Bioorganic & Medicinal Chemistry 23 (2015) 6602–6611

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Adenosine analogs as inhibitors of tyrosyl-tRNA synthetase: Design, synthesis and antibacterial evaluation

Wei Wei [a], Wei-Kang Shi [a], Peng-Fei Wang [b], Xiao-Tong Zeng [a], Pan Li [a], Ji-Rong Zhang [a], Qian Li [a], Zhi-Ping Tang [a], Jia Peng [a], Lang-Zhou Wu [a], Mei-Qun Xie [a], Chan Liu [a], Xian-Hui Li [b], Ying-Chun Wang [a], Zhu-Ping Xiao [a,c,*], Hai-Liang Zhu [a,c,*]

[a] College of Chemistry and Chemical Engineering, Jishou University, Jishou 416000, PR China
[b] School of Medicine, Jishou University, Jishou 416000, PR China
[c] State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, PR China

ARTICLE INFO

Article history:
Received 20 August 2015
Revised 8 September 2015
Accepted 9 September 2015
Available online 11 September 2015

Keywords:
Adenosine analogs
Tyrosyl-tRNA synthetase inhibitors
Antibacterial
Molecular docking

ABSTRACT

Herein we describe the synthesis and evaluation of a series of adenosine analogs 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–activity relationship analysis 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.

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 launched 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 diffuseness 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 novel scaffold.

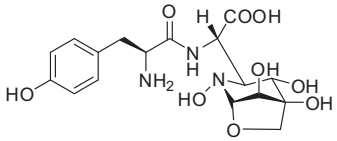
Aminoacyl-tRNA synthetases (aaRSs) are a kind of key enzymes which catalyze the transfer of amino acids to their cognate tRNAs in the process of protein synthesis.[3–5] These enzymes are essential for protein synthesis, and this process was forced to terminate when these enzymes were restrained.[6,7] 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 analogs 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.

It is well known that the TyrRS acts as other tRNA synthetase enzymes by a two-step mechanism. In the first stage, the enzyme recognizes 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 analogs 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 analogs **b1**–**b14** and **c1**–**c25**. They were subsequently evaluated for biological activities against a representative Gram-positive organism

* Corresponding authors.
E-mail addresses: xiaozhuping2005@163.com (Z.-P. Xiao), zhuhl@nju.edu.cn (H.-L. Zhu).

http://dx.doi.org/10.1016/j.bmc.2015.09.018
0968-0896/



Scheme 1. Structure of SB-219383.

(*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

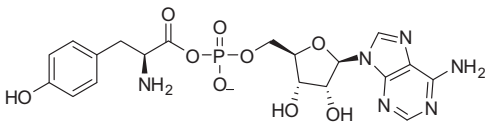
All chemicals (reagent grade) used were purchased from Aldrich (U.S.A) and Sinopharm Chemical Reagent Co., Ltd (China). Separation of the compounds by column chromatography was carried out with silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co., Ltd (China). The quantity of silica 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).

2.1.2. General procedure for preparation of compounds c

A solution of dichloromethane containing compounds **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 compounds **c** (Scheme 3) in yields of 65–80%.



Scheme 2. Structure of tyrosyl adenylate.

2.1.3. ((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyl tetrahydrofuro[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 = 8.3 Hz, 2H, ArH); 7.39 (s, 2H, NH_2); 7.47 (d, J = 8.2 Hz, 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-dimethyl tetrahydrofuro[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 = 2.2 Hz, 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 = 1.9 Hz, 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-dihydroxy tetrahydrofuran-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 = 11.6 Hz, 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 = 2.6 Hz, 1H, CH^{purine}); 8.30 (d, J = 3.2 Hz, 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-dihydroxy tetrahydrofuran-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 = 11.8 Hz, 1H, CH); 4.67 (t, J = 4.9 Hz, 1H, CH); 5.42 (d, J = 5.4 Hz, 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 = 5.0 Hz, 1H, CH^{purine}); 8.32 (d, J = 3.2 Hz, 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-dimethyl tetrahydrofuro[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 = 2.2 Hz, 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-dihydroxy tetrahydrofuran-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 = 1.9 Hz, 1H, CH); 4.27–4.34 (m, 2H, CH_2); 4.41 (d, J = 11.9 Hz, 1H, CH); 4.65 (d, J = 4.0 Hz, 1H, CH); 5.26 (d, J = 5.2 Hz, 1H, OH); 5.54 (d, J = 4.8 Hz, 1H, OH); 5.97 (d, J = 1.7 Hz,