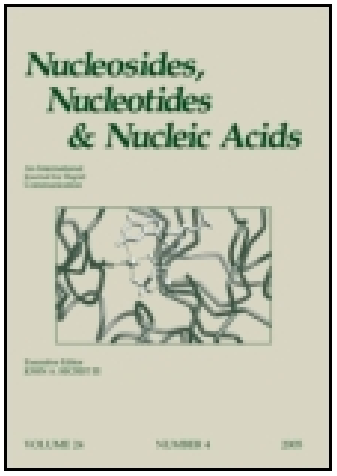

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Synthesis of Novel Peptidyl Adenosine Antibiotic Analogs

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SYNTHESIS OF NOVEL PEPTIDYL ADENOSINE ANTIBIOTIC ANALOGS

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□ *A small library of peptidyl adenosine antibiotic analogs was synthesized, under the Pilot Scale Library Program of the NIH Roadmap initiative, from 2′,3′-O-isoproylideneadenosine-5′-carboxylic acid* ***2*** *in excellent yield. The coupling of the amino terminus of L-2-aminophenylbutyric methyl ester to a free 5′-carboxylic acid moiety of* ***2*** *followed by sodium hydroxide treatment led to carboxylic acid analog* ***4****. Hydrolysis of this latter gave unprotected nucleoside analog* ***5****. Intermediate* ***4*** *served as the precursor for the preparation of novel peptidyl adenosine analogs* ***6–18*** *in good yields and high purity through peptide coupling reactions to diverse amine derivatives. No marked anticancer and antimalaria activity was noted on preliminary cellular testing; however these analogs should be useful candidates for other types of biological activity.*

Keywords Adenosine peptides; antibiotic analogs; general biological activities

INTRODUCTION

The functional complexity and varied three-dimensional characteristics of the natural products have been key elements behind their propensity to produce wide ranging and interesting biological activities. In fact, as of 1997 approximately 60% of the anti-cancer and anti-infective drugs that were on the market or in clinical trials were natural products, or were derived from or attributed to natural product templates.[1] Not surprisingly therefore, natural products and their analogs remain a source of inspiration in the search

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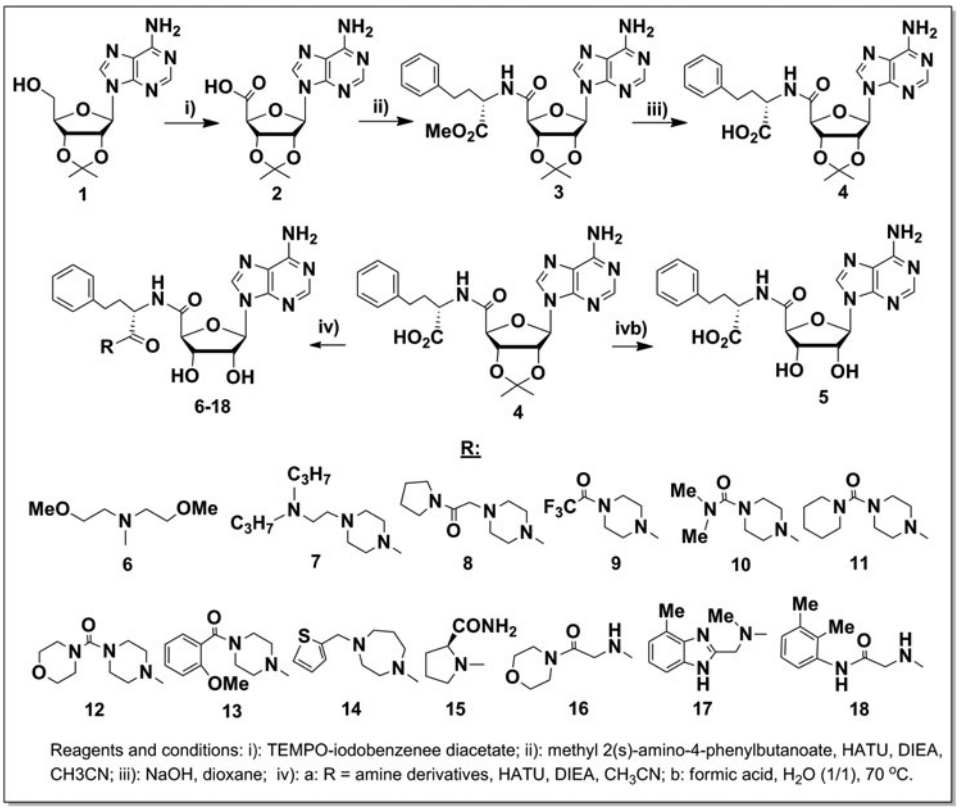
for novel bioactive ligands and molecular probes. Natural nucleoside antibiotics, for example, demonstrate potent activities such as protein synthesis inhibition, glycosyltransferase inhibition, and methyltransferase inhibition, among others.[2–5] More recently, there has been a trend to develop approaches for the synthesis and screening of this class of compounds, that do not exhibit typical antimetabolite activities based on nucleoside phosphorylation and incorporation into nucleoside metabolic pathways.[6–10] In many respects, the nucleoside scaffolds offer an opportunity for numerous diverse and directionally oriented substitutions in order to probe for activity against diverse protein binding sites. Furthermore, many of these basic scaffolds are not well represented in commercial chemical space, including the Molecular Library Small Molecule Repository.

For the last four decades, there has been a great deal of interest in the isolation and synthesis of nucleoside amino acids and peptides as overviewed in previous publications.[11–17] Most syntheses of nucleoside peptides have involved either the coupling of an amino[11,12,18] or hydroxyl[19] group of a nucleoside to the carboxyl group of a blocked amino acid, or displacement of a leaving group on a nucleoside by the amino group of an amino acid. On the other hand, oxidation of the 5′-hydroxymethylene of nucleosides to 5′-carboxylates is an essential step in the preparation of a number of biologically active molecules.[14a–e] For example, the binding of 5′-carboxylic acid ester and amide adenosine derivatives with adenosine receptors has been reported,[15–17] as exemplified by 5′-*N*-ethylcarboxamido adenosine,[14a] a high-affinity, non-selective adenosine receptor agonist. Usually, the affinity for adenosine receptors increases when the 5′-hydroxyl moiety is replaced with a 5′-*N*-ethylcarboxamido group.[14c] 5′-Modified adenosine analogues are also reported as growth inhibitors of multidrug resistant *Plasmodium falciparum*[20], inhibitors of trypanosomal glycolytic enzymes and inhibitors of specific enzymes[21] involved in trypanosomal polyamine synthesis.[22] Hence, we have designed and prepared diverse nucleoside antibiotic-like small molecule libraries, under the Pilot Scale Library Program of the NIH Roadmap Initiative, to probe specific or general biological activities. Very recently, we have reported an initial phase of this program and certain of the described analogs have shown interesting multiple biological activities in the preliminary screening.[23] In continuation of this effort, we herein report a facile synthesis of a new small peptidyl adenosine analog library (**6–18)** (Scheme 1) derived from carboxylic acid intermediate **4**.

RESULTS AND DISCUSSION

As depicted in Scheme 1, 2′,3′-*O*-isoproylideneadenosine-5′-carboxylic acid **2** was prepared in 92% yield and high purity according to the method



SCHEME 1 Structures of compounds 5–18.

previously described[24] using catalytic amounts of 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) and [bis(acetoxy)iodo]benzene (BAIB) in a 1:1 acetonitrile–water solvent system. When this intermediate was coupled to L-2-aminophenylbutyric methyl ester, compound **3** (Scheme 1) was formed in 81% yield. Saponification with NaOH gave **4**, and subsequent treatment with 50% formic acid removed the isopropylidene blocking group and quantitatively afforded carboxylic acid derivative **5**. The carboxylic acid group of **4** was designed as a site of further diversification through robust peptide coupling chemistry in order to prepare the targeted compounds **6-18** (Scheme 1). The resulting nucleoside peptides were expected to show reasonable stability to dissolution, storage, and screening as evidenced by similar aminoacyl functions found in various nucleoside antibiotics such as puromycin, gougerotin, amicetin, and blasticidin S**,** all known inhibitors of protein synthesis. Thus, the coupling of carboxylic acid **4** to 13 different amines using HATU/DIEA in acetonitrile in a parallel fashion on a Radleys 12-place carousel reaction station at a 0.5 mmol scale followed with acid-mediated deprotection and purification led to the targeted analogs **6–18** in good yield and high purity.

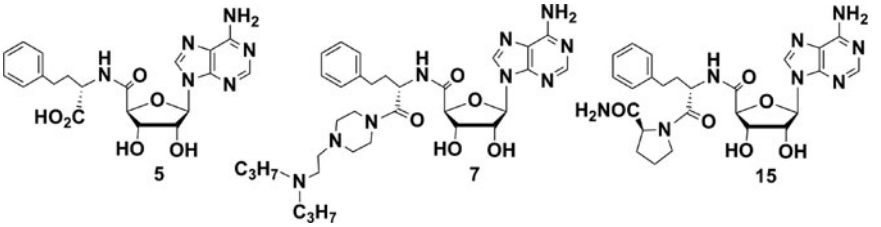


FIGURE 1 Active compounds.

Biological Evaluation

All 14 synthesized compounds **5–18** were screened in vitro against five human tumor cell lines (HT29 colon, PC3 prostate, MDA-MB-231 breast, leukemia, and human brain tumor). No appreciable antitumor activity or cytotoxicity was seen at compound concentrations lower than 100 μM. Also, no inhibition was detected when compounds **5–18** were screened against *Plasmodium falciparum* (cause of malaria) strain 3D7 at a fixed concentration of 7 μM. In addition, analogs **5–18** have been submitted in the Molecular Libraries Probe Production Centers Network (MLPCN) to be screened against a wide range of biological assays (see www.ncbi.nlm.nih.gov/pcsubstance search term Robert Reynolds). Certain analogs (Figure 1) exhibited interesting activities in MLPCN primary screens—for example, adenosine dipeptides **5** (SID: 134214897) and **15** (SID: 134214904) were found to be inhibitors of Dengue virus type 2 by using cytopathic effect assay. Compound **7** (SID: 134214910) was identified as an inhibitor of Sirtuin 5 (Sirt-5) which is a promising target for treating several human diseases.

Biological Assays

The anti-tumor assays were performed following procedures previously described.[23,25] The antimalaria assay was realized using the protocol published by Guiguemde et al.[26]

CONCLUSION

In conclusion, we have synthesized a small library adenosine antibiotic-like analogs derived from 2′,3′-*O*-isopropylideneadenosine-5′-carboxylic acid using parallel solution phase methods in good yields and high purity. No marked anticancer and antimalaria activity or cytotoxicity was witnessed and all analogs have been submitted for screening in the MLPCN and preliminary screening has indicated a variety of interesting activities although full evaluation of the synthesized targets is still under way.

Experimental

The exact mass spectral data were obtained with an Agilent LC-MSTOF or with Bruker BIOTOF II by electrospray ionization (ESI). ^{1}H-NMR spectra were recorded on a Nicolet NT-300 NB spectrometer operating at 300.635 MHz or on Agilent/Varian MR-400 spectrometer operating at 399.930 MHz. Chemical shifts in $CDCl_3$ and Me_2SO-d_6 are expressed in parts per million downfield from tetramethylsilane (TMS). Chemical shifts (δ) listed for multiplets were measured from the approximate centers, and relative integrals of peak areas agreed with those expected for the assigned structures. Melting points were automatically determined on Opti Melt apparatus and are uncorrected. Determination of percent purity were obtained by HPLC (column type: Vydac C18) using an Agilent 1100 LC equipped with a diode array UV detector and monitored at multiple wavelengths. Flash column chromatography was performed via the Biotage Isolera One flash purification system using silica-packed SNAP cartridge, KP-Sil.

2',3'-O-Isopropylideneadenosine-5'-carboxylic acid 2

BAIB (220 mmol), TEMPO (20 mmol), and a 2′,3′-*O*-isopropylideneadenosine (100 mmol) were combined in a reaction vessel, and to this mixture was added 200 mL of a 1:1 acetonitrile–water solution. The reaction mixture was stirred for 3 hours. The resulting precipitate was filtered, triturated sequentially with diethyl ether and acetone, and dried in vacuo. Yield 92%, white solid, mp 250–253°C dec. ^{1}H NMR (400 MHz, Me_2SO-d_6): 12.79 (br s, OH), 8.24 (s, H2), 8.08 (s, H8), 7.25 (s, NH_2), 6.32 (br s, H1'), 5.51–5.53 (m, H2'), 4.45–4.46 (m, H3'), 4.67 (d, H4', J = 1.7 Hz), 1.52 and 1.35 (2s, $2CH_3$). FABMS (M+H) calculated for $C_{13}H_{15}N_5O_5$.H was 322.1146 found 322.1152.

(2S)-2-[[(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-O-isopropylideneoxolane-2-carbonyl]-amino]-4-phenylbutanoic acid 4

To a solution of HATU (0.5 mmol) and the carboxylic acid **2** (0.75 mmol) in acetonitrile (5 mL) was added methyl L-2-amino-4-phenylbutanoate (0.5 mmol) followed by DIEA (0.75 mmol) under argon at room temperature. The reaction mixture was stirred for 2 hours (TLC monitored), pre-adsorbed on silica gel and purified using the Biotage automated flash chromatography system. The obtained intermediate **3** was treated with an aqueous solution of NaOH (1N, 1.5 mL) in dioxane and stirred vigorously for 1 hour whereby TLC showed no remaining starting material. The solvent was removed in vacuo at ~40°C bath temperature, water was added to the residue and the desired product **4** was collected as a white solid by precipitation with 1N HCl solution. Yield 78%. ^{1}H NMR (400 MHz, Me_2SO-d_6): 12.7 (br s, CO_2H), 8.33 (s, H8), 8.08 (s, H2), 8.01–8.06 (m, NHCO), 7.31 (br s, NH_2), 7.07–7.27(m, 5H, Ph), 6.34 (d, H1', J = 2 Hz), 5.38–5.4 (dd,

H3', $J = 2.4$ and 6.3 Hz), 5.31–5.34 (dd, H2', $J = 2.3$ and 6.2 Hz), 4.62 (d, H4', $J = 2.4$ Hz), 3.95–3.98 (m, CHCO), 2.26–2.33 (m, CH_2), 1.63–1.65 and 1.72–1.76 (2m, CH_2), 1.35 and 1.57 (2s, $2CH_3$). FABMS (M + H) calculated for $C_{23}H_{26}N_6O_6$.H was 483.1986 found 483.1982.

(2S)-2-[[(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxyoxolane-2-carbonyl]amino]-4-phenylbutanoic acid 5

The intermediate **4** (0.25 mmol) and 4 mL of formic acid (50%) were heated to 70°C and stirred vigorously for 2 hours whereby TLC showed no remaining starting material. The solvent was removed in vacuo at ~60°C bath temperature, silica gel was added to the residue dissolved in methanol and the mixture was concentrated, and chromatographed to give **5** as a white powder. Yield 82%, mp 149–152°C. HPLC 94%, $t_R = 9.1$ minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.04 (br s, NH), 8.39 (s, H2), 8.08 (s, H8), 7.41 (br s, NH_2), 7.16–7.29 (m, 5H, Ph), 5.99 (d, H1', $J = 7.9$ Hz), 5.84 (br s, OH2'), 5.61 (d, OH3', $J = 6.2$ Hz), 4.68–4.72 (m, H2'), 4.1 (d, H4', $J = 1.2$ Hz), 4.27–4.31 (m, CHCO), 4.18–4.19 (m, H3'), 2.63–2.67 (m, CH_2), 1.98–2.03 and 2.11–2.15 (2m, CH_2). FABMS (M + H) calculated for $C_{20}H_{22}N_6O_6$.H was 443.1673 found 443.1678.

General Procedure for Preparation of Compounds: 6–18

To a solution of HATU (0.5 mmol) and the carboxylic acid **4** (0.75 mmol) in acetonitrile (5 mL) was added selected amine (0.5 mmol) followed by DIEA (0.75 mmol) under argon in a 12-place carousel reaction station at room temperature. The reaction mixture was stirred for 30–180 minutes (TLC monitored), pre-adsorbed on silica gel, and purified using the Biotage automated flash chromatography system. The obtained pure intermediate (0.25 mmol) and 4 mL of formic acid (50%) in a Radleys' reaction tube was heated to 70°C and stirred vigorously for 2 hours whereby TLC showed no remaining starting material. The solvent was removed in vacuo at ~60°C bath temperature, silica gel was added to the residue dissolved in methanol and the mixture was concentrated, and chromatographed to give the desired product.

(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-N-[(2S)-1-[bis(2-methoxyethyl)amino]-1-oxo-4-phenylbutan-2-yl]-3,4-dihydroxyoxolane-2-carboxamide 6

Yield 84%, white solid, mp 65–67°C. HPLC 91%, $t_R = 12.4$ minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.4 (d, NH, $J =$ 7 Hz), 8.34 (s, H2), 8.19 (s, H8), 7.43 (br s, NH_2), 7.13–7.29 (m, 5H, Ph), 5.96 (d, H1', $J = 7.8$ Hz), 5.83 and 5.59 (2d, OH2', $J = 4.3$ Hz and OH3', $J =$ 6.2 Hz), 4.92–4.97 (m, CHCO), 4.66–4.70 (m, H2'), 4.4 (d, H4', $J = 0.8$ Hz), 4.15–4.17 (m, H3'), 3.36–3.64 (m, $2OCH_2CH_2N$), 3.22 and 3.17 (2s, $2CH_3$),

2.49–2.67 (m, CH_2), 1.95–2.04 (m, CH_2). FABMS (M + H) calculated for $C_{26}H_{35}N_7O_7$.H was 558.2670 found 558.2671.

(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-N-[(2S)-1-[4-[2-(dipropylamino)ethyl]piperazin-1-yl]-1-oxo-4-phenylbutan-2-yl]-3,4-dihydroxyoxolane-2-carboxamide 7

Yield 79%, white solid, mp 76–80°C. HPLC 99%, $t_R = 11$ minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.4 (d, NH, J = 8.7 Hz), 8.35 (s, H2), 8.14 (s, H8), 7.43 (br s, NH_2), 7.18–7.29 (m, 5H, Ph), 5.97 (d, H1', $J = 7.8$ Hz), 5.86 and 5.63 (2br s, OH2' and OH3'), 4.83–4.88 (m, CHCO), 4.66–4.69 (m, H2'), 4.39 (d, H4', $J = 1.1$ Hz), 4.15–4.16 (m, H3'), 3.13 (br m, $2CH_2N$), 2.52–2.63 (m, CH_2), 2.85–2.84 (m, $2NCH_2CH_2N$, $2CH_2N$ and $2CH_2NCO$), 1.99–2.04 (m, CH_2), 1.3–1.39 (m, $2CH_2$), 0.81 (2t, $2CH_3$, $J = 7.4$ Hz). FABMS (M + H) calculated for $C_{32}H_{47}N_9O_5$.H was 638.3772 found 638.3766.

(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxy-N-[(2S)-1-oxo-1-[4-(2-oxo-2-pyrrolidin-1-ylethyl)piperazin-1-yl]-4-phenylbutan-2-yl]oxolane-2-carboxamide 8

Yield 72%, white solid, mp 117–120°C. HPLC 97%, $t_R = 11.6$ minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.36 (d, NH, $J = 9$ Hz), 8.36 (s, H2), 8.14 (s, H8), 7.43 (br s, NH_2), 7.18–7.3 (m, 5H, Ph), 5.97 (d, H1', $J = 8.2$ Hz), 5.83 and 5.6 (2d, OH2', $J = 4.3$ Hz and OH3', J = 6.6 Hz), 4.84–4.89 (m, CHCO), 4.66–4.71 (m, H2'), 4.4 (d, H4', $J = 0.8$ Hz), 4.16–4.18 (m, H3'), 3.35–3.42 (m, $3CH_2N$), 3.25 (t, CH_2, $J = 6.6$ Hz), 3.06 (s, CH_2), 2.63 (t, CH_2, $J = 7.6$ Hz), 2.34–2.45 (m, $2NCH_2$), 1.69–2.03 (m, $3CH_2$). FABMS (M + H) calculated for $C_{30}H_{39}N_9O_6$.H was 622.3096 found 622.3093.

(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxy-N-[(2S)-1-oxo-4-phenyl-1-[4-(2,2,2-trifluoroacetyl)piperazin-1-yl]butan-2-yl]oxolane-2-carboxamide 9

Yield 81%, white solid, mp 114–117°C. HPLC 97%, $t_R = 12.6$ minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.42 (m, NH), 8.36 (s, H2), 8.13 (s, H8), 7.43 (br s, NH_2), 7.17–7.3 (m, 5H, Ph), 5.98 (d, H1', $J = 5.5$ Hz), 5.83 and 5.6 (2d, OH2', $J = 4.3$ Hz and OH3', J = 6.3 Hz), 4.9–4.93 (m, CHCO), 4.66–4.71 (m, H2'), 4.42 (d, H4', $J = 1.1$ Hz), 4.17–4.19 (m, H3'), 3.97–3.54 (m, $2NCH_2CH_2N$), 2.6–2.7 (m, CH_2), 1.9–2.1 (m, CH_2). FABMS (M + H) calculated for $C_{26}H_{29}F_3N_8O_6$.H was 607.2234 found 607.2233.

4-[(2S)-2-[[(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxyoxolane-2-carbonyl]amino]-4-phenylbutanoyl]-N,N-dimethylpiperazine-1-carboxamide 10

Yield 74%, white solid, mp 121–124°C. HPLC 97%, $t_R = 11.4$ minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.45 (d, NH, $J = 8.6$ Hz), 8.35 (s, H2), 8.14 (s, H8), 7.43 (br s, NH_2), 7.18–7.3 (m, 5H, Ph), 5.97 (d, H1', $J = 7.9$ Hz), 5.83 and 5.6 (2d, OH2', $J = 3.9$ Hz and OH3', $J = 6.6$ Hz), 4.86–4.91 (m, CHCO), 4.65–4.7 (m, H2'), 4.41 (d, H4', $J = 1.1$ Hz), 4.16–4.19 (m, H3'), 3.39–3.44 (m, $2NCH_2$), 2.92–3.09 (m, $2NCH_2$), 2.72 (s, $2CH_3$), 2.62 (t, CH_2, $J = 8.5$ Hz), 1.9–2.06 (m, CH_2). FABMS (M + H) calculated for $C_{27}H_{35}N_9O_6$.H was 582.2783 found 582.2780.

(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxy-N-[(2S)-1-[piperdine-4-carbonyl)-piperazin-1-yl]-1-oxo-4-phenylbutan-2-yl]oxolane-2-carboxamide 11

Yield 80%, white solid, mp 127–131°C. HPLC 96%, $t_R = 11.4$ minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.43 (d, NH, $J = 8.6$ Hz), 8.35 (s, H2), 8.14 (s, H8), 7.43 (br s, NH_2), 7.18–7.3 (m, 5H, Ph), 5.97 (d, H1', $J = 8.3$ Hz), 5.83 and 5.6 (2d, OH2', $J = 3.9$ Hz and OH3', $J = 6.6$ Hz), 4.85–4.91 (m, CHCO), 4.65–4.7 (m, H2'), 4.41 (d, H4', $J = 1.2$ Hz), 4.16–4.19 (m, H3'), 3.36–3.43 (m, $2NCH_2$), 2.92–3.11 (m, $4NCH_2$), 2.62 (t, CH_2, $J = 8.5$ Hz), 1.89–2.09 (m, CH_2), 1.44–1.52 (m, $3CH_2$). FABMS (M + H) calculated for $C_{30}H_{39}N_9O_6$.H was 622.3096 found 622.3092.

(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxy-N-[(2S)-1-[4-(morpholine-4-carbonyl)-piperazin-1-yl]-1-oxo-4-phenylbutan-2-yl]oxolane-2-carboxamide 12

Yield 67%, white solid, mp 118–121°C. HPLC 97%, $t_R = 11.4$ minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.46 (d, NH, $J = 8.7$ Hz), 8.35 (s, H2), 8.14 (s, H8), 7.43 (br s, NH_2), 7.18–7.3 (m, 5H, Ph), 5.97 (d, H1', $J = 7.9$ Hz), 5.83 and 5.6 (2d, OH2', $J = 4.3$ Hz and OH3', $J = 6.3$ Hz), 4.86–4.91 (m, CHCO), 4.65–4.69 (m, H2'), 4.41 (d, H4', $J = 1.1$ Hz), 4.16–4.19 (m, H3'), 3.54 (t, $2OCH_2$, $J = 4.6$ Hz), 3.36–3.43 (m, $2NCH_2$), 3.12 (t, $2NCH_2$, $J = 4.6$ Hz), 3–3.05 (m, $2NCH_2$), 2.62 (t, CH_2, $J = 8.5$ Hz), 1.9–2.08 (m, CH_2). FABMS (M + H) calculated for $C_{29}H_{37}N_9O_7$.H was 624.2888 found 624.2885.

(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxy-N-[(2S)-1-[4-(2-methoxybenzoyl)-piperazin-1-yl]-1-oxo-4-phenylbutan-2-yl]oxolane-2-carboxamide 13

Yield 68%, white solid, mp 132–135°C. HPLC 96%, $t_R = 12.5$ minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.41–9.47 (m, NH), 8.36 (s, H2), 8.13 (s, H8), 7.43 (br s, NH_2), 6.89–7.4 (m, 9H, Ph), 5.98 (d, H1', $J = 7.8$ Hz), 5.82–5.85 (m, OH2'), 5.6 (d, OH3', $J = 6.3$ Hz),

4.85–4.93 (m, CHCO), 4.66–4.71 (m, H2'), 4.37–4.43 (m, H4'), 4.15–4.19 (m, H3'), 3–3.8 (m, $4NCH_2$ and CH_3), 2.54–2.64 (m, CH_2), 2.06 (m, CH_2). FABMS (M + H) calculated for $C_{32}H_{36}N_8O_7$.H was 645.2779 found 645.2776.

(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxy-N-[(2S)-1-oxo-4-phenyl-1-[4-(thiophen-2-ylmethyl)-1,4-diazepan-1-yl]butan-2-yl]oxolane-2-carboxamide 14

Yield 77%, white solid, mp 98–101°C. HPLC 97%, t_R = 12.5 minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.45–9.51 (2d, NH, J = 8.6 Hz), 8.35 (s, H2), 8.18 (s, H8), 7.44 (br s, NH_2), 7.34–7.39 (2dd, 1H, thiophene, J = 1.2 Hz and 5.1 Hz), 7.15–7.29 (m, 5H, Ph), 5.84–6.95 (m, 2H, thiophene), 5.97 (d, H1', J = 7.8 Hz), 5.84 and 5.6 (2d, OH2', J = 4.3 Hz and OH3', J = 6.7 Hz), 4.84–4.89 (m, CHCO), 4.66–4.71 (m, H2'), 4.4 (s, H4'), 4.16–4.18 (m, H3'), 3.64–3.74 (m, NCH_2), 3.4–3.48 (m, $2NCH_2$), 2.4–2.68 (m, $2NCH_2$ and CH_2), 1.96–2.04 (m, CH_2), 1.61–1.68 (m, CH_2). FABMS (M + H) calculated for $C_{30}H_{36}N_8O_5S$.H was 621.2602 found 621.2598.

(2S)-1-[(2S)-2-[[(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxyoxolane-2-carbonyl]-amino]-4-phenylbutanoyl]pyrrolidine-2-carboxamide 15

Yield 73%, white solid, mp 156–160°C. HPLC 96%, t_R = 10.4 minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.32 (d, NH, J = 8.6 Hz), 8.35 (s, H2), 8.16 (s, H8), 7.42 (br s, NH_2), 7.16–7.29 (m, 5H, Ph, and NH), 6.87 (br s, NH), 5.96 (d, H1', J = 7.8 Hz), 5.82 and 5.6 (2d, OH2', J = 4.3 Hz and OH3', J = 6.6 Hz), 4.71–4.76 (m, CHCO), 4.63–4.68 (m, H2'), 4.4 (d, H4', J = 1.2 Hz), 4.16–4.18 (m, H3' and CHCO), 3.37–3.52 (m, $2CH_2$), 2.66–2.67 (m, NCH_2), 1.75–2.11 (m, $3CH_2$). FABMS (M + H) calculated for $C_{25}H_{30}N_8O_6$.H was 539.2361 found 539.2361.

(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxy-N-[(2S)-1-[(2-morpholin-4-yl-2-oxoethyl)amino]-1-oxo-4-phenylbutan-2-yl]oxolane-2-carboxamide 16

Yield 69%, white solid, mp 143–146°C. HPLC 96%, t_R = 10.3 minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.04 (d, NH, J = 8.2 Hz), 8.38 (s, H2), 8.14 (t, NH, J = 5.5 Hz), 8.1 (s, H8), 7.39 (br s, NH_2), 7.16–7.27 (m, 5H, Ph), 5.98 (d, H1', J = 7.9 Hz), 5.98 and 5.58 (2d, OH2', J = 3.9 Hz and OH3', J = 6.6 Hz), 4.69–4.73 (m, CHCO), 4.52–4.58 (m, H2'), 4.41 (d, H4', J = 1.2 Hz), 4.18–4.2 (m, H3'), 3.85–4 (2dd, $COCH_2$, J = 5.5 Hz and 16.9 Hz), 3.4–3.52 (m, $4CH_2N$), 2.6–2.67 (m, CH_2), 1.91–2.1 (m, CH_2). FABMS (M + H) calculated for $C_{26}H_{32}N_8O_7$.H was 569.2466 found 569.2460.

(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxy-N-[(2S)-1-[methyl-[(6-methyl-1H-benzimidazol-2-yl)methyl]amino]-1-oxo-4-phenylbutan-2-yl]oxolane-2-carboxamide 17

Yield 75%, white solid, mp 151–154°C. HPLC 94%, $t_R = 13.7$ minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 12.02–12.3 (2d, NH, $J = 10.9$ Hz), 9.33–9.48 (2d, NHCO, $J = 9$ Hz), 8.28 (s, H2), 8.17 (s, H8), 6.92–7.47 (m, 8H, Ph and NH_2), 5.98 (d, H1', $J = 7.5$ Hz), 5.84–5.86 (m. OH2'), 5.61 (d, OH3', $J = 6.2$ Hz), 4.47–5.05 (m, CHCO, CH_2N, and H2'), 4–4.43 (2d, H4', $J = 1.2$ Hz), 4.15–4.21 (m, H3'), 3.04 and 2.89 (2s, CH_3), 2.51–2.68 (m, CH_2), 2.36–2.42 (m, CH_3), 1.96–2.09 (m, CH_2). FABMS (M + H) calculated for $C_{30}H_{33}N_9O_5$.H was 600.2677 found 600.2676

(2S,3S,4R,5R)-5-(6-aminopurin-9-yl)-N-[(2S)-1-[[2-(2,3-dimethylanilino)-2-oxoethyl]-amino]-1-oxo-4-phenylbutan-2-yl]-3,4-dihydroxyoxolane-2-carboxamide 18

Yield 71%, white solid, mp 149–152°C. HPLC 98%, $t_R = 13$ minutes, phosphate buffer/MeOH. ^{1}H NMR (400 MHz, Me_2SO-d_6): 9.26 (s, NH), 9.05 (d, NH, $J = 8.2$ Hz), 8.4–8.43 (m, NH), 8.36 (s, H2), 8.1 (s, H8), 7.39 (s, NH_2), 6.98–7.28 (m, 8H, Ph), 5.99 (d, H1', $J = 7.8$ Hz), 5.79 and 5.58 (2d, OH2', $J = 4.3$ Hz and OH3', $J = 6.3$ Hz), 4.69–4.73 (m, H2'), 4.5–4.56 (m, CHCO), 4.42 (d, H4', $J = 1.1$ Hz), 4.19–4.21 (m, H3'), 3.83–3.95 (m, $COCH_2$), 2.63–2.65 (m, CH_2), 2.25 (m, CH_3), 2.06–2.17 (m, CH_2 and CH_3). FABMS (M + H) calculated for $C_{30}H_{34}N_8O_6$.H was 603.2674 found 603.2669.

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