7 Hz, 2H); 8.20 (s, 1H); 8.35 (s, 1H); 8.53 (t, J = 5.8 Hz, 1H); EIMS m/z 540 (M^+). Anal. Calcd for $C_{25}H_{25}FN_6O_7$: C, 55.55; H, 4.66; F, 3.51; N, 15.55; Found: C, 55.50; H, 4.67; F, 3.51; N, 15.57.

2.1.24. N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)meth-yl)-2-(4-(4-fluorophenyl)-5-oxo-2,5-dihydrofuran-3-yloxy)acetamide ***(d10)***

White powder, 47.9%, mp 184–186 °C; ^{1}H NMR (DMSO-d_6): 3.52 (t, J = 5.8 Hz, 2H); 4.03 (q, J = 5.3 Hz, 1H); 4.13 (q, J = 4.5 Hz, 1H); 4.72 (q, J = 5.8 Hz, 1H); 4.87 (s, 2H); 5.04 (s, 2H); 5.35 (d, J = 4.9 Hz, 1H); 5.51 (d, J = 6.2 Hz, 1H); 5.90 (d, J = 6.0 Hz, 1H); 7.22 (t, J = 9.0 Hz, 2H); 7.34 (s, 2H); 7.90 (dd, J = 8.8 Hz, J = 5.8 Hz, 2H); 8.20 (s, 1H); 8.36 (s, 1H); 8.62 (t, J = 5.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 41.50; 65.52; 69.20; 71.75; 73.15; 83.54; 88.42; 100.27; 115.33 (d, J = 21.1 Hz); 119.92; 126.27 (d, J = 3.2 Hz); 129.43 (d, J = 7.9 Hz); 140.77; 149.62; 153.04; 156.62; 161.38 (d, J = 245.0 Hz); 166.92; 172.47; 175.00; EIMS m/z 500 (M^+). Anal. Calcd for $C_{22}H_{21}FN_6O_7$: C, 52.80; H, 4.23; F, 3.80; N, 16.79; Found: C, 52.84; H, 4.21; F, 3.76; N, 16.78.

2.1.25. N-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-d]- [1,3]dioxol-4-yl)methyl)-3-bromobenzamide ***(c11)***

White powder, 63.2%, mp 181–182 °C; ^{1}H NMR (DMSO-d_6): 1.32

(s, 3H); 1.54 (s, 3H); 3.47–3.61 (m, 2H); 4.31 (td, J = 6.1 Hz, J = 3.4 Hz, 1H); 5.07 (dd, J = 6.4 Hz, J = 3.4 Hz, 1H); 5.49 (dd, J = 6.4 Hz, J = 2.6 Hz, 1H); 6.17 (d, J = 2.6 Hz, 1H); 7.36 (s, 2H); 7.44 (t, J = 7.9 Hz, 1H); 7.74 (ddd, J = 8.0 Hz, J = 2.1 Hz, J = 1.0 Hz, 1H); 7.84 (dt, J = 7.8 Hz, J = 1.3 Hz, 1H); 8.01 (t, J = 1.9 Hz, 1H); 8.10 (s, 1H); 8.34 (s, 1H); 8.79 (t, J = 5.7 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.75; 27.50; 41.99; 82.32; 83.50; 84.69; 89.41; 113.98; 119.73; 122.10; 126.91; 130.39; 131.05; 134.50; 136.79; 140.60; 149.26; 153.15; 156.59; 165.63; EIMS m/z 488 (M^+). Anal. Calcd for $C_{20}H_{21}BrN_6O_4$: C, 49.09; H, 4.33; Br, 16.33; N, 17.17; Found: C, 49.13; H, 4.33; Br, 16.32; N, 17.15.

2.1.26. *N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)meth-yl)-3-bromobenzamide* ***(d11)***

White powder, 43.6%, mp 166–167 °C; ^{1}H NMR (DMSO-d_6): 3.6s (bs, 2H); 4.10 (bs, 1H); 4.20 (bs, 1H); 4.77 (bs, 1H); 5.33 (s, 1H); 5.51 (bs, 1H); 5.89 (bs, 1H); 7.33 (s, 2H); 7.44 (t, J = 7.8 Hz, 1H); 7.74 (d, J = 8.0 Hz, 1H); 7.86 (d, J = 8.7 Hz, 1H); 8.04 (d, J = 1.9 Hz, 1H); 8.08 (s, 1H); 8.36 (s, 1H); 8.87 (bs, 1H); EIMS m/z 448 (M^+). Anal. Calcd for $C_{17}H_{17}BrN_6O_4$: C, 45.45; H, 3.81; Br, 17.79; N, 18.71; Found: C, 45.48; H, 3.81; Br, 17.78; N, 18.69.

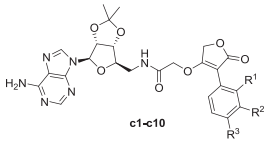
2.2. Extraction of the TyrRS and enzyme assay

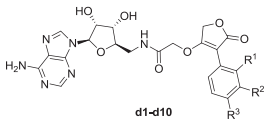
TyrRS was over-expressed in *E. coli* and purified to near homogeneity (~98% as judged by SDS-PAGE) using standard purification procedures. TyrRS activity was measured by aminoacylation using modifications to previously described methods [15]. The assays were performed at 37 °C in a mixture containing (final concentrations) 100 mM Tris/Cl pH 7.9, 50 mM KC1, 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: 1.48–2.22TBq/mmol), 10 μM carrier). TyrRS (0.2 nM) was preincubated 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.

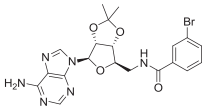
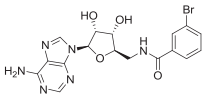
2.3. Antimicrobial activity

The antibacterial activities of the synthesized compounds were tested against Gram-positive bacterial strain (*S. aureus* ATCC 6538, penicillin G sodium as positive control) and two Gram-negative bacterial strains (*E. coli* ATCC 8739, ciprofloxacin as positive control *and P. aeruginosa* ATCC 9027, ciprofloxacin 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 [16]. 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 μL 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 on SpectraMax® Plus384) should be controlled under 1.0–2.0 to achieve reliable results. The observed MIC_{50}s were presented in Table 2.

Table 1
In vitro inhibitory activity data of the synthesized compounds against TyrRS and .





entry	R^1	R^2	R^3	IC_{50} (μM)
c1	Cl	H	Cl	83.8 ± 5.0
c2	Cl	H	H	24.3 ± 1.5
c3	H	Cl	H	3.8 ± 0.19
c4	H	H	Cl	22.7 ± 1.3
c5	H	OCH_2CH_3	OCH_2CH_3	>100
c6	H	H	Br	92.6 ± 6.1
c7	H	Cl	Cl	33.4 ± 1.1
c8	H	OCH_3	OCH_3	>100
c9	H	F	H	56 ± 3.7
c10	H	H	F	>100
d1	Cl	H	Cl	26.1 ± 1.3
d2	Cl	H	H	7.6 ± 0.2
d3	H	Cl	H	0.61 ± 0.04
d4	H	H	Cl	5.2 ± 0.3
d5	H	OCH_2CH_3	OCH_2CH_3	>100
d6	H	H	Br	29.1 ± 1.8
d7	H	Cl	Cl	9.1 ± 0.4
d8	H	OCH_3	OCH_3	>100
d9	H	F	H	16.4 ± 1.0
d10	H	H	F	31.7 ± 2.0
c11				>100
d11				94.5 ± 6.2

2.4. Assays for in vitro toxicity

Compounds were assayed in HeLa cells to determine the potential toxic effect in vitro. Cells (about 5 × 10^4 cells/mL) were cultured on 96-well plate and incubated at 37 °C with 5% CO_2 overnight and then were treated with selected compounds at different concentrations for 24 h. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to evaluate the cell viability. The absorbance of each well was measured at 570 nm on a microplate reader (SpectraMax® Plus384). The viability index of untreated cells (cells plus medium) was tested as control. Survival ratios are expressed in percentages with respect to untreated cells. EC_{50} values were determined from replicates of 6 wells from at least three independent experiments.

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

Compound	MIC_{50} (μg/mL)		
	A	B	C
c1	>100	90.9	>100
c2	15.8	25.6	30.3
c3	2.8	4.4	7.1
c4	20.3	21.4	39.6
c5	>100	>100	>100
c6	98.6	>100	>100
c7	60.2	63.7	90.7
c8	>100	>100	>100
c9	83.6	82.8	>100
c10	>100	>100	>100
d1	29.4	33.6	55.4
d2	8.8	10.2	17.3
d3	0.07	0.51	1.2
d4	4.6	6.8	13.7
d5	>100	>100	>100
d6	46.6	40.5	75.1
d7	10.5	13.6	20.7
d8	>100	>100	>100
d9	13.9	15.2	23.7
d10	39.2	47.4	72.7
c11	>100	>100	>100
d11	>100	>100	>100
ciprofloxacin	0.1	0.64	/
penicillin G sodium	/	/	0.57

(A) *E. coli* ATCC 8739; **(B)** *P. aeruginosa* ATCC 9027; **(C)** *S. aureus* ATCC 6538.

2.5. Protocol of docking study

The X-ray structure of TyrRS from *E. coli* was downloaded from the Protein Data Bank (PDB code: 1vbm) [17] and was modified by adding hydrogen atoms and removing water as well as cocrystallized substrate (tyrosinyl adenylate) using SYBYL-X version 2.1.1 software suite (Tripos, Inc. St. Louis, MO) [18]. The active site was defined as all the amino acid residues confined within a 5 Å radius sphere centered about tyrosinyl adenylate, and the composite structure without original ligand was utilized as the in silico model for docking studies. Default parameters and values within the minimization dialogue were used except where otherwise mentioned. The docked conformations of ligands were evaluated and ranked using Surflex-Dock and four scoring functions implemented in the CSCORE software module within the SYBYL-X environment. The CSCORE module allowed consensus scoring that integrated multiple well-known scoring functions such as ChemScore, D-Score, G-Score and PMF-Score to evaluate docked ligand conformations [19].

3. Results and discussion

3.1. Chemistry

Twenty-two 3-arylfuran-2(5*H*)-one-adenosine were synthesized by the route outlined in Scheme 3. Compounds **b1**-**b11** were prepared by hydrolysis of the corresponding esters (**1**) that were prepared by esterification of the 3-aryl-4-hydroxyfuran-2(5*H*)-one with ethyl bromoacetate, and compound **3** was prepared by hydrazinolysis of compound **2** which were prepared under Mitsunobu conditions. Subsequently, condensation of a specific compound **b** with **3** in CH_2Cl_2 gave the corresponding compound **c**. Deprotection of **c1**-**c11** furnished compounds **d1**-**d11** in the presence of trifluoroacetic acid.

3.2. Inhibitory activities against TyrRS

Compounds **c1**-**c11** and **d1**-**d11**, bearing different substituent in the 3-arylfuran-2(5*H*)-one moiety, were synthesized and evaluated for their inhibition against TyrRS, and IC_{50}s are presented in Table 1. In general, compounds **d1**-**d10** showed more active than the corresponding 2′,3′-isopropylidene analogues (**c1**-**c11**), most likely due to the isopropylidene blocked interactions between hydrogen-bond donors and acceptors. The 3-chloro derivative **d3** exhibited submicromolar potency, with IC_{50} of 0.51 μM against TyrRS. Replacement of the chlorine atom with a fluorine atom (**d9**) resulted in 30-fold higher IC_{50} Values. Similarly, replacement of the chlorine atom at the 4-position with a fluorine or bromine atom significantly reduced the potency. The corresponding chlorine analogues at the *ortho*- (**d2**) and *para*- (**d4**) positions led to a 10-fold decrease in potency. The introduction of another chlorine atom at the 4-position in the derivative **d3** led to compound **d7**. This new molecule had a decreased potency by more than 1 log unit. Replacement of the chlorine with a methoxyl or ethoxyl group led to inactive compounds (compounds **d8** and **d5**), and was supported by a pairwise comparison between **d7** and **d8** (**d7** *vs* **d5**), most likely due to steric factors given the narrowness of the binding pocket. Finally, replacement of side chain containing 3-arylfuran-2(5*H*)-one moiety with a benzoyl fragment (**c11** and **d11**) was detrimental to biochemical activity, leading to IC_{50} values greater than 100 μM.

3.3. Bacterial growth inhibition

All compounds were evaluated against three representative pathogenic bacterial strains, including a Gram-positive organism (*S. aureus* ATCC 6538) and two Gram-negative organisms (*E. coli* ATCC 8739*; P. aeruginosa* ATCC 9027) and results are presented in Table 2. Three derivatives (**c3**, **d3** and **d4**) were characterized by a good inhibition activity against *E. coli* and *P. aeruginosa*, showing MIC_{50} values lower than 7 μg/mL. Compound **d3**, characterized by a chlorine atom in 3-position of the benzene ring, showed the best antibacterial efficacy with MIC_{50} value of 0.07 μg/mL against *E. coli* and 0.51 μg/mL against *P. aeruginosa* respectively, which has comparable activity to that of marketed antibiotics ciprofloxacin.

3.4. Cytotoxic assays

After the evaluation of the synthesized compounds as antibacterial agents, the potent compounds were selected to ascertain on HeLa cells for their cytotoxicity, and the results are shown in Table 3. None of the derivatives showed significant toxicity to the HeLa human tumor cell line. Notably, the most active antibacterial agent, compound **d3**, shows EC_{50} over 100 μM, which is more than 700-fold higher than its MIC_{50} value (0.07 μg/mL = 0.136 μM). Compound **d3** were thus considered as noncytotoxic.

3.5. Molecular docking

The most active compound **d3** identified in enzyme assays and the inactive compound **d8** were selected to perform molecular docking, and alignments for **d3** and **d8** in the active sites are shown in Fig. 1. The planar adenosine moieties of compounds **d3** and **d8** arrange in a similar orientation, and occupy an opening pocket, the adenosine binding pocket of tyrosyladenylate. While significant

Table 3
Cytotoxicity data of selected compounds.

Compound	EC_{50} (μM)	Compound	EC_{50} (μM)
c3	>100	**d4**	>100
d2	98.3 ± 6.9	**d7**	>100
d3	>100	**d9**	80.2 ± 4.7

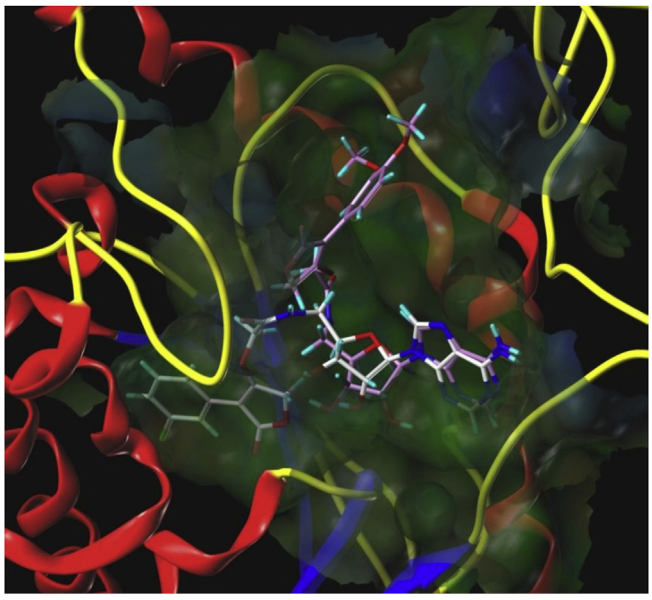


Fig. 1. Comparison of binding modes for compounds **d3** (white) and **d8** (light violet). This figure was made using SYBYL. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

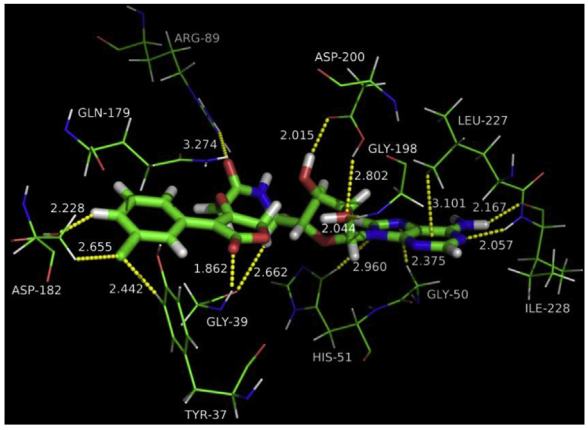


Fig. 2. Binding mode of compound **d3** in TyrRS active site. This figure was made using PyMol.

changes in ribose side-chain conformation are observed, most likely as a result of the changing spatial environment imparted by 3,4-dimethoxy groups of 3-arylfuran-2(5*H*)-one moiety. The ribose side-chain of **d8** extends outside of the active pocket, leading to a marked decrease of binding affinities in comparison with **d3** (Fig. 1). The absence of significant interactions between **d8** and TyrRS may attribute to the inactivity of **d8**. On the other hand, the ribose side-chain of **d3** occupies the tyrosine binding pocket of tyrosyladenylate, and multiple hydrogen bonds, including nonclassical hydrogen bonds, are formed between the 3-arylfuran-2(5*H*)-one moiety and Tyr37, Gly39 and Asp182 (Fig. 2). In addition, strong hydrophobic interactions are observed between benzene ring in 3-arylfuran-2(5*H*)-one and Gln179. Compound **d3** was therefore endowed with excellent potency.

4. Conclusions

By application of a ligand-based search for novel TyrRS inhibitors, 22 compounds were synthesized to experimentally assess their inhibitory potency against TyrRS and selected microorganisms. In comparison with adenosine containing 3-arylfuran-2(5*H*)-ones, protection of 2′,3′-OHs by isopropylidene led to a significant decrease in potency against TyrRS. The inhibitor bearing a 3-chlorophenylfuran-2(5*H*)-one moiety (**d3**) with an IC_{50} in submicromolar range showed excellent antibacterial potency compared to that of the marketed antibiotics ciprofloxacin, suggesting a promising scaffold for development of efficient antibacterial drugs and a promising candidate for further structure optimizations. Molecular dockings performed on **d3** and **d8** revealed an interaction pattern explaining the high affinity **d3**.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.03.074.

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