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Discovery of bacterial NAD$^+$-dependent DNA ligase inhibitors: Improvements in clearance of adenosine series

Suzanne S. Stokes *, Madhusudhan Gowravaram, Hoan Huynh, Min Lu, George B. Mullen, Brendan Chen, Robert Albert, Thomas J. O'Shea, Michael T. Rooney, Haiqing Hu, Joseph V. Newman, Scott D. Mills

Infection Innovative Medicines Unit, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451, USA

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ABSTRACT

Optimization of clearance of adenosine inhibitors of bacterial NAD$^+$-dependent DNA ligase is discussed. To reduce Cytochrome P-450-mediated metabolic clearance, many strategies were explored; however, most modifications resulted in compounds with reduced antibacterial activity and/or unchanged total clearance. The alkyl side chains of the 2-cycloalkoxyadenosines were fluorinated, and compounds with moderate antibacterial activity and favorable pharmacokinetic properties in rat and dog were identified.

Over the past three decades, there has been an increase in infections caused by antibiotic-resistant pathogens and a concomitant decrease in the number of new antibacterial compounds in the drug development pipeline.[1] This confluence of circumstances has resulted in the urgent need for new antibacterial drugs.

The discovery and preliminary SAR of an adenosine series of NAD$^+$-dependent DNA ligase (LigA) inhibitors with antibacterial activity (Fig. 1) was previously described.[2] NAD$^+$-dependent DNA ligases are essential enzymes for DNA replication, repair, and recombination in all bacterial species.[3] Because of their essential nature and distinct structure compared to human homologs, LigA represents a potentially valuable target for identifying novel and selective antibacterial agents.[4] The adenosine series of LigA inhibitors was identified using high-throughput screening (HTS) and subsequently optimized for antibacterial activity against the Gram positive pathogens *Streptococcus pneumoniae* (*S. pneumoniae*) and *Staphylococcus aureus* (*S. aureus*) by modification of the 2-position of the adenine ring and the 3′- and 5′-substituents on the ribose.[2]

Adenosine analogs were identified with antibacterial activity and some drug-like physical properties such as high solubility and low plasma protein binding. These compounds had small or medium-sized alkyl rings attached via an ether to the 2-position of the adenine, and the sugar was 5′-deoxyribose or 5′-deoxy-5′-fluororibose. However, these 2-alkoxy-5′-deoxyadenosines were rapidly cleared in rat pharmacokinetic studies (Table 1), with

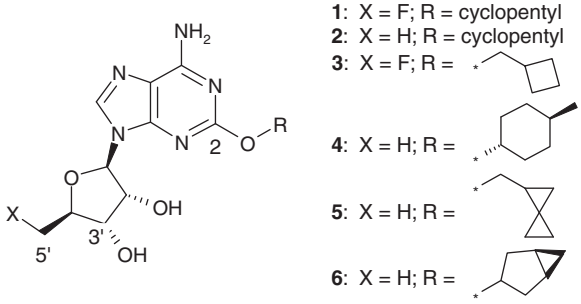

Figure 1. Examples of antibacterial adenosine LigA inhibitors, **1**–**6**. Substituents at 2-position of adenine ring and 5′ of the ribose are noted.

Table 1
In vivo rat clearance and antibacterial activity of adenosine analogs **1**–**6**

Compd	Clearance, rat ml/min/kg (%HBF[a])	MIC Spn[b] (μg/ml)
1	83 (>100)	4
2	43 (62)	2
3	73 (>100)	2
4	89 (>100)	1
5	96 (>100)	1
6	50 (72)	2

Clearance was determined by measuring the concentration of **1**–**6** in rat plasma over time after iv dosing of compounds at 3 mg/kg.
[a] Percent of hepatic blood flow (100% = 70 ml/min/kg).
[b] Minimum inhibitory concentration against *S. pneumoniae* D39, as described in Ref. 2c.

* Corresponding author.
E-mail address: suzanne.stokes@astrazeneca.com (S.S. Stokes).

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clearance approaching hepatic blood flow indicating the compounds were rapidly cleared from systemic circulations of the animals. Evaluation of several analogs in bile duct cannulated rats indicated that biliary clearance was negligible, and in vitro studies indicated that compounds of the adenosine series were stable in whole blood. Consistent with observations from microsome- and hepatocyte-stability experiments, metabolite identification studies demonstrated that oxidative metabolism of the cycloalkyl substituent was the major metabolic hotspot (Fig. 2). Interestingly, no deglycosylation and no cleavage of the ether–adenine linkage were detected. Also, only minor amounts of products from glucuronidation and oxidation of the adenine core were observed. During an experiment to determine efficacy of **3** in a mouse model of lung infection with *S. pneumoniae*, in vivo exposure was dramatically increased when the animals were pre-treated with aminobenzotriazole (ABT), a mechanism-based inactivator of Cytochrome P-450 (CYP) (Fig. 3).[5] The difference in reduction of Colony Forming Units (CFU) in the lungs of animals with and without the ABT pre-treatment is presumably due to the difference in clearance between the two groups. This is another indication that clearance was due to CYP-mediated metabolism. Subsequently, a chemistry effort was undertaken to reduce CYP-mediated oxidative metabolism of the cycloalkyl side chain.

General strategies to reduce CYP-mediated metabolism include removing or sterically blocking the vulnerable site, reducing hydrogen-abstraction potential of the site, or reducing affinity of the compound for the CYP active site by decreasing lipophilicity.[6] All of these approaches were pursued in this series. Table 2 shows examples of 2-alkoxyadenosine analogs designed to reduce clearance due to oxidative metabolism of the 2-position cycloalkyl substituent. Replacement of a carbon from the cyclopentyl groups of **1** and **2** by an oxygen resulted in **7** which was less lipophilic (Log*D* −0.06 for **7** compared to Log*D* 1.5 for **1**).[7] For **9**, a methyl substituent was incorporated on the cyclopropylmethoxy side chain of **8** to sterically block access to the CYP active site. However, in vivo total clearance was not improved for **7** and **9** compared to **2** and **8**, respectively. In some cases, total clearance was reduced by fluorination of the cycloalkyl ring. Incorporation of 4-trifluoromethyl cyclohexanol at the 2-position resulted in **10** [relative stereochemistry is *trans*, *cis* isomers not investigated] which exhibited a threefold reduction in clearance compared to the non-fluorinated methylcyclohexyl-containing **4**. Difluorination of the cyclohexyl ring resulted in **11** which also exhibited a lower clearance. However, there were also analogs for which fluorination of the side chain did not result in a reduction of total clearance compared to the non-halogenated versions. Examples include **12**, containing a trifluoromethyl substituent designed to block oxidation of the methylene carbon of cyclopropane methanol, and the tetrafluorocyclobutane methanol-containing **13**. Total clearance was equivalent for those compounds compared to the non-halogenated

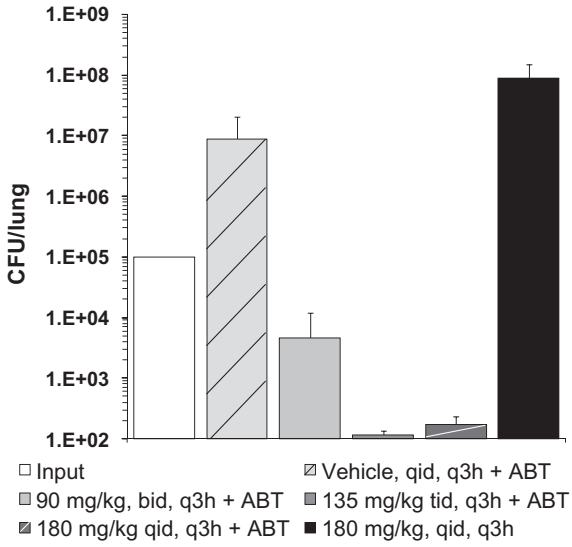


Figure 3. In vivo activity of **3** on *S. pneumoniae* in the lungs of immunocompetent mice (for experimental details, see Ref. 2c). Results are plotted as means; error bars represent the standard errors of the means. Dosing regimens are given in mg/kg per day (bid = 2*x* per day, tid = 3*x* per day, qid = 4*x* per day; q3 h = 4*x* per day every 3 h). ABT administered as a 100 mg/kg oral dose 2 h prior to treatment.

analogs **8** and **3**. To reduce metabolic liability from the methylene carbon, it was removed in **14** and **15**. For these compounds, fluorination of the cycloalkyl group resulted in reduced clearance. However, many of these modifications also resulted in decreased antibacterial activity (**7**, **10**, **11, 13–15** compared to the non-halogenated parents **1–4, 8**).

The major contributors to clearance of the adenosines were hepatic metabolism and renal clearance. To study the relationship between clearance and lipophilicity, renal clearance was measured and compared to total clearance. Initial studies suggested that renal clearance accounted for a greater proportion of the total clearance for compounds with Log*D* <1.5, such as **7** compared to **2**. For example, for **14**, renal clearance accounted for 70% of the total clearance, and Log*D* = 1.3. However, SAR for antibacterial activity indicated that compounds which balance antibacterial activity and drug-like physical properties have Log*D* 1.5–2.5;[2a] therefore, these more polar analogs were not pursued. In some of the fluorinated compounds, it is possible that metabolic switching occurred such that oxidation of the cycloalkyl ring no longer accounted for the major metabolites. Another mechanism, such as glucuronidation or oxidation of the adenine core may have become more prevalent. Metabolites from oxidation of the core and glucuronide conjugates were also more prevalent in the qualitative metabolite identification studies in compounds with halogenated

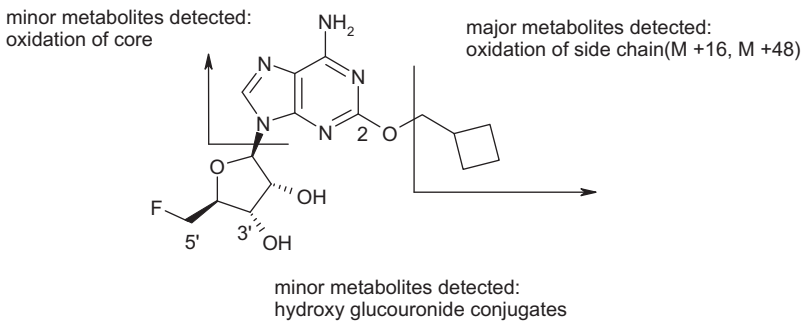
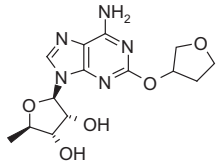
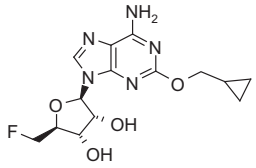
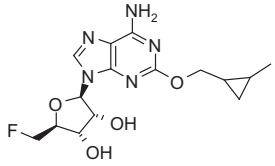
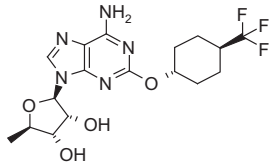
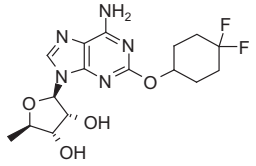
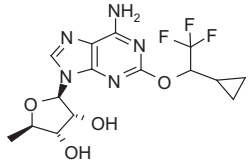
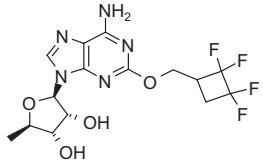
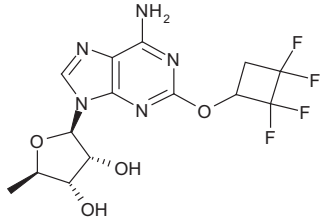
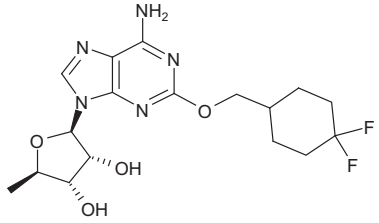


Figure 2. Qualitative metabolite identification study of **3**. Samples were collected and pooled from the rat pharmacokinetic assay and analyzed by LC/MS/MS. Data shown are representative of metabolite identification studies run with other 2-alkoxy-5′-deoxyadenosines from this series.

Table 2
Antibacterial activity, Log*D*, total clearance, and renal clearance of **7**–**15**, designed to reduce CYP-mediated oxidation of the 2-position side chain

Compd	Structure	MIC *S. pneumoniae* (μg/ml)	Log*D* (ACDlogD7.4[a])	total clearance Cl (ml/min/kg)	Renal clearance Cl_r (ml/min/kg) (% total)
7		16	−0.06	67	13 (19%)
8		8	(−0.2)	49	13 (27%)
9		4	(0.3)	115	11 (10%)
10		2	2.2	27	2 (7%)
11		8	1.4	33	15 (45%)
12		8	1.8	73	5 (7%)
13		4	1.4	85	17 (20%)
14		16	1.3	23	16 (70%)
15		16	1.7	58	8 (14%)

[a] Calculated Log*D* using commercially available software package ACD labs from Advanced Chemistry Development.

2-position side chains [data not shown]. However, further quantitative experiments would be necessary to determine the actual mechanism of clearance in these compounds.

Compound **10** (Fig. 4) demonstrated sufficient antibacterial activity and metabolic stability such that it was chosen for further evaluation in rat and dog. Pharmacokinetic analyses were performed determining dog clearance to be lower than rat clearance (20% vs 39% hepatic blood flow), and oral bioavailability of **10** was high: 88% and 86% in dog and rat, respectively. For the adenosine series, in general, trends for mouse, rat, and dog clearance rates were the same. Testing of **10** in an animal model of infection, without the addition of ABT, should lead to a reduction in colony forming units thus demonstrating its efficacy. However, other directions for this project were pursued before this experiment could be performed.

Synthesis of the antibacterial adenosine LigA inhibitors is described in Schemes 1 and 2. Compounds **1**–**15** were synthesized as described previously from commercially available 2-chloroadenosine (Scheme 1).[2,8] Protection, tosylation, nucleophilic displacement with either hydride or fluoride, displacement using an alkoxide, and final deprotection yielded the desired compounds **1**–**15**. Most alcohols that were used as precursors to the 2-alkoxy substituents were either available commercially or made by reduction of the corresponding ester or carboxylic acid.

Synthesis of 4,4-difluorocyclohexanol (Scheme 2) was made by mono-protection of 1,4-cyclohexanediol and oxidation of the remaining alcohol to yield **18**. Fluorination of the resulting ketone gave a mixture of **19** and **20**. In order to separate the desired **19** from the co-eluting side product **20** resulting from elimination of HF, dihydroxylation of the olefin was performed. This resulted in a diol which was significantly more polar than the difluoro intermediate **19**, so the two components could be easily separated by flash chromatography. Deprotection resulted in the desired alcohol **21** which could then be used in the subsequent displacement reaction of the previously described 2-chloroadenosine intermediate **17**.

In conclusion, the adenosine class of LigA inhibitors derived from an HTS screen was optimized for potency, physical properties, and clearance. The adenosine class represents the first series reported to show efficacy in an animal model of infection due to inhibition of bacterial DNA ligase, thereby validating LigA as a useful target for antibacterial drug discovery. The major issue in this series was the high clearance due to CYP-mediated metabolism of the 2-alkoxy substituent or high renal clearance for polar analogs. Although many strategies were pursued to address the metabolism, fluorination of the cycloalkyl moiety was the most successful way to decrease total clearance without a significant loss of antibacterial activity; this was more successful for compounds without a methylene between the ether and cycloalkyl ring. Through this approach, **10** was designed and synthesized which exhibited good in vivo stability in both rat and dog with high oral bioavailability. To obtain adenosine analogs with potential clinical utility, these

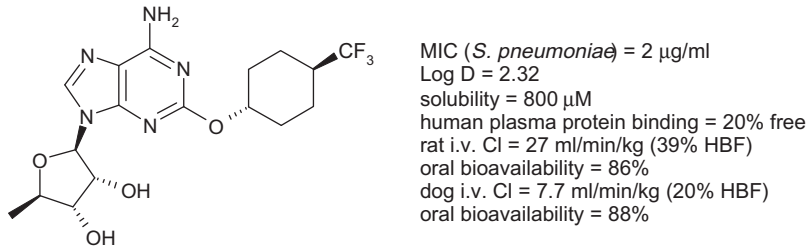
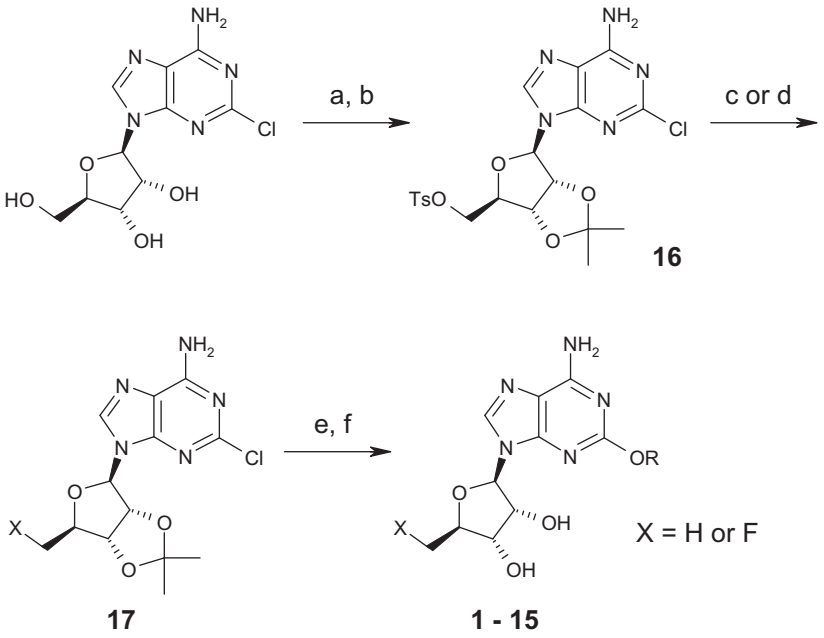
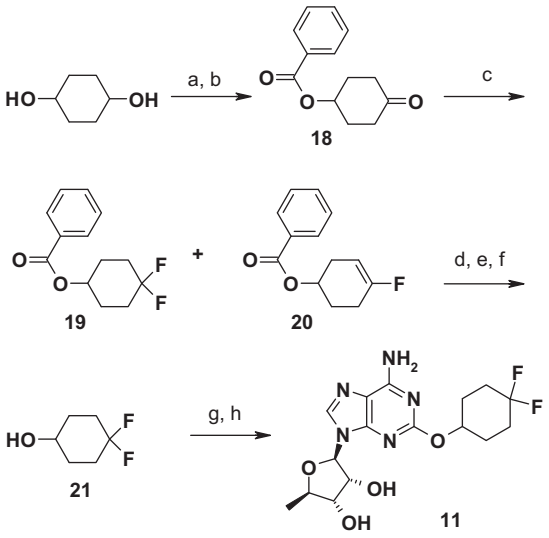


Figure 4. Profile of compound **10** including antibacterial activity, physical properties, and pharmacokinetic properties.



Scheme 1. Synthesis of 2-alkoxy-5′-deoxyadenosines **1**–**15**. Reagents and conditions: (a) 2,2-Dimethoxypropane, pTsOH, acetone, 45 °C (85%); (b) TsCl, pyridine, −10 °C (85%); (c) $LiEt_3BH$, THF (74%); (d) TBAF, CH_2Cl_2 (55%); (e) ROH, NaOH, THF, 75 °C (10–85%); (f) AcOH or HCO_2H, H_2O, 85 °C (80–90%).



Scheme 2. Synthesis of 4,4-difluorocyclohexanol analog **11**. Reagents and conditions: (a) Benzoyl chloride, NEt_3, CH_2Cl_2 (99%); (b) PCC, mol sieves, CH_2Cl_2 (83%); (c) DAST, CH_2Cl_2, 0 °C [not isolated]; (d) OsO_4 (aq), acetone/H_2O; (e) SiO_2 column chromatography (78% over 2 steps); (f) KOH (aq), THF (99%); (g) NaOH, **17**, THF, 75 °C; (h) HCO_2H, H_2O, 85 °C.

compounds require further improvements in potency while maintaining in vivo clearance which results in sufficient exposure to treat infections.

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7. The 5′fluoro substituent does not alter the physical properties; **1** and **2** have nearly identical *c*log*D* values. Therefore comparison with either compound is valid.
8. For experimental details and ^{1}HNMR data, see Cavero-Tomas, M.; Gowravaram, M.; Huynh, H., Ni, H.; Stokes, S. Patent WO 2006040558.