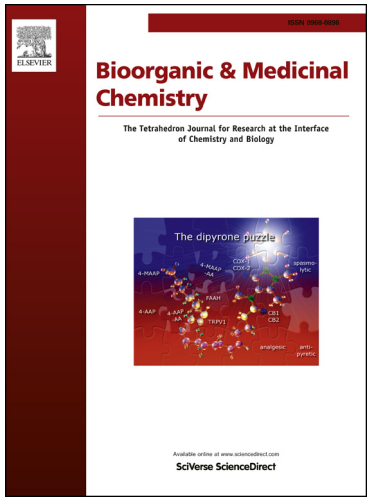

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Adenosine analogues as inhibitors of tyrosyl-tRNA synthetase: design, synthesis and antibacterial evaluation

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Abstract

Herein we describe the synthesis and evaluation of a series of adenosine analogues for in vitro antibacterial activity against *Staphylococcus aureus*, *Escherichia coli and Pseudomonas aeruginosa*. Out of these compounds, compound **c6** has much stronger antibacterial potency against *Pseudomonas aeruginosa* than ciprofloxacin, and was determined to target tyrosyl-tRNA synthetase with IC_{50} of 0.8±0.07 μM. Structure-activtiy relationship analysises suggested that introduction of a fluorine atom at the 3′-position of benzene ring of the phenylacetyl moiety significantly increased affinities to the enzyme. In comparison with isopropylidene analogs, 2′,3′-deprotected compounds displayed higher inhibitory activity. Molecular dockings provided an explanation for observations in biological assays.

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Keywords: adenosine analogues; tyrosyl-tRNA synthetase inhibitors; antibacterial; molecular docking;

1. Introduction

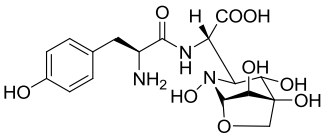
Infectious disease is the second major cause of death worldwide and the third leading cause of death in developed countries [1]. Since the introduction of the first sulfonamide and penicillin in 1935 and 1940, many synthetic and natural antibiotics have been lauched and have saved millions of lives. The ability of bacteria to evade any form of established therapy has become apparent, causing a rapid emergence and worldwide diffussness of pathogens resistant to one or more antibiotics. Despite the impressive therapeutic successes of antibiotics throughout recent decades, acute infectious diseases account for 25% of deaths worldwide, killing 13-17 million people per year [2]. Therefore, there is a pressing need for antiinfective drugs with a new mechanism or/and a novle scaffold.

Aminoacyl-tRNA synthetases (aaRSs) are a kind of key enzymes which catalyze the transfer of amino acids to their cognate tRNAs in the process of protein synthesis [3-5]. These enzymes are essential for protein synthesis， and this process was forced to terminate when these enzymes were restrained [6-7]. A selective inhibition of aaRSs in bacteria is feasible on account of the different functions between prokaryotes aaRSs and eucaryon. This concept is proven by the success of the broad-spectrum antibacterial drug mupirocin, which targets the bacterial isoleucyl-tRNA synthetase [8-10]. Therefore, aaRSs inhibitors as a new type of antibacterial agents have been receiving significant attention.

To our knowledge, previous studies have identified a few inhibitors against bacterial TyrRS including the naturally compound SB-219383 (Scheme 1) and several synthetic

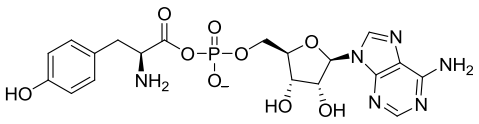
compounds. Although SB-219383 and its semi-synthetic analogues exhibit IC_{50s}<1 nM against *Staphylococcus aureus* tyrosyl-tRNA synthetase (TyrRS), they show very weak *in vitro* activity against bacterial intact cells such as *Staphylococci* and *Streptococci* [11-13]. This encourages us to search the new antiinfective drugs targeting TyrRS.

Scheme 1 structure of SB-219383



It is well known that the TyrRS acts as other tRNA synthetase enzymes by a two-step mechanism. In the first stage, the enzyme recognises a tyrosine and activates it by reaction with ATP to produce a tyrosyl adenylate (Scheme 2). In the second step, the enzyme catalyzes the transfer of the tyrosine onto its cognate tRNA to form the desired product. Therefore, a series of analogues of adenosine which was similar to the tyrosyl-adenylate intermediate have been selected as a potential target in a structure-based drug design approach. In this context, the side-chain of the tyrosyl-adenylate intermediate was modified to afford analogues **b1**-**b14** and **c1**-**c25**. 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.

Scheme 2 structure of tyrosyl adenylate



2. Materials and methods

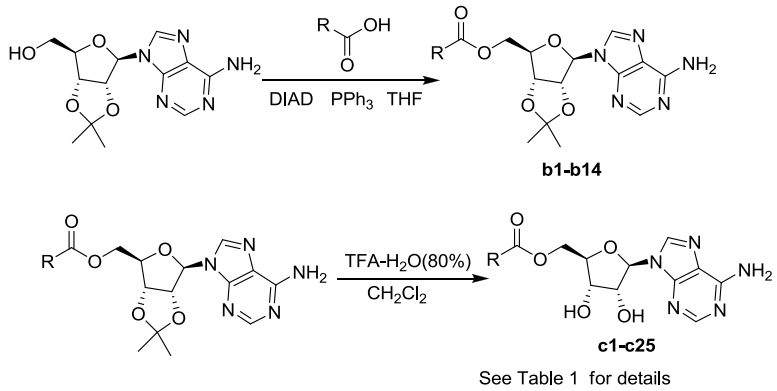
2.1. Chemistry

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 (200-300 mesh) purchased from Qingdao Haiyang Chemical Co.,Ltd (China). The quantity of silica gel used was 30-60 times the weight charged on the column. Then, the eluates were monitored using thin-layer chromatography (TLC) using silica gel GF254 plates from Qingdao Haiyang Chemical Co., Ltd (China) with an UV lamp (254 nm). Melting points (uncorrected) were determined on a XT4 MP apparatus (Taike Corp., Beijing, China). EI mass spectra were obtained on a Waters GCT mass spectrometer, and NMR spectra were recorded on Bruker AV-400 and AV600 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 b

Triphenylphosphine (1.5 mmol), 2',3'-*O*-isopropylideneadenosine (1.0 mmol), and an appropriately substituted benzeneacetic acids (1.0 mmol) were dissolved in anhydrous THF (20 mL). After the solution was cooled in an ice bath, diisopropyl azodicarboxylate (DIAD 2.0 mmol) was added dropwise, and the resulted mixture was stirred at room temperature for several hours (monitored by TLC). Evaporation to dryness and flash chromatography (AcOEt/petroleum ether, from 5/1 to 3/1) afforded compounds **b** (Scheme 3).

Scheme 3 Synthetic route of analogues of adenosine



2.1.2. General procedure for preparation of compounds c

A solution of dichloromethane containing compound **b** (0.40 mmol) in 1.5 mL of 80% aqueous trifluoroacetic acid was stirred at room temperature until complete by TLC. Saturated sodium bicarbonate (30 mL) was added to neutralize trifluoroacetic acid. The solution was 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 compound **c** (Scheme 3) in yields of 65%-80%.

2.1.3.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1, 3]dioxol-4-yl)methyl 2-(3-bromophenyl)acetate (b1)

White powder, 63.1%, mp 174-176°C; ^{1}H NMR (DMSO-d_6): 1.33 (s, 3H, CH_3); 1.54 (s, 3H, CH_3); 3.56-3.67 (m, 2H, CH_2); 4.16-4.20 (m, 1H, CH); 4.26-4.30 (m, 1H, CH); 4.36-4.39 (m, 1H, CH); 5.01-5.04 (m, 1H, CH); 5.42-5.44 (m, 1H, CH); 6.19 (s, 1H, CH); 7.16 (d, *J*=8.3Hz, 2H, ArH); 7.39 (s, 2H, NH_2); 7.47 (d, *J*=8.2Hz, 2H, ArH); 8.17 (s, 1H, CH ^{purine}); 8.30 (s, 1H, CH ^{purine}); EIMS m/z 503 (M^+). Anal. Calcd for $C_{21}H_{22}BrN_5O_5$: C, 50.01; H, 4.40; Br, 15.84; N, 13.89; Found: C, 50.17; H, 4.42; Br, 15.78; N, 13.84.

2.1.4.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1, 3]dioxol-4-yl)methyl 2-(3-chlorophenyl)acetate (b2)

White powder, 82.4%, mp 144-146°C; ^{1}H NMR (DMSO-d_6): 1.33 (s, 3H, CH_3); 1.55 (s, 3H, CH_3); 3.64-3.74 (m, 2H, CH_2); 4.18-4.22 (m, 1H, CH); 4.28-4.32 (m, 1H, CH); 4.36-4.40 (m, 1H, CH); 5.03-5.05 (m, 1H, CH); 5.43-5.45 (m, 1H, CH); 6.19 (d, *J*=2.2Hz, 1H, CH); 7.20-7.22 (m, 1H, ArH); 7.37 (s, 2H, NH_2); 7.52-7.55 (m, 3H, ArH); 8.17 (d, *J*=1.9Hz, 1H, CH ^{purine}); 8.30 (s, 1H, CH ^{purine}); EIMS m/z 459 (M^+). Anal. Calcd for $C_{21}H_{22}ClN_5O_5$: C, 54.85; H, 4.82; Cl, 7.71; N, 15.23; Found: C, 54.67; H, 4.82; Cl, 7.74; N, 15.29.

2.1.5.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl) methyl 2-(3-bromophenyl)acetate (c1)

White powder, 55.4%, mp 168-170°C; ^{1}H NMR (DMSO-d_6): 3.72 (s, 2H, CH_2); 4.10 (s, 1H, CH); 4.26 (s, 2H, CH_2); 4.36 (d, *J*=11.6Hz, 1H, CH); 4.66 (s, 1H, CH); 5.38 (s, 1H, OH); 5.57 (s, 1H, OH); 5.91 (d, 1H, CH); 7.24-7.46 (m, 6H, ArH and NH_2); 8.14 (d, *J*=2.6Hz, 1H, CH ^{purine}); 8.30 (d, *J*=3.2Hz, 1H, CH ^{purine}); EIMS m/z 463 (M^+). Anal. Calcd for $C_{18}H_{18}BrN_5O_5$: C, 46.57; H, 3.91; Br, 17.21; N, 15.08; Found: C, 46.63; H, 3.90; Br, 17.27; N, 15.04.

2.1.6.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl) methyl 2-(3-chlorophenyl)acetate (c2)

White powder, 72.8%, mp 167-168°C; ^{1}H NMR (DMSO-d_6): 3.69 (s, 2H, CH_2); 4.11 (s, 1H, CH); 4.22-4.30 (m, 2H, CH_2); 4.38 (d, *J*=11.8Hz, 1H, CH); 4.67 (t, *J*=4.9Hz, 1H, CH); 5.42 (d, *J*=5.4Hz, 1H, OH); 5.59-5.63 (m, 1H, OH); 5.93 (s, 1H, CH); 7.19-7.22 (m, 1H, ArH); 7.31-7.33 (m, 5H, ArH and NH_2); 8.16 (d, *J*=5.0Hz, 1H, CH ^{purine}); 8.32 (d, *J*=3.2Hz, 1H, CH ^{purine}); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 40.02; 64.90; 70.70; 73.28; 81.89; 88.17; 119.63; 127.31; 128.69; 129.83; 130.55; 133.29; 137.11; 140.22; 149.83; 153.15; 156.58; 171.15; EIMS m/z 419 (M^+). Anal. Calcd for $C_{18}H_{18}ClN_5O_5$: C, 51.50; H, 4.32; Cl, 8.44; N, 16.68; Found: C, 51.34; H, 4.33; Cl, 8.47; N, 16.72.

2.1.7.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 2-(4-fluorophenyl)acetate (b3)

White powder, 80.5%, mp 154-156°C; ^{1}H NMR (DMSO-d_6): 1.33 (s, 3H, CH_3); 1.54 (s, 3H, CH_3); 3.57-3.67 (m, 2H, CH_2); 4.16-4.20 (m, 1H, CH); 4.26-4.30 (m, 1H, CH); 4.37-4.40 (m, 1H, CH); 5.02-5.04 (m, 1H, CH); 5.42-5.44 (m, 1H, CH); 6.18 (d, *J*=2.2Hz, 1H, CH); 7.07-7.13 (m, 2H, ArH); 7.21-7.25 (m, 2H, ArH); 7.38 (s, 2H, NH_2); 8.17 (s, 1H, CH ^{purine}); 8.30 (s, 1H, CH ^{purine}); EIMS m/z 443 (M^+). Anal. Calcd for $C_{21}H_{22}FN_5O_5$: C, 56.88; H, 5.00; F, 4.28; N, 15.79; Found: C, 56.97; H, 5.01; F, 4.27; N, 15.73.

2.1.8.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 2-(4-fluorophenyl)acetate (c3)

White powder, 62.1%, mp 172-174°C; ^{1}H NMR (DMSO-d_6): 3.64 (s, 2H, CH_2); 4.18 (d, *J*=1.9Hz, 1H, CH); 4.27-4.34 (m, 2H, CH_2); 4.41 (d, *J*=11.9Hz, 1H, CH); 4.65 (d, *J*=4.0Hz, 1H, CH); 5.26 (d, *J*=5.2Hz, 1H, OH); 5.54 (d, *J*=4.8Hz, 1H, OH); 5.97 (d, *J*=1.7Hz, 1H, CH); 6.99-7.03 (m, 2H, ArH); 7.06 (s, 2H, NH_2); 7.24 (t, *J*=6.0Hz, 2H, ArH); 8.11 (s, 1H, CH ^{purine}); 8.18 (s, 1H, CH ^{purine}); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 40.02; 64.45; 70.59; 73.64; 81.78; 88.68; 115.23; 115.42; 119.89; 130.13; 131.26; 131.33; 139.56; 149.64; 152.96; 156.43; 160.52; 171.04; EIMS m/z 403 (M^+). Anal. Calcd for $C_{18}H_{18}FN_5O_5$: C, 53.60; H, 4.50; F, 4.71; N, 17.36; Found: C, 53.42; H, 4.51; F, 4.72; N, 17.41.

2.1.9.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 2-(4-bromophenyl)acetate (b4)

White powder, 50.5%, mp 136-138°C; ^{1}H NMR (DMSO-d_6): 1.33 (s, 3H, CH_3); 1.54 (s, 3H, CH_3); 3.56-3.67 (m, 2H, CH_2); 4.16-4.20 (m, 1H, CH); 4.26-4.30 (m, 1H, CH); 4.36-4.39 (m, 1H, CH); 5.01-5.04 (m, 1H, CH); 5.42-5.44 (m, 1H, CH); 6.19 (s, 1H, CH); 7.16 (d, *J*=8.3Hz, 2H, ArH); 7.39 (s, 2H, NH_2); 7.47 (d, *J*=8.2Hz, 2H, ArH); 8.17 (s, 1H, CH ^{purine}); 8.30 (s, 1H, CH ^{purine}); EIMS m/z 503 (M^+). Anal. Calcd for $C_{21}H_{22}BrN_5O_5$: C, 50.01; H, 4.40; Br, 15.84; N, 13.89; Found: C, 50.18; H, 4.39; Br, 15.79; N, 13.85.

2.2.0.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 2-(4-chlorophenyl)acetate (b5)

White powder, 66.3%, mp 160-162°C; ^{1}H NMR (DMSO-d_6): 1.33 (s, 3H, CH_3); 1.54 (s, 3H, CH_3); 3.58-3.68 (m, 2H, CH_2); 4.16-4.21 (m, 1H, CH); 4.26-4.30 (m, 1H, CH); 4.36-4.40 (m, 1H, CH); 5.02-5.04 (m, 1H, CH); 5.42-5.44 (m, 1H, CH); 6.18 (d, *J*=2.2Hz, 1H, CH); 7.22 (d, *J*=8.4Hz, 2H, ArH); 7.34 (d, *J*=8.4Hz, 2H, ArH); 7.39 (s, 2H, NH_2); 8.17 (s, 1H, CH ^{purine}); 8.30 (s, 1H, CH ^{purine}); EIMS m/z 459 (M^+). Anal. Calcd for $C_{21}H_{22}ClN_5O_5$: C, 54.85; H, 4.82; Cl, 7.71; N, 15.23; Found: C, 54.67; H, 4.82; Cl, 7.74; N, 15.29.

2.2.1.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 2-(4-bromophenyl)acetate (c4)

White powder, 72.7%, mp 113-115°C; ^{1}H NMR (DMSO-d_6): 3.70 (s, 2H, CH_2); 4.12 (s, 1H, CH); 4.22-4.26 (m, 2H, CH_2); 4.34-4.37 (m, 1H, CH); 4.63 (t, *J*=4.8Hz, 1H, CH); 5.46 (d, *J*=5.5Hz, 1H, OH); 5.62 (d, *J*=5.7Hz, 1H, OH); 5.94 (d, *J*=4.6Hz, 1H, CH); 7.20 (d, *J*=8.2Hz, 2H, NH_2); 7.48 (d, *J*=8.1Hz, 2H, ArH); 8.33 (m, 3H, ArH and CH ^{purine}); 8.48 (s, 1H, CH ^{purine}); EIMS m/z 463 (M^+). Anal. Calcd for $C_{18}H_{18}BrN_5O_5$: C, 46.57; H, 3.91; Br, 17.21; N, 15.08; Found: C, 46.41; H, 3.91; Br, 17.27; N, 15.13.

2.2.2.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 2-(4-chlorophenyl)acetate (c5)

White powder, 68.3%, mp 86-88°C; ^{1}H NMR (DMSO-d_6): 3.70 (d, *J*=2.1Hz, 2H, CH_2); 4.07-4.11 (m, 1H, CH); 4.21-4.29 (m, 2H, CH_2); 4.34-4.38 (m, 1H, CH); 4.65-4.68 (m, 1H, CH); 5.37 (d, *J*=5.4Hz, 1H, OH); 5.57 (d, *J*=5.7Hz, 1H, OH); 5.91 (d, *J*=4.9Hz, 1H, CH); 7.25 (d, *J*=8.5Hz, 2H, NH_2); 7.32-7.36 (m, 4H, ArH); 8.16 (m, 1H, CH ^{purine}); 8.31 (s, 1H, CH ^{purine}); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 39.62; 64.79; 70.69; 73.27; 81.89; 88.18; 119.63; 128.69; 128.69; 131.76; 131.76; 132.04; 133.71; 140.22; 149.82; 153.12; 156.55; 171.24; EIMS m/z 419 (M^+). Anal. Calcd for $C_{18}H_{18}ClN_5O_5$: C, 51.50; H, 4.32; Cl, 8.44; N, 16.68; Found: C, 51.31; H, 4.33; Cl, 8.47; N, 16.73.

2.2.3.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 2-(3-fluorophenyl)acetate (b6)

White powder, 79.8%, mp 177-179°C; ^{1}H NMR (DMSO-d_6): 1.33 (s, 3H, CH_3); 1.54 (s, 3H, CH_3); 3.64-3.74 (m, 2H, CH_2); 4.22 (d, *J*=6.1Hz, 1H, CH); 4.31 (d, *J*=11.2Hz, 1H, CH); 4.39 (s, 1H, CH); 5.03 (s, 1H, CH); 5.39 (d, *J*=5.6Hz, 1H, CH); 6.20 (s, 1H, CH); 7.10-7.18 (m, 2H, ArH); 7.25-7.32 (m, 2H, ArH); 7.39 (s, 2H, NH_2); 8.17-8.19 (m, 1H, CH ^{purine}); 8.29 (s, 1H, CH ^{purine}); EIMS m/z 443 (M^+). Anal. Calcd for $C_{21}H_{22}FN_5O_5$: C, 56.88; H, 5.00; F, 4.28; N, 15.79; Found: C, 56.69; H, 5.01; F, 4.28; N, 15.85.

2.2.4.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 2-(4-hydroxyphenyl)acetate (b7)

White powder, 61.1%, mp 168-170°C; ^{1}H NMR (DMSO-d_6): 1.33 (s, 3H, CH_3); 1.54 (s, 3H, CH_3); 3.51 (d, *J*=3.7Hz, 2H, CH_2); 4.16-4.20 (m, 1H, CH); 4.26-4.30 (m, 1H, CH); 4.36-4.39 (m, 1H, CH); 5.00-5.03 (m, 1H, CH); 5.39-5.41 (m, 1H, CH); 6.18 (d, *J*=2.4Hz, 1H, CH); 6.58-6.64 (m, 3H, ArH); 7.05 (t, *J*=8.2Hz, 1H, ArH); 7.36 (s, 2H, NH_2); 8.17 (s, 1H, CH ^{purine}); 8.28 (s, 1H, CH ^{purine}); 9.35 (s, 1H, OH); EIMS m/z 441 (M^+). Anal. Calcd for $C_{21}H_{23}N_5O_6$: C, 57.14; H, 5.25; N, 15.86; Found: C, 57.33; H, 5.21; N, 15.80.

2.2.5.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 2-(2-hydroxyphenyl)acetate (b8)

White powder, 83.5%, mp 142-144°C; ^{1}H NMR (DMSO-d_6): 1.32 (s, 3H, CH_3); 1.54 (s, 3H, CH_3); 3.51 (s, 2H, CH_2); 4.16-4.20 (m, 1H, CH); 4.23-4.27 (m, 1H, CH); 4.38-4.41 (m, 1H, CH); 4.95-4.97 (m, 1H, CH); 5.21-5.23 (m, 1H, CH); 6.15 (d, *J*=2.6Hz, 1H, CH); 6.67 (t, *J*=7.4Hz, 1H, ArH); 6.78 (d, *J*=7.6Hz, 1H, ArH); 6.99 (d, *J*=7.4Hz, 1H, ArH); 7.03-7.07 (m, 1H, ArH); 7.35 (s, 2H, NH_2); 8.17 (s, 1H, CH ^{purine}); 8.22 (s, 1H, CH ^{purine}); 9.51 (s, 1H, OH); EIMS m/z 441 (M^+). Anal. Calcd for $C_{21}H_{23}N_5O_6$: C, 57.14; H, 5.25; N, 15.86; Found: C, 57.33; H, 5.21; N, 15.83.

2.2.6.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl) methyl 2-(3-fluorophenyl)acetate (c6)

White powder, 45.7%, mp 158-159°C; ^{1}H NMR (DMSO-d_6): 3.74 (s, 2H, CH_2); 4.11 (s, 1H, CH); 4.23-4.27 (m, 2H, CH_2); 4.36-4.39 (m, 1H, CH); 4.66 (d, *J*=3.4Hz, 1H, CH); 5.39 (d, *J*=4.2Hz, 1H, OH); 5.58 (d, *J*=4.5Hz, 1H, OH); 5.92-5.93 (m, 1H, CH); 7.08 (t, *J*=8.2Hz, 3H, ArH); 7.32-7.36 (m, 3H, ArH and NH_2); 8.16 (d, *J*=2.0Hz, 1H, CH ^{purine}); 8.30 (d, *J*=1.1Hz, 1H, CH ^{purine}); EIMS m/z 403 (M^+). Anal. Calcd for $C_{18}H_{18}FN_5O_5$: C, 53.60; H, 4.50; F, 4.71; N, 17.36; Found: C, 53.46; H, 4.51; F, 4.71; N, 17.41.

2.2.7.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d] [1,3]dioxol-4-yl)methyl 2-(3-hydroxyphenyl)acetate (b9)

White powder, 81.3%, mp 172-174°C; ^{1}H NMR (DMSO-d_6): 1.33 (s, 3H, CH_3); 1.54 (s, 3H, CH_3); 3.51 (d, *J*=3.7Hz, 2H, CH_2); 4.16-4.20 (m, 1H, CH); 4.26-4.30 (m, 1H, CH); 4.36-4.39 (m, 1H, CH); 5.00-5.03 (m, 1H, CH); 5.39-5.41 (m, 1H, CH); 6.18 (d, *J*=2.4Hz, 1H, CH); 6.58-6.64 (m, 3H, ArH); 7.05 (t, *J*=8.2Hz, 1H, ArH); 7.36 (s, 2H, NH_2); 8.17 (s, 1H, CH ^{purine}); 8.28 (s, 1H, CH ^{purine}); 9.35 (s, 1H, OH); EIMS m/z 441 (M^+). Anal. Calcd for $C_{21}H_{23}N_5O_6$: C, 57.14; H, 5.25; N, 15.86; Found: C, 57.34; H, 5.21; N, 15.81.

2.2.8.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 2-(4-hydroxyphenyl)acetate (c7)

White powder, 39.8%, mp 137-139°C; ^{1}H NMR (DMSO-d_6): 3.58 (s, 2H, CH_2); 4.12 (s, 1H, CH); 4.19-4.25 (m, 2H, CH_2); 4.33-4.37 (m, 1H, CH); 4.64 (s, 1H, CH); 5.39 (m, 1H, OH); 5.58 (s, 1H, OH); 5.92 (d, *J*=4.7Hz, 1H, CH); 6.61-6.65 (m, 3H, ArH); 7.07 (t, *J*=7.6Hz, 1H, ArH); 7.41 (s, 2H, NH_2); 8.17 (s, 1H, CH ^{purine}); 8.33 (s, 1H, CH ^{purine}); 9.39 (s, 1H, OH); EIMS m/z 401 (M^+). Anal. Calcd for $C_{18}H_{19}N_5O_6$: C, 53.86; H, 4.77; N, 17.45; Found: C, C, 53.69; H, 4.78; N, 17.51.

2.2.9.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 2-(2-hydroxyphenyl)acetate (c8)

White powder, 40.3%, mp 125-127°C; ^{1}H NMR (DMSO-d_6): 3.55 (s, 2H, CH_2); 4.10 (s,

1H, CH); 4.19-4.25 (m, 2H, CH_2); 4.31-4.37 (m, 1H, CH); 4.65 (s, 1H, CH); 5.39 (m, 1H, OH); 5.58 (s, 1H, OH); 5.91 (d, *J*=4.7Hz, 1H, CH); 6.62-6.65 (m, 3H, ArH); 7.06 (t, *J*=7.6Hz, 1H, ArH); 7.42 (s, 2H, NH_2); 8.19 (s, 1H, CH ^{purine}); 8.33 (s, 1H, CH ^{purine}); 9.37 (s, 1H, OH); EIMS m/z 401 (M^+). Anal. Calcd for $C_{18}H_{19}N_5O_6$: C, 53.86; H, 4.77; N, 17.45; Found: C, 53.71; H, 4.78; N, 17.49.

2.3.0.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 2-(3-hydroxyphenyl)acetate (c9)

White powder, 57.3%, mp 112-114°C; ^{1}H NMR (DMSO-d_6): 3.57 (s, 2H, CH_2); 4.10 (s, 1H, CH); 4.19-4.25 (m, 2H, CH_2); 4.33-4.37 (m, 1H, CH); 4.64 (s, 1H, CH); 5.39 (m, 1H, OH); 5.58 (s, 1H, OH); 5.91 (d, *J*=4.7Hz, 1H, CH); 6.62-6.65 (m, 3H, ArH); 7.06 (t, *J*=7.6Hz, 1H, ArH); 7.41 (s, 2H, NH_2); 8.17 (s, 1H, CH ^{purine}); 8.32 (s, 1H, CH ^{purine}); 9.37 (s, 1H, OH); EIMS m/z 401 (M^+). Anal. Calcd for $C_{18}H_{19}N_5O_6$: C, 53.86; H, 4.77; N, 17.45; Found: C, 53.95; H, 4.76; N, 17.39.

2.3.1.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 2-p-tolylacetate (b10)

White powder, 64.5%, mp 83-85°C; ^{1}H NMR (DMSO-d_6): 1.32 (s, 3H, CH_3); 1.54 (s, 3H, CH_3); 2.25 (s, 3H, CH_3); 3.54 (d, *J*=3.1Hz, 2H, CH_2); 4.16-4.20 (m, 1H, CH); 4.26-4.29 (m, 1H, CH); 4.38 (s, 1H, CH); 5.00 (d, *J*=2.9Hz, 1H, CH); 5.38 (d, *J*=4.8Hz, 1H, CH); 6.17 (d, *J*=2.4Hz, 1H, CH); 7.06 (s, 4H, ArH); 7.38 (s, 2H, NH_2); 8.17 (d, *J*=5.7Hz, 1H, CH ^{purine}); 8.27 (d, *J*=3.2Hz, 1H, CH ^{purine}); EIMS m/z 439 (M^+). Anal. Calcd for $C_{22}H_{25}N_5O_5$: C, 60.13; H, 5.73; N, 15.94; Found: C, 60.32; H, 5.71; N, 15.89.

2.3.2.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 2-(3,4-dichlorophenyl)acetate (b11)

White powder, 50.8%, mp 90-92°C; ^{1}H NMR (DMSO-d_6): 1.33 (s, 3H, CH_3); 1.55 (s, 3H, CH_3); 3.64-3.74 (m, 2H, CH_2); 4.18-4.22 (m, 1H, CH); 4.28-4.32 (m, 1H, CH); 4.36-4.40 (m, 1H, CH); 5.03-5.05 (m, 1H, CH); 5.43-5.45 (m, 1H, CH); 6.19 (d, *J*=2.2Hz, 1H, CH); 7.20-7.22 (m, 1H, ArH); 7.37 (s, 2H, NH_2); 7.52-7.55 (m, 2H, ArH); 8.17 (d, *J*=1.9Hz, 1H, CH ^{purine}); 8.30 (s, 1H, CH ^{purine}); EIMS m/z 493 (M^+). Anal. Calcd for $C_{21}H_{21}Cl_2N_5O_5$: C,

51.02; H, 4.28; Cl, 14.34; N, 14.17; Found: C, 51.20; H, 4.27; Cl, 14.31; N, 14.13.

2.3.3.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 2-(3,4-dichlorophenyl)acetate (c11)

White powder, 70.7%, mp 172-174°C; ^{1}H NMR (DMSO-d_6): 3.71 (d, *J*=2.6Hz, 2H, CH_2); 4.08-4.12 (m, 1H, CH); 4.22-4.27 (m, 2H, CH_2); 4.34-4.37 (m, 1H, CH); 4.63 (t, *J*=5.0Hz, 1H, CH); 5.37 (d, *J*=5.4Hz, 1H, OH); 5.57 (d, *J*=5.7Hz, 1H, OH); 5.91 (d, *J*=4.9Hz, 1H, CH); 7.19-7.21 (m, 1H, ArH); 7.48-7.51 (m, 2H, ArH); 7.66 (s, 2H, NH_2); 8.21 (m, 1H, CH ^{purine}); 8.37 (s, 1H, CH ^{purine}); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 39.12; 64.90; 70.64; 73.41; 81.98; 88.29; 119.53; 130.07; 130.41; 130.77; 131.22; 131.97; 135.77; 140.77; 149.59; 151.73; 155.40; 170.94; EIMS m/z 453 (M^+). Anal. Calcd for $C_{18}H_{17}Cl_2N_5O_5$: C, 47.59; H, 3.77; Cl, 15.61; N, 15.42; Found: C, 47.47; H, 3.78; Cl, 15.67; N, 15.45.

2.3.4.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 2-p-tolylacetate (c10)

White powder, 70.4%, mp 80-82°C; ^{1}H NMR (DMSO-d_6): 2.26 (s, 3H, CH_3); 3.62 (s, 2H, CH_2); 4.10 (s, 1H, CH); 4.20-4.27 (m, 2H, CH_2); 4.34 (d, *J*=11.2Hz, 1H, CH); 4.65 (d, *J*=4.2Hz, 1H, CH); 5.40 (s, 1H, OH); 5.60 (s, 1H, OH); 5.90-5.93 (m, 1H, CH); 7.09 (s, 4H, ArH); 7.34 (s, 2H, NH_2); 8.15-8.17 (m, 1H, CH ^{purine}); 8.28 (t, *J*=2.2Hz, 1H, CH ^{purine}); EIMS m/z 399 (M^+). Anal. Calcd for $C_{19}H_{21}N_5O_5$: C, 57.14; H, 5.30; N, 17.53; Found: C, 57.33; H, 5.29; N, 17.47.

2.3.5.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 2-(3,4-dimethoxyphenyl)acetate (b12)

White powder, 80.5%, mp 132-134°C; ^{1}H NMR (DMSO-d_6): 1.33 (s, 6H, CH_3); 1.55 (s, 6H, CH_3); 3.64-3.74 (m, 2H, CH_2); 4.18-4.22 (m, 1H, CH); 4.28-4.32 (m, 1H, CH); 4.36-4.40 (m, 1H, CH); 5.03-5.05 (m, 1H, CH); 5.43-5.45 (m, 1H, CH); 6.19 (d, *J*=2.2Hz, 1H, CH); 7.20-7.22 (m, 1H, ArH); 7.37 (s, 2H, NH_2); 7.52-7.55 (m, 2H, ArH); 8.17 (d, *J*=1.9Hz, 1H, CH ^{purine}); 8.30 (s, 1H, CH ^{purine}). Anal. Calcd for $C_{23}H_{27}N_5O_7$: C, 56.90; H, 5.61; N, 14.43; Found: C, 56.83; H, 5.62; N, 14.47.

2.3.6.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 2-(3,4-dimethoxyphenyl)acetate (c12)

White powder, 88.3%, mp 162-164°C; ^{1}H NMR (DMSO-d_6): 1.32 (s, 3H, CH_3); 1.53 (s, 3H, CH_3); 3.60-3.70 (m, 2H, CH_2); 4.18-4.29 (m, 2H, CH_2); 4.39-4.42 (m, 1H, CH); 4.96-4.98 (m, 1H, CH); 5.29-5.31 (m, 1H, CH); 5.46 (d, *J*=5.5Hz, 1H, OH); 5.62 (d, *J*=5.7Hz, 1H, OH); 6.15 (d, *J*=1.9Hz, 1H, CH); 7.23 (d, *J*=8.4Hz, 2H, NH_2); 7.32-7.34 (m, 3H, ArH); 8.11 (s, 1H, CH ^{purine}); 8.25 (s, 1H, CH ^{purine}); EIMS m/z 445 (M^+). Anal. Calcd for $C_{20}H_{23}N_5O_7$: C, 53.93; H, 5.20; N, 15.72; Found: C, 53.75; H, 5.21; N, 15.66.

2.3.7.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 2-(2-chlorophenyl)acetate (b13)

White powder, 54.8%, mp 155-156°C; ^{1}H NMR (DMSO-d_6): 1.32 (s, 3H, CH_3); 1.54 (s, 3H, CH_3); 3.54 (d, *J*=3.1Hz, 2H, CH_2); 4.16-4.20 (m, 1H, CH); 4.26-4.29 (m, 1H, CH); 4.38 (s, 1H, CH); 5.00 (d, *J*=2.9Hz, 1H, CH); 5.38 (d, *J*=4.8Hz, 1H, CH); 6.17 (d, *J*=2.4Hz, 1H, CH); 7.06 (s, 4H, ArH); 7.38 (s, 2H, NH_2); 8.17 (d, *J*=5.7Hz, 1H, CH ^{purine}); 8.27 (d, *J*=3.2Hz, 1H, CH ^{purine}); EIMS m/z 459 (M^+). Anal. Calcd for $C_{21}H_{22}ClN_5O_5$: C, 54.85; H, 4.82; Cl, 7.71; N, 15.22; Found: C, 54.97; H, 4.81; Cl, 7.69; N, 15.18.

2.3.8.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 2-(2-chlorophenyl)acetate (c13)

White powder, 77.1%, mp 185-187°C; ^{1}H NMR (DMSO-d_6): 3.83 (d, *J*=3.6Hz, 2H, CH_2); 4.09-4.12 (m, 1H, CH); 4.23-4.28 (m, 2H, CH_2); 4.37-4.41 (m, 1H, CH); 4.60-4.64 (m, 1H, CH); 5.38 (d, *J*=5.4Hz, 1H, OH); 5.56 (d, *J*=5.9Hz, 1H, OH); 5.91 (d, *J*=5.2Hz, 1H, CH); 7.27-7.34 (m, 4H, ArH and NH_2); 7.37-7.40 (m, 1H, ArH); 7.43-7.46 (m, 1H, ArH); 8.16 (m, 1H, CH ^{purine}); 8.26 (s, 1H, CH ^{purine}); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 38.69; 64.95; 70.74; 73.34; 82.00; 88.02; 119.62; 127.68; 129.54; 129.57; 132.60; 132.96; 134.17; 140.06; 149.87; 153.16; 156.57; 170.45; EIMS m/z 419 (M^+). Anal. Calcd for $C_{18}H_{18}ClN_5O_5$: C, 51.50; H, 4.32; Cl, 8.44; N, 16.68; Found: C, 51.67; H, 4.32; Cl, 8.42; N, 16.62.

2.3.9.((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl 2-(2-fluorophenyl)acetate (b14)

White powder, 78.9%, mp 104-106°C; ^{1}H NMR (DMSO-d_6): 1.33 (s, 3H, CH_3); 1.54 (s, 3H, CH_3); 3.64-3.74 (m, 2H, CH_2); 4.22 (d, *J*=6.1Hz, 1H, CH); 4.31 (d, *J*=11.2Hz, 1H, CH); 4.39 (s, 1H, CH); 5.03 (s, 1H, CH); 5.39 (d, *J*=5.6Hz, 1H, CH); 6.20 (s, 1H, CH); 7.10-7.18 (m, 2H, ArH); 7.25-7.32 (m, 2H, ArH); 7.39 (s, 2H, NH_2); 8.17-8.19 (m, 1H, CH ^{purine}); 8.29 (s, 1H, CH ^{purine}); EIMS m/z 443 (M^+). Anal. Calcd for $C_{21}H_{22}FN_5O_5$: C, 56.88; H, 5.00; F, 4.28; N, 15.79; Found: C, 56.72; H, 5.00; F, 4.29; N, 15.84.

2.4.0.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 2-(2-fluorophenyl)acetate (c14)

White powder, 67.3%, mp 186-187°C; ^{1}H NMR (DMSO-d_6): 3.76 (s, 2H, CH_2); 4.10-4.14 (m, 1H, CH); 4.25-4.29 (m, 2H, CH_2); 4.38-4.42 (m, 1H, CH); 4.63-4.67 (m, 1H, CH); 5.41 (d, *J*=5.4Hz, 1H, OH); 5.60 (d, *J*=5.8Hz, 1H, OH); 5.94 (d, *J*=5.1Hz, 1H, CH); 7.12-7.20 (m, 2H, ArH); 7.31-7.35 (m, 4H, ArH and NH_2); 8.18 (m, 1H, CH ^{purine}); 8.29 (s, 1H, CH ^{purine}); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 34.22 (*J*=2.61Hz); 64.98; 70.72; 73.35; 81.97; 88.08; 115.56 (*J*=21.37Hz); 119.62; 121.93 (*J*=16.00Hz); 124.80 (*J*=3.49Hz); 129.76 (*J*=8.18Hz); 132.44 (*J*=4.03Hz); 140.09; 149.86; 153.17; 156.58; 161.10 (*J*=243.37Hz); 170.62; EIMS m/z 403 (M^+). Anal. Calcd for $C_{18}H_{18}FN_5O_5$: C, 53.60; H, 4.50; F, 4.71; N, 17.36; Found: C, 53.81; H, 4.49; F, 4.70; N, 17.32.

2.4.1.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl acetate (c15)

White powder, 65,8%, mp 198-201°C; ^{1}H NMR (DMSO-d_6): 0.84 (t, *J*=7.4Hz, 3H, CH_3); 4.07-4.10 (m, 1H, CH); 4.17-4.22 (m, 1H, CH); 4.25-4.29 (m, 1H, CH); 4.33-4.37 (m, 1H, CH); 4.66-4.70 (m, 1H, CH); 5.42 (d, *J*=4.8Hz, 1H, OH); 5.63 (d, *J*=5.7Hz, 1H, OH); 5.91 (d, *J*=4.8Hz, 1H, CH); 7.35 (s, 2H, NH_2); 8.16 (s, 1H, CH ^{purine}); 8.33 (s, 1H, CH ^{purine}); EIMS m/z 309 (M^+). Anal. Calcd for $C_{12}H_{15}N_5O_5$: C, 46.60; H, 4.89; N, 22.64; Found: C, 46.53; H, 4.89; N, 22.72.

2.4.2.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)met

hyl butyrate (c16)

White powder, 57.3%, mp 125-127°C; ^{1}H NMR (DMSO-d_6): 0.84 (t, *J*=7.4Hz, 3H, CH_3); 1.46-1.55 (m, 2H, CH_2); 2.26-2.29 (m, 2H, CH_2); 4.07-4.10 (m, 1H, CH); 4.17-4.22 (m, 1H, CH); 4.25-4.29 (m, 1H, CH); 4.33-4.37 (m, 1H, CH); 4.66-4.70 (m, 1H, CH); 5.42 (d, *J*=4.8Hz, 1H, OH); 5.63 (d, *J*=5.7Hz, 1H, OH); 5.91 (d, *J*=4.8Hz, 1H, CH); 7.35 (s, 2H, NH_2); 8.16 (s, 1H, CH ^{purine}); 8.33 (s, 1H, CH ^{purine}); EIMS m/z 337 (M^+). Anal. Calcd for $C_{14}H_{19}N_5O_5$: C, 49.85; H, 5.68; N, 20.76; Found: C, 49.97; H, 5.66; N, 20.72.

2.4.3.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)met

hyl hexanoate (c17)

White powder, 64.3%, mp 164-166°C; ^{1}H NMR (DMSO-d_6): 0.81 (t, *J*=6.9Hz, 3H, CH_3); 1.15-1.28 (m, 4H, $2CH_2$); 1.44-1.52 (m, 2H, CH_2); 2.26-2.30 (m, 2H, CH_2); 4.06-4.09 (m, 1H, CH); 4.17-4.21 (m, 1H, CH); 4.25-4.28 (m, 1H, CH); 4.32-4.36 (m, 1H, CH); 4.66-4.70 (m, 1H, CH); 5.40 (d, *J*=5.5Hz, 1H, OH); 5.61 (d, *J*=5.8Hz, 1H, OH); 5.91 (d, *J*=4.9Hz, 1H, CH); 7.34 (s, 2H, NH_2); 8.15 (s, 1H, CH ^{purine}); 8.32 (s, 1H, CH ^{purine}); EIMS m/z 365 (M^+). Anal. Calcd for $C_{16}H_{23}N_5O_5$: C, 52.59; H, 6.34; N, 19.17; Found: C, 52.64; H, 6.32; N, 19.11.

2.4.4.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)met

hyl octanoate (c18)

White powder, 70.3%, mp 101-103°C; ^{1}H NMR (DMSO-d_6): 0.83 (t, *J*=6.9Hz, 3H, CH_3); 1.20 (s, 8H, $4CH_2$); 1.46-1.49 (m, 2H, CH_2); 2.28 (t, *J*=7.1Hz, 2H, CH_2); 4.06-4.09 (m, 1H, CH); 4.17-4.22 (m, 1H, CH); 4.25 (d, *J*=3.6Hz, 1H, CH); 4.31-4.35 (m, 1H, CH); 4.67 (d, *J*=4.1Hz, 1H, CH); 5.40 (d, *J*=3.8Hz, 1H, OH); 5.60 (d, *J*=4.4Hz, 1H, OH); 5.90 (d, *J*=4.9Hz, 1H, CH); 7.34 (s, 2H, NH_2); 8.15 (s, 1H, CH ^{purine}); 8.32 (s, 1H, CH ^{purine}); EIMS m/z 393 (M^+). Anal. Calcd for $C_{18}H_{27}N_5O_5$: C, 54.95; H, 6.92; N, 17.80; Found: C, 54.77; H, 6.94; N, 17.85.

2.4.5.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)met

hyl decanoate (c19)

White powder, 61.6%, mp 94-96°C; ^{1}H NMR (DMSO-d_6): 0.84 (t, *J*=6.8Hz, 3H, CH_3); 1.20 (s, 12H, $6CH_2$); 1.46-1.54 (m, 2H, CH_2); 2.28 (t, *J*=14.3Hz, 2H, CH_2); 4.06-4.09 (m, 1H, CH); 4.17-4.21 (m, 1H, CH); 4.25 (s, 1H, CH); 4.31-4.35 (m, 1H, CH); 4.67 (d, *J*=3.1Hz, 1H, CH); 5.40 (s, 1H, OH); 5.60 (d, *J*=3.5Hz, 1H, OH); 5.91 (d, *J*=4.9Hz, 1H, CH); 7.39 (s, 2H, NH_2); 8.16 (s, 1H, CH ^{purine}); 8.33 (s, 1H, CH ^{purine}); EIMS m/z 421 (M^+). Anal. Calcd for $C_{20}H_{31}N_5O_5$: C, 56.99; H, 7.41; N, 16.62; Found: C, 56.81; H, 7.39; N, 16.58.

2.4.6.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)met

hyl benzoate (c20)

White powder, 80.3%, mp 140-142°C; ^{1}H NMR (DMSO-d_6): 4.23-4.26 (m, 1H, CH); 4.45-4.49 (m, 2H, CH_2); 4.59-4.63 (m, 1H, CH); 4.79 (d, *J*=4.4Hz, 1H, CH); 5.51 (d, *J*=4.4Hz, 1H, OH); 5.69 (d, *J*=4.9Hz, 1H, OH); 5.95 (d, *J*=4.7Hz, 1H, CH); 7.35 (s, 2H, NH_2); 7.52 (t, *J*=7.7Hz, 2H, ArH); 7.67 (t, *J*=7.4Hz, 1H, ArH); 7.95 (d, *J*=7.2Hz, 2H, ArH); 8.12 (s, 1H, CH ^{purine}); 8.32 (s, 1H, CH ^{purine}); EIMS m/z 371 (M^+). Anal. Calcd for $C_{17}H_{17}N_5O_5$: C, 54.98; H, 4.61; N, 18.86; Found: C, 54.77; H, 4.62; N, 18.91.

2.4.7.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)met

hyl 4-chlorobenzoate (c21)

White powder, 60.1%, mp 122-124°C; ^{1}H NMR (DMSO-d_6): 4.23-4.27 (m, 1H, CH); 4.46-4.51 (m, 2H, CH_2); 4.61-4.65 (m, 1H, CH); 4.78-4.82 (m, 1H, CH); 5.50 (d, *J*=5.6Hz, 1H, OH); 5.67 (d, *J*=5.6Hz, 1H, OH); 5.96 (d, *J*=4.6Hz, 1H, CH); 7.37 (s, 2H, NH_2); 7.59 (d, *J*=8.6Hz, 2H, ArH); 7.94 (d, *J*=8.6Hz, 2H, ArH); 8.13 (s, 1H, CH ^{purine}); 8.33 (s, 1H, CH ^{purine}); EIMS m/z 405 (M^+). Anal. Calcd for $C_{17}H_{16}ClN_5O_5$: C, 50.32; H, 3.97; Cl, 8.74; N, 17.26; Found: C, 50.15; H, 3.98; Cl, 8.76; N, 17.31.

2.4.8.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)met

hyl 3-chlorobenzoate (c22)

White powder, 80.1%, mp 120-121°C; ^{1}H NMR (DMSO-d_6): 4.21-4.25 (m, 1H, CH);

4.30-4.50 (m, 2H, CH_2); 4.60-4.64 (m, 1H, CH); 4.76-4.80 (m, 1H, CH); 5.46 (d, *J*=5.5Hz, 1H, OH); 5.62 (d, *J*=5.7Hz, 1H, OH); 5.93 (d, *J*=4.8Hz, 1H, CH); 7.33 (s, 2H, NH_2); 7.56 (t, *J*=7.9Hz, 1H, ArH); 7.75-7.77 (m, 1H, ArH); 7.88-7.92 (m, 2H, ArH); 8.08 (s, 1H, CH ^{purine}); 8.32 (s, 1H, CH ^{purine}); EIMS m/z 405 (M^+). Anal. Calcd for $C_{17}H_{16}ClN_5O_5$: C, 50.32; H, 3.97; Cl, 8.74; N, 17.26; Found: C, 50.52; H, 3.96; Cl, 8.71; N, 17.20.

2.4.9.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 3-fluorobenzoate (c23)

White powder, 85.6%, mp 139-141°C; ^{1}H NMR (DMSO-d_6): 4.24 (d, *J*=3.4Hz, 1H, CH); 4.47-4.51 (m, 2H, CH_2); 4.61 (d, *J*=11.8Hz, 1H, CH); 4.79 (d, *J*=4.8Hz, 1H, CH); 5.48 (d, *J*=4.8Hz, 1H, OH); 5.64 (d, *J*=4.9Hz, 1H, OH); 5.94 (d, *J*=4.1Hz, 1H, CH); 7.34 (s, 2H, NH_2); 7.52-7.60 (m, 2H, ArH); 7.71 (d, *J*=9.2Hz, 1H, ArH); 7.78 (d, *J*=7.2Hz, 1H, ArH); 8.10 (s, 1H, CH ^{purine}); 8.32 (s, 1H, CH ^{purine}); EIMS m/z 389 (M^+). Anal. Calcd for $C_{17}H_{16}FN_5O_5$: C, 52.44; H, 4.14; F, 4.88; N, 17.99; Found: C, 52.56; H, 4.14; F, 4.87; N, 17.92.

2.5.0.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 4-fluorobenzoate (c24)

White powder, 76.4%, mp 98-100°C; ^{1}H NMR (DMSO-d_6): 4.23-4.26 (m, 1H, CH); 4.44-4.50 (m, 2H, CH_2); 4.60-4.63 (m, 1H, CH); 4.81 (t, *J*=4.9Hz, 1H, CH); 5.48 (d, *J*=4.8Hz, 1H, OH); 5.64 (d, *J*=4.9Hz, 1H, OH); 5.96 (d, *J*=4.6Hz, 1H, CH); 7.33-7.37 (m, 4H, ArH and NH_2); 7.99-8.02 (m, 2H, ArH); 8.12 (s, 1H, CH ^{purine}); 8.32 (s, 1H, CH ^{purine}); EIMS m/z 389 (M^+). Anal. Calcd for $C_{17}H_{16}FN_5O_5$: C, 52.44; H, 4.14; F, 4.88; N, 17.99; Found: C, 52.31; H, 4.15; F, 4.88; N, 18.05.

2.5.1.((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl 4-bromobenzoate (c25)

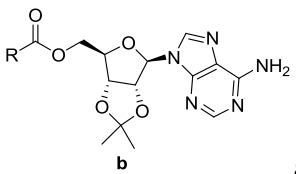
White powder, 67.5%, mp 204-206°C; ^{1}H NMR (DMSO-d_6): 4.20-4.24 (m, 1H, CH); 4.43-4.48 (m, 2H, CH_2); 4.59-4.63 (m, 1H, CH); 4.75-4.79 (m, 1H, CH); 5.46 (d, *J*=5.6Hz, 1H, OH); 5.63 (d, *J*=5.6Hz, 1H, OH); 5.93 (d, *J*=4.6Hz, 1H, CH); 7.35 (s, 2H, NH_2); 7.73 (d, *J*=8.4Hz, 2H, ArH); 7.85 (d, *J*=8.4Hz, 2H, ArH); 8.11 (s, 1H, CH ^{purine}); 8.31 (s, 1H, CH ^{purine}); EIMS m/z 449 (M^+). Anal. Calcd for $C_{17}H_{16}BrN_5O_5$: C, 45.35; H, 3.58; Br, 17.75; N, 15.55;

Found: C, 45.46; H, 3.57; Br, 17.70; N, 15.52.

2.2. Extraction of the TyrRS and enzyme assay

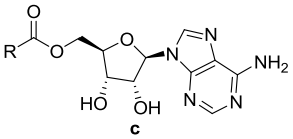
S. aureus TyrRS was over-expressed in *E. coli* and purified to near homogeneity (~98% as judged by SDS-PAGE) using standard purification procedures. TyrRS activity was measured by aminoacylation using modifications to previously described methods [17]. The assays were performed at 37°C in a mixture 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.

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

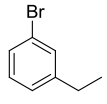
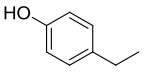


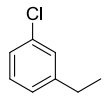
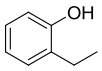
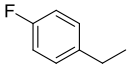
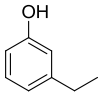
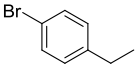
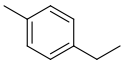
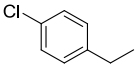
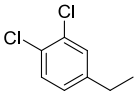
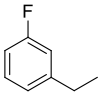
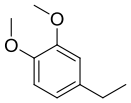
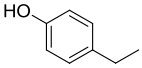
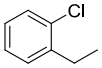
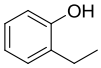
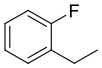
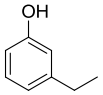
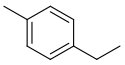
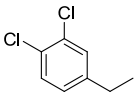
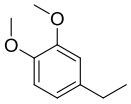
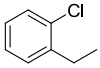
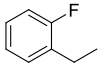
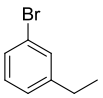
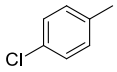
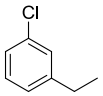
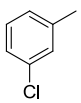
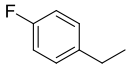
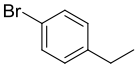
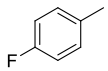
b

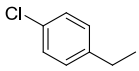
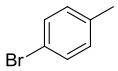
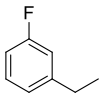
and



c

entry	R	IC_{50} (μM)	entry	R	IC_{50} (μM)
b1		3.7±0.4	**c7**		23±0.1

b2		6.3±0.1	**c8**		44±1.2
b3		7.1±0.4	**c9**		9.3±0.6
b4		5.6±0.2	**c10**		15±0.7
b5		8.4±0.2	**c11**		97±0.5
b6		1.1±0.3	**c12**		>100
b7		19±1.1	**c13**		47±0.1
b8		55±1.3	**c14**		40±0.5
b9		9.6±0.3	**c15**	CH_3	
b10		17±0.1	**c16**		65±0.7
b11		>100	**c17**		77±2.2
b12		>100	**c18**		93±1.5
b13		47±1.1	**c19**		>100
b14		44±0.7	**c20**		39±0.2
c1		3.3±0.2	**c21**		43±0.3
c2		6.0±0.1	**c22**		28±0.1
c3		7.7±0.5	**c23**		11±0.1
c4		5.2±0.4	**c24**		51±0.4

c5		8.1±0.7	**c25**		62±0.2
c6		0.8±0.07			

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 [14]. 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 3mg 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.

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

Compound	MIC_{50} (μg/mL)		
	A	B	C
b1	40.6	38.3	18.8
b2	27.8	44.9	22
b3	76.2	＞100	24.8
b4	＞100	＞100	21.2
b5	15.2	7.2	30.6
b6	60.3	＞100	12.9
b7	44.6	＞100	17.4
b8	37.2	＞100	23
b9	18.9	＞100	25.9
b10	27	＞100	14.1
b11	34	40.2	19.3
b12	47.3	＞100	30.1
b13	29	＞100	28
b14	25.7	＞100	25.5
c1	38.1	＞100	27.4
c2	20.2	49.2	35.1
c3	＞100	＞100	26.1
c4	88.9	42.2	19.3
c5	7.7	13.8	19.9
c6	1.3	9.2	0.08
c7	9.8	44.3	25.9
c8	15.9	37.8	26
c9	37	＞100	27.3
c10	16.4	47.4	11.8
c11	19.4	48.5	21.4
c12	16.5	＞100	10.2

c13	7.4	＞100	26.7
c14	23.6	51.4	35.8
c15	77.6	＞100	58.2
c16	＞100	＞100	87.3
c17	＞100	＞100	＞100
c18	＞100	＞100	＞100
c19	＞100	＞100	＞100
c20	33.7	67.4	21.9
c21	28.1	＞100	19.3
c22	11.3	9.4	8.7
c23	5.3	20.4	3.5
c24	24.8	＞100	11.2
c25	10.1	＞100	22.7
ciprofloxacin	0.67	/	0.1
penicillin G sodium	/	0.54	/

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

2.4. Protocol of docking study

The automated docking studies were carried out using AutoDock version 4.2. First, AutoGrid component of the program pre-calculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. A grid box of 65×75×75 Å size (x, y, 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 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 nine adenosine analogues were synthesized by the routes outlined in Scheme 3. Compound **b** was prepared by esterification of the corresponding acid with 2′,3′-*O*-isopropylideneadenosine and Compound **c** was prepared by the deprotection of isopropylidene group from compound **b** [15-16]. All of these target compounds were first reported and fully characterized by elementary analyses, EIMS, ^{1}H NMR and ^{13}C NMR.

3.2. Inhibitory activities of the adenosine analogues against TyrRS from *S. aureus*

All synthesized compounds (**b1**-**b14** and **c1**-**c25**) were tested for inhibitory activity against TyrRS from *S. aureus* and IC_{50} values are presented in Table 1. In the initial phase of our studies, we focused our efforts on introduction of varies substituents to the benzene ring of the phenylacetyl moiety (**b1**-**b14** and **c1**-**c14**). *Meta*-fluorine containing compounds (**b6** and **c6**) showed IC_{50} values of 1.1 and 0.8 μM, being the most potent member in their own series, and substitution with chloro, bromo or hydroxy groups led to a slight decrease in activity. In comparison with substituents at the 3-position, introduction of chloro, fluoro, brom or hydroxy substituents at the 2-position or 4-position led to a significant reduction in

potency. Modification at the 2-position or 4-position therefore seems to be detrimental to enzyme inhibition. Introduction two substituents (**b11**, **b12**, **c11** and **c12**) on the aryl ring resulted in significant attenuation of the level of TyrRS inhibition, indicating that these substituents may interfere the binding of ligand molecules to the enzyme. We then turned our attention to exploring the structure-activity relationship between 2′,3′-deprotected compounds (**c1** to **c14**) and 2′,3′-protected compounds (**b1** to **b14**). The compounds (**c1** to **c14**) with two hydroxyl groups at the 2′-position and 3′-position of the ribose exerted the higher inhibitory activity against *S. aureus* TyrRS than the corresponding parent compounds (**b1** to **b14**). This suggested that the inversion of hydrogen-bond donors and acceptors caused by the isopropylidene group blocks the hydrogen-bonding interactions between ligand and receptor, leading to a drease in binding energy. Therefore, the deprotected compounds seem to be more suitable for further research.

Finally, replacement of the substituted phenylacetyl (**c5**, **c2**, **c6**, **c3** and **c4**) with corresponding substituted benzoyl (**c21** to **c25**) resulted in some attenuation of potency. Introduction of a fluorine atom at 3-position or 4-position in benzene ring led to a slight increase in potency with respect to the corresponding chlorine or bromine analogues (**c22** vs **c23** and **c24** vs **c25**), which suggest that it is advisable to maintaining a fluorine atom at an appropriate position .

Compounds (**c15** to **c19**) with R^1 being an aliphatic group showed weak activity against TyrRS and the activities decrease as carbon chain increase. Out of tested compounds, compound **c6** was proved as the most potent, having IC_{50} of 0.8±0.07 μM.

3.3. Antibacterial activity

All compounds were tested against a representative Gram-positive organism (*S. aureus* ATCC 6538) and two Gram-negative organisms (*E. coli* ATCC 8739; *P. aeruginosa* ATCC 9027), and the results are presented in Table 2. In general, some of the evaluated compounds exhibited good activities against Gram-negative bacteria, especially against *P. aeruginosa* ATCC 9027, and all are inactive against Gram-positive bacteria. Compound **c6** is the most potent compound in all of the synthetic compounds, which is close to or slightly over that of marketed antibiotics (ciprofloxacin). The compounds with strong inhibition against TyrRS also exhibit good antibacterial activities, especially against *P. aeruginosa* ATCC 9027, which shows that their inhibition of TyrRS may lead to the antibacterial activity.

3.4. Molecular docking

Docking study was undertaken using the AutoDock version 4.2 in order to gain insight into the binding mode of compound **c6**. The binding mode of compound **c6** within the binding site of the TyrRS complex structure (1jij.pdb) [16] is shown in Fig. 1 and Fig. 2.

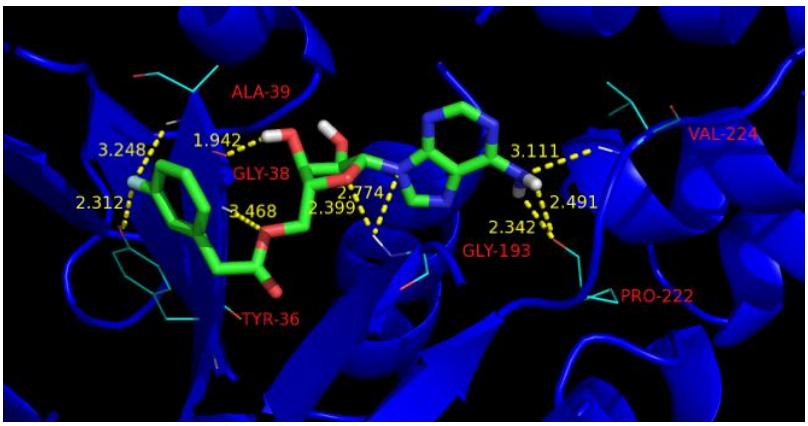


Fig. 1 Binding mode of compound **c6** with TyrRS from *S. aureus*. For clarity, only interacting residues were labeled. Hydrogen bonding interactions are shown in dash. This figure was made using PyMol.

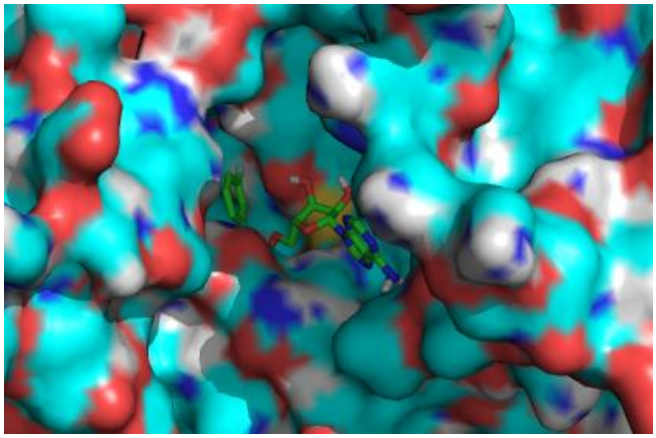


Fig. 2 Binding mode of compound **c6** with TyrRS. The enzyme is shown as surface; while **c6** docked structures are shown as sticks. This figure was made using PyMol.

In the binding model, adenosine moiety of **c6** is in the vicinity of the entrance cavity, which formed hydrogen-bonds with surrounding residues such as Gly193, Pro222 and Val224. Oxygen atom of ribose-ring moiety is involved in hydrogen bond formation with hydrogen of the NH_2 group of Gly193 (distance = 2.399 Å), while nitrogen atom in adenine interacts with hydrogen of the NH_2 group of Gly193 at distances of 2.774 Å. The NH_2 of the adenine strongly contributes to complex stabilization by forming three hydrogen bonds with hydrogen atoms offered by the NH_2 group of Val224 (distance = 3.111 Å) and oxygen atom of Pro222 residue, having H···O bond lengths of 2.342 Å and 2.491 Å. Additionally, the hydroxy group of the ribose ring makes a strong interaction with the backbone amino groups of Gly38. Moreover, an H-bond (1.942 Å) formed between saturated oxygen and hydrogen of NH_2 group of Gly38 further stabilizes the complex. The part of benzene ring is located at the bottom of the binding site. The fluorine atom of benzene ring interacted with Ala39 and Tyr36 at a distance of 3.248 Å and 2.312 Å. The number of hydrogen bonds formed has the most crucial role in complex stabilization, the nine hydrogen bonds assure stable complex formation and explain the high inhibitory activity of this compound. Therefore, changing the

position of fluorine atom or replacing it with other substituent group significantly reduced the enzyme inhibitory activity.

4. Conclusions

In this work, we synthesized a novel class of adenosine analogues, which have distinctive structural difference from other antimicrobial agents. These novel compounds are modified from the tyrosyl-adenylate intermediate. Their biological activities were tested against TyrRS from *S. aureus* and microorganism. Summarizing the results of tables 1-2, we can conclude that compound **c6** have much stronger antibacterial potency against *P. aeruginosa* ATCC 9027 than ciprofloxacin and good inhibitory activity with IC_{50} of 0.8±0.07 μM against TyrRS. Additionally, we also performed docking of **c6** into *S. aureus* TyrRS binding pocket. The docking model indicated that introduction of a fluorine atom at the 3′-position of benzene ring of the phenylacetyl moiety significantly increased affinities to the enzyme that may explain the excellent inhibitory activity of compound **c6**. Thus compound **c6** can be considered for developing into a potent antibacterial agent.

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Adenosine analogues as inhibitors of tyrosyl-tRNA synthetase: design synthesis, and antibacterial evaluation

Wei Wei, Wei-Kang Shi, Peng-Fei Wang, Xiao-Tong Zeng, Pan Li, Ji-Rong Zhang, Qian Li, Zhi-Ping Tang, Jia Peng, Lang-Zhou Wu, Mei-Qun Xie, Chan Liu, Xian-Hui Li, Yin-Chun Wang, Zhu-Ping Xiao, and Hai-Liang Zhu