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Synthesis and Pharmacological Evaluation of Dual Acting Antioxidant A_{2A} Adenosine Receptor Agonists

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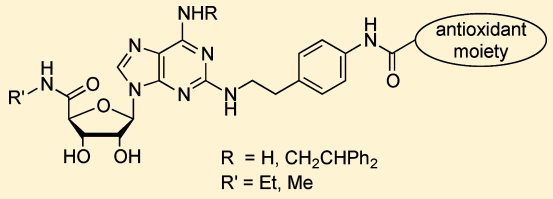
ABSTRACT: A series of adenosine-5′-*N*-alkylcarboxamides and N^6-(2,2-diphenylethyl)adenosine-5′-*N*-alkylcarboxamides bearing antioxidant moieties in the 2-position were synthesized from the versatile intermediate, O^6-(benzotriazol-1-yl)-2-fluoro-2′,3′-*O*-isopropylideneinosine-5′-*N*-alkylcarboxamide (**1**). These compounds were evaluated as A_{2A} adenosine receptor (A_{2A}R) agonists in a cAMP accumulation assay, and a number of potent and selective agonists were identified. Three of these compounds were evaluated further in an ischemic injury cell survival assay and a reactive oxygen species (ROS) production assay whereby **15b** and **15c** were shown to reduce ROS activity and cell death due to ischemia.



INTRODUCTION

Adenosine is a ubiquitous endogenous nucleoside that mediates a number of physiological processes.[1,2] These effects are mediated through binding interaction with adenosine receptors (ARs) which are members of the G protein-coupled receptor (GPCR) family. There are known to be four distinct AR subtypes, which are termed A_1, A_{2A}, A_{2B}, and A_3. The A_1 and A_3Rs couple to G_i inhibiting adenylate cyclase activity, thereby prohibiting cyclic AMP production. Conversely, the A_{2A} and A_{2B}Rs couple to G_s and mediate the stimulation of adenylate cyclase, promoting cyclic AMP production. Adenosine helps to regulate a wide array of physiological processes and is involved in cancer[1] and cardioprotection.[2] Specifically, a great deal of interest has centered on the A_{2A} receptor and its role in inflammation.[3−5]

Recently, the emergence of GPCR crystal structure elucidation and the discovery that the seven transmembrane (7TM) topology is common between various family A GPCRs, such as the CXCR4 chemokine receptor,[6] D_3 dopamine receptor,[7] and β_1 and β_2 adrenergic receptors,[8,9] has led to a paradigm shift in GPCR drug discovery. Specifically, the illumination of the A_{2A}R's crystal structure with a bound antagonist (ZM241385) has been reported.[10] This initial report led to increased efforts to crystallize the receptor with other ligands, specifically A_{2A}R selective agonists. Subsequently, the A_{2A}R selective agonist UK-432,097 has been used to probe the internal structure of the active site.[11] A number of pharmacologically important agonists displaying A_{2A}R selectivity have been synthesized and are shown in Figure 1. YT-146 and DPMA have shown cardioprotective effects,[12,13] whereas CGS-21680 has been used for studying neuronal transmission.[14−16] The drug candidate UK-432,097 was developed by Pfizer for the treatment of chronic obstructive pulmonary disease but unfortunately gave poor efficacy results in phase II clinical trials.[17] Apadenoson (ATL-146e) and Binodenoson (MRE-0470), which are both highly selective A_{2A}R agonists, are being trialled for myocardial perfusion imaging, while Sonedenoson (MRE-0094) has displayed promise for diabetic foot ulcers and wound healing.[18] The A_{2A}R agonist Regadenoson, licensed as Lexiscan, is a coronary vasodilator which has found application diagnostically as a radionuclide myocardial perfusion imaging agent.[19] It is currently the only selective agonist on the market.

Selective A_{2A}R agonists have considerable promise in the prevention and treatment of ischemia-reperfusion injury.[20] After a myocardial infarction, the recruitment of immune cells to the damaged myocardium and the death of cardiomyocytes are two of the most important steps in the pathogenesis of the disease. A_{2A}R activation inhibits neutrophil accumulation and reduces infarct size,[21] and A_{2A}R agonists are therefore of interest as therapeutic agents post myocardial infarction. Despite this promise, most inflammatory agents that have been evaluated for the therapy of IR injury in clinical studies have failed. Accordingly, it has been suggested that new strategies to augment adenosine-mediated protective effects may be more effective.[20] Free radical production is well established to increase massively during cardiac ischemia and reperfusion, with a subsequent loss of cardiomyocytes. The use of antioxidants as a strategy to reduce the loss of cardiomyocytes has been of significant interest.[22,23] The combination of A_{2A}R agonist and antioxidant functionalities is conceptually attractive because it combines a mechanism to inhibit both immune cell recruitment and direct loss of

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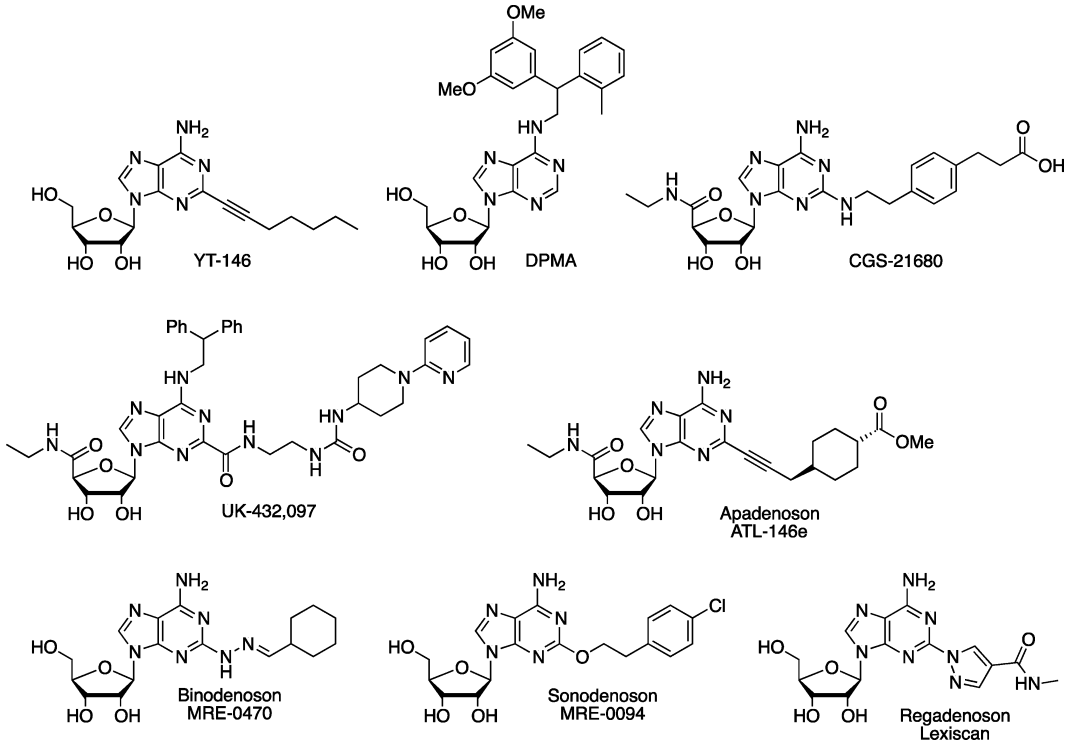
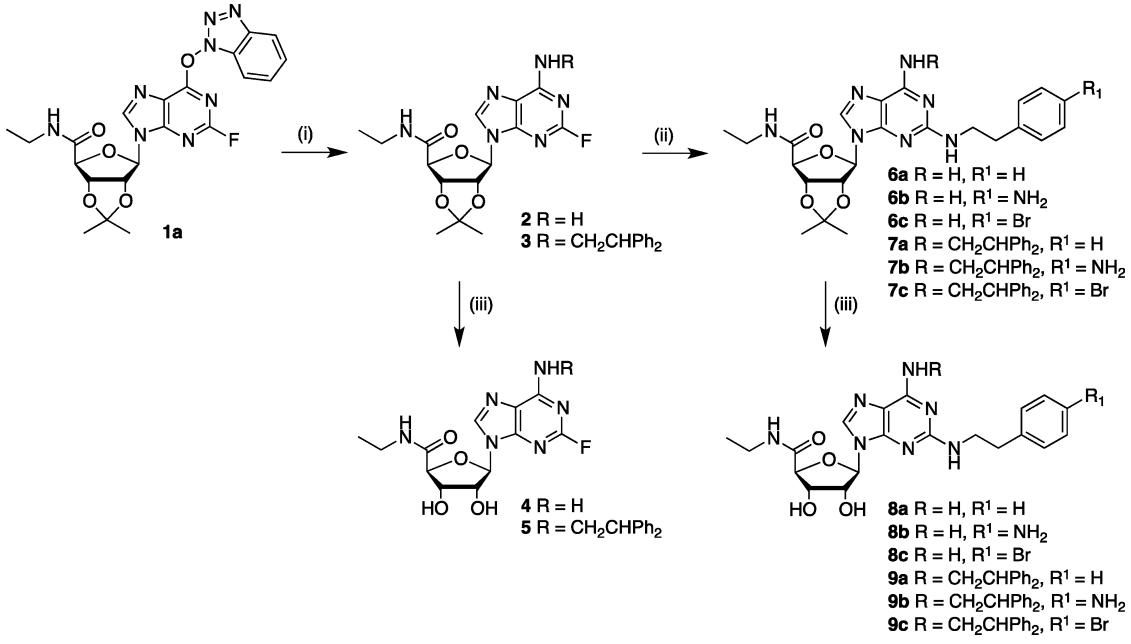


Figure 1. Pharmacologically important $A_{2A}R$ agonists.

Scheme 1[a]



[a]Reagents and conditions: (i) RNH_2, DIPEA, MeCN, 25 °C; (ii) RNH_2, DIPEA, EtOH, 70 °C; (iii) 1M HCl, MeCN, 50 °C.

cardiomyocytes. Accordingly, the central aim of this study is to prepare dual acting agents which act as both $A_{2A}R$ selective agonists and antioxidants.

The structure−activity relationships to date have suggested three key points of the adenosine molecule that can be modified to elicit $A_{2A}R$ selectivity, namely the 2-, 6-, and 5′-positions. We have previously worked on the synthesis of potent and selective A_1R[24] and A_3R[25] agonists and have now expanded our approach to include $A_{2A}R$ agonists. As demonstrated in Figure 1, $A_{2A}R$ selectivity can be enhanced when bulky aromatic groups are incorporated in the 2-positon. Less tolerance is exhibited at the N^6-position apart from the inclusion of a 2,2-diphenylethyl moiety as UK-432,097 demonstrates. The addition of a 5′-alkylcarboxamido group, typically a methyl or an ethyl substituent, can also lead to enhanced $A_{2A}R$ selectivity. Recently, we have reported convergent syntheses of highly substituted 2-, 6-, and/or 5′-substituted adenosines. With these modifications in mind, we

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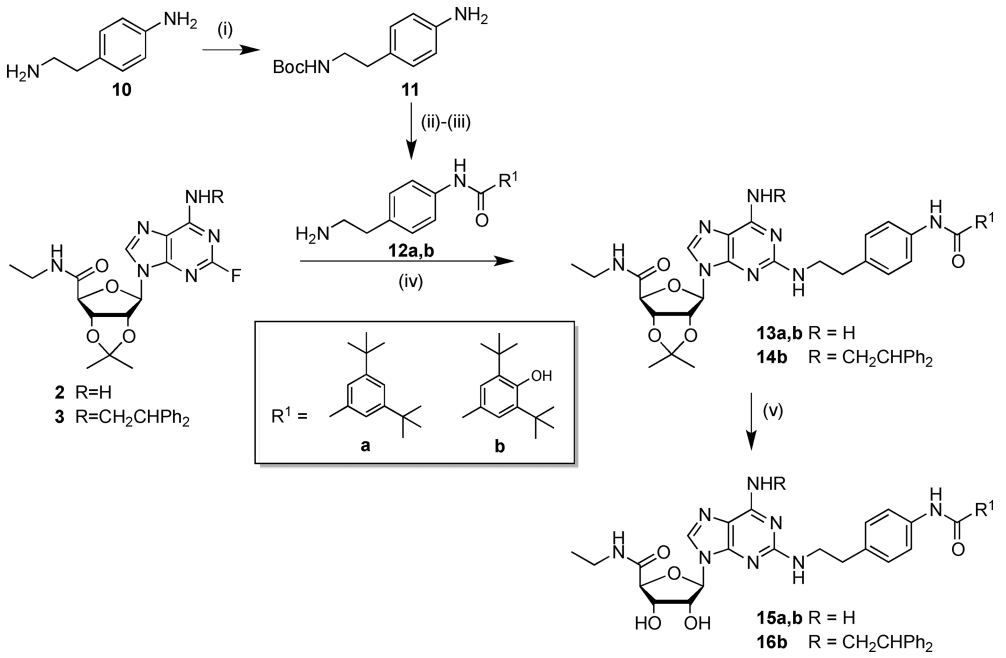
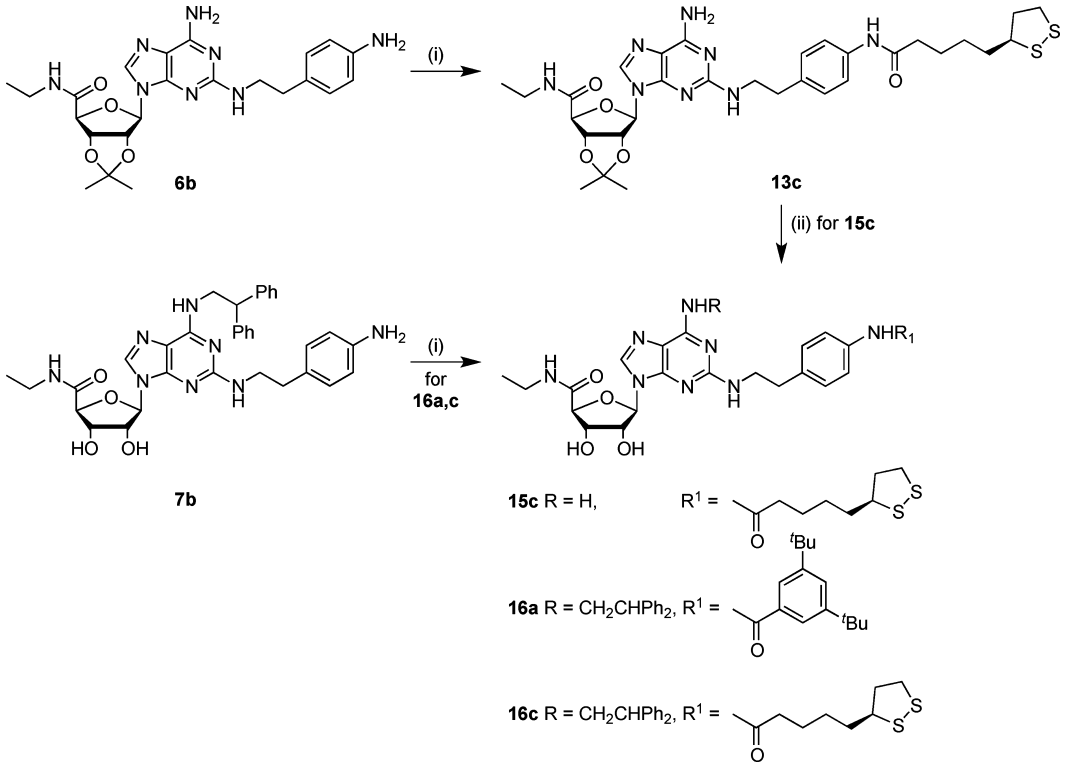
Scheme 2[a]



[a]Reagents and conditions: (i) Boc_2O, CH_2Cl_2, 0 °C; (ii) RCO_2H, EDCI, HOBt, DIPEA, DMF, 25 °C; (iii) TFA, CH_2Cl_2, 25 °C; (iv) **2** or **3**, DIPEA, EtOH, 70 °C; (v) 1M HCl, MeCN, 50 °C.

Scheme 3[a]



[a]Reagents and conditions: (i) **6b** or **7b**, RCO_2H, EDCI, HOBt, DIPEA, DMF, 25 °C; (ii) 1M HCl, MeCN, 50 °C.

have sought to incorporate this knowledge into a novel series of adenosine-5′-*N*-alkylcarboxamides and N^6-(2,2-diphenylethyl)-adenosine-5′-*N*-alkylcarboxamides, which possess antioxidant moieties in the 2-position. To this end, we have incorporated an analogue of the recognized phenolic antioxidant, BHT,[26] which is able to break radical chain reactions through atom transfer as well as a lipoic acid analogue that can mediate in glutathione redox couples.[27,28] These antioxidant moieties are likely to emulate many of the natural pathways which negate free radicals as seen with vitamin E, resveratrol-type compounds and endogenous lipoic acid.

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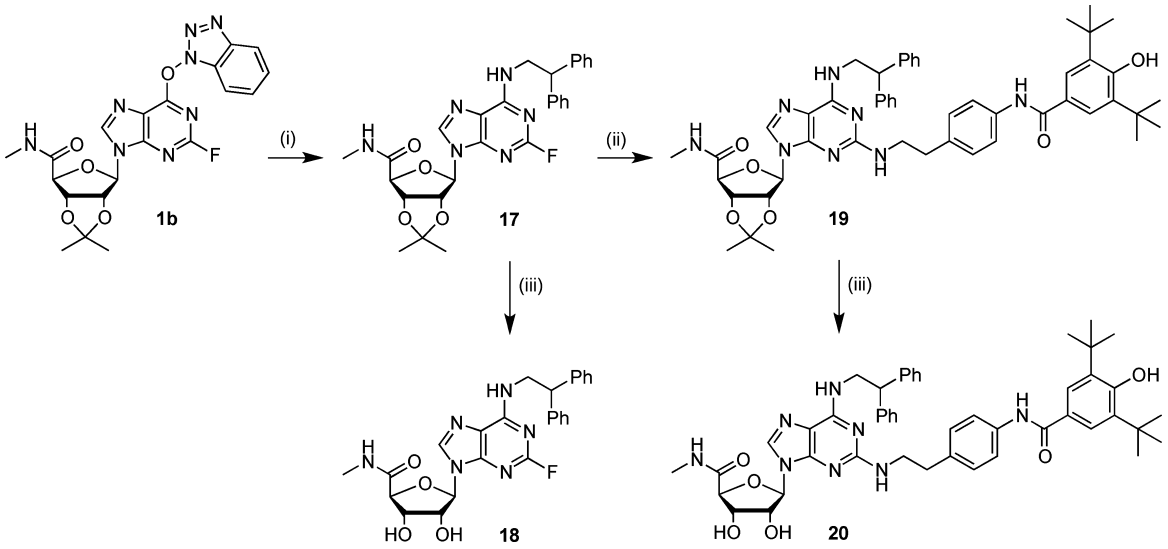
Scheme 4[a]



[a]Reagents and conditions: (i) 2,2-diphenylethylamine, DIPEA, MeCN, 25 °C; (ii) **12b**, DIPEA, MeCN, 150 °C; (iii) 1M HCl, MeCN, 50 °C.

RESULTS AND DISCUSSION

Chemistry. The starting materials used in this study, O^6-(benzotriazol-1-yl)-2-fluoro-2′,3′-*O*-isopropylideneinosine-5′-*N*-alkylcarboxamides (**1a** and **1b**), were synthesized in six steps from guanosine.[29,30] The key features of these syntheses include a BOP-mediated coupling and subsequent halogenation via diazotization. The highly versatile intermediate **1a** was first subjected to N^6-substitution with either ammonia or 2,2-diphenylethylamine in the presence of DIPEA in MeCN to afford **2** and **3**, respectively (Scheme 1). The isopropylidene groups on these molecules were deprotected with hydrochloric acid at 50 °C to afford the 2-fluoro analogues **4** and **5**.

We also envisaged utilizing a hydrophobic alkyl amine, namely 4-(2-aminoethyl)aniline as a functionalized linker in the 2-position to incorporate known antioxidant groups. First, the 2-position of **2** and **3** were reacted with a small series of *p*-substituted *p*-anilinophenethylamines and deprotected with HCl to give the adenosine-5′-*N*-ethylcarboxamides **8a**−**c** and **9a**−**c** (Scheme 1).

Second, a series of phenethylamine linked amines containing antioxidant moieties were synthesized and coupled to **2** or **3**. Reaction with Boc anhydride of the ethylamine portion of 4-(2-aminoethyl)aniline in CH_2Cl_2 was carried out to give the Boc-protected amine (**11**) (Scheme 2). This was then coupled with the appropriate benzoic acid with EDCI, HOBt, and DIPEA in DMF to give the corresponding amides, which were subsequently treated with TFA to produce the amines **12a** and **12b**. These primary amines included known antioxidant groups utilized by our group in the past.[29,30] Reaction of the substituted amines **12a** and **12b** with 2-fluoroadenosine-5′-*N*-ethyl carboxamides **2** and **3**, followed by deprotection of the isopropylidene group, afforded **15a**, **15b**, and **16b**. Substitution at the 2-position was achieved with a range of simple and structurally diverse amines.

This sequence was unsuccessful for the preparation of **16a**, and an alternative route was sought. The inclusion of the lipoic acid moiety into the analogous amide was also problematic, therefore the more convergent approach from the 2-(2-(4-aminophenyl))ethyl substituted adenosine-5′-*N*-ethylcarboxamides **6b** and **7b** was employed (Scheme 3).

In addition to the 5′-*N*-ethylcarboxamido compounds, two novel 5′-*N*-methylcarboxamides (**18** and **20**) containing the N^6-(2,2-diphenylethylamino) group were synthesized (Scheme 4) and their properties studied. The influence of the 5′-*N*-methylcarboxamide group was examined to determine any impact on the selectivity arising from the incorporation of this minor structural change. This synthetic sequence was achieved in a similar fashion to that of the 5′-*N*-ethylcarboxamides (Scheme 4).

cAMP Assays. Compound potency was assessed using a cAMP accumulation assay in stably transfected CHO cell lines, each overexpressing one of the human A_1, A_{2A}, A_{2B}, and A_3Rs. The results of these assays are described in Table 1.

In general, the series of 2-substituted adenosine-5′-*N*-ethylcarboxamides (compounds **4**, **8a**−**c**, and **15a**−**c**) proved to be more potent agonists at the A_{2A}R than the corresponding N^6-(2,2-diphenylethyl)adenosine-5′-*N*-ethylcarboxamide series (**5**, **9a**−**c**, and **16a**−**c**). All members of the adenosine-5′-*N*-ethylcarboxamide series had EC_{50} values less than 50 nM, and five of the seven compounds in this series had EC_{50} values less than 15 nM (Table 1, entries 1−7). More specifically, 2-fluoroadenosine-5′-*N*-ethylcarboxamide (**4**) showed good potency with an EC_{50} of 5.6 nM at the A_{2A}R but relatively poor selectivity versus the other receptor subtypes. Substitution of the 2-fluoro with a 2-phenylethylamino (compound **8a**) or *p*-aminophenylethylamino moiety (**8b**) reduced the potency at the A_{2A}R but significantly improved receptor subtype selectivity. Significantly, **8b** retained reasonable potency (14 nM) and selectivity (~10-fold vs A_3R and ~24-fold vs A_1R) selectivity. The amide derivatives of **8b**, compounds **15a**−**c**, also proved to be potent and selective A_{2A}R agonists. Notably, the compounds bearing antioxidant functionality in the 2-position (compounds **15b** and **15c**) demonstrated very good potency for the A_{2A}R, with EC_{50} values of 14 and 6.6 nM, respectively. These compounds also exhibited high selectivity for A_{2A}R relative to the A_1R (284- and 324-fold, respectively) and reasonable selectivity relative to the A_3R (~18-fold in both cases).

As noted above, the N^6-(2,2-diphenylethyl)adenosine-5′-*N*-ethylcarboxamide series (**5**, **9a**−**c**, and **16a**−**c**) were slightly less

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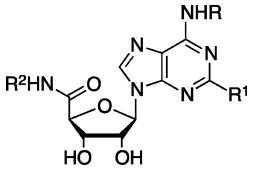
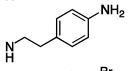
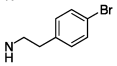
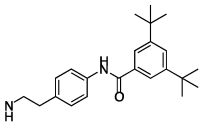
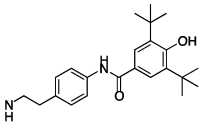
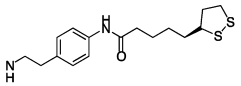
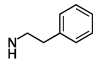
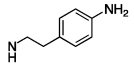
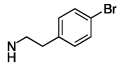
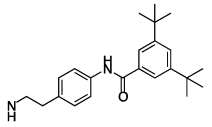
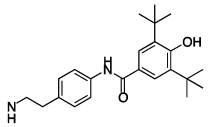
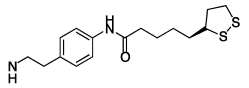
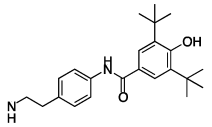
Table 1. Evaluation of the Adenosine Derivatives in cAMP Accumulation Assays at all Receptor Subtypes



	No.	R	R¹	R²	IC_{50} - A_1R (nM)	EC_{50} - $A_{2A}R$ (nM)	EC_{50} - $A_{2B}R$ (nM)	IC_{50} - A_3R (nM)	A_{2A}/A_1	A_{2A}/A_3
1	**4**	H	F	Et	0.36 ± 0.02	5.6 ± 0.4	2.3 ± 0.5	23 ± 5	0.06	4.1
2	**8a**	H		Et	112 ± 43	12 ±2	145 ± 57	104 ± 85	9.3	8.6
3	**8b**	H		Et	326 ± 159	14 ± 2	126 ± 14	141 ± 86	23.6	10.3
4	**8c**	H		Et	2082 ± 1492	42 ± 3	355 ± 83	259 ± 29	49.3	6.2
5	**15a**	H		Et	2324 ± 905	45 ± 4	1489 ± 64	60097 ± 8654	58.2	336
6	**15b**	H		Et	3990 ± 1425	14 ± 2	6069 ± 4401	756 ± 102	284	18.2
7	**15c**	H		Et	2136 ± 150	6.6 ± 0.7	739 ± 251	121 ± 18	324	18.3
8	**5**	CH_2CHPh_2	F	Et	10 ± 2.4	0.85 ± 0.65	14 ± 3	8.0 ± 1.6	12.2	9.4
9	**9a**	CH_2CHPh_2		Et	1007 ± 105	66 ± 52	1173 ± 338	120 ± 37	15.3	1.8
10	**9b**	CH_2CHPh_2		Et	1184 ± 247	6.3 ± 1.5	257 ± 71	33 ± 2	188	5.2
11	**9c**	CH_2CHPh_2		Et	428 ± 70	30.9 ± 13.2	296± 45	149 ± 18	13.8	3.6
12	**16a**	CH_2CHPh_2		Et	2208±190	366 ± 263	2279 ± 494	1390 ± 198	101	3.8
13	**16b**	CH_2CHPh_2		Et	5251 ± 2525	712 ± 205	40310 ± 13861	1407 ± 1350	7.4	2.0
14	**16c**	CH_2CHPh_2		Et	3914 ± 1381	89.7± 22.2	12160 ± 7980	113 ± 44	43.7	1.3
15	**18**	CH_2CHPh_2	F	Me	122 ± 11	2.02 ± 1.02	120 ± 16	7.04 ± 0.85	60.4	3.5
16	**20**	CH_2CHPh_2		Me	413267 ± 67326	2242 ± 1021	6694 ± 1354	1721 ± 681	184	0.77

potent at the $A_{2A}R$ (Table 1, entries 8−15). The *p*-aminophenethylamino compound **9b** showed very good potency at the $A_{2A}R$ (EC_{50} = 6.3 nM), however the phenethylamino (**9a**) and *p*-bromophenethylamino (**9c**) analogues were less potent, with half-maximal inhibitory concentrations of 66 and 31 nM, respectively. The selectivity of **9b** was markedly better as well with over 188-fold selectivity (A_{2A}/A_1) but no significant increase over the A_3R (A_{2A}/A_3 = 5.2). The compound **16a** and the antioxidant containing molecules **16b** and **16c** had relatively modest potency and selectivity for the $A_{2A}R$. The compound incorporating the 5′-*N*-methylcarboxamide moiety (**18**) produced a highly potent compound, exhibiting a 2.02 nM EC_{50} at the $A_{2A}R$. This compound showed some selectivity versus the A_1R (60-fold) but very modest selectivity relative versus the A_3R (3.5-fold).

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In summary, a number of 2-substituted adenosine 5′-*N*-alkylcarboxamides (**8a−c** and **15a−c**) and N^6-(2,2-diphenylethyl), 2-substituted adenosine 5′-*N*-alkylcarboxamides (**9a−c** and **16a−c**) were synthesized and their pharmacology explored. The N^6-amino series (**8a−c** and **15a−c**) showed overall greater selectivity and potency when a functionalized linker joined to an antioxidant group was attached. The N^6-2,2-diphenylethylamino series (**9a−c** and **16a−c**) pharmacology was less straightforward, however **9b** was the most active compound at the $A_{2A}R$, demonstrating an EC_{50} of 6.3 nM. The retention of the 2-fluoro groups (**4**, **5**, **18**) demonstrated highly potent compounds, in particular **5** had an EC_{50} of 0.85 nM at the $A_{2A}R$.

Molecular Modeling. The most potent $A_{2A}R$ ligands (**8a**, **8b**, and **15b**) identified in this study were docked into the crystal structure of the $A_{2A}R$ (PDB ID: 3QAK, Figure 2A)[11]

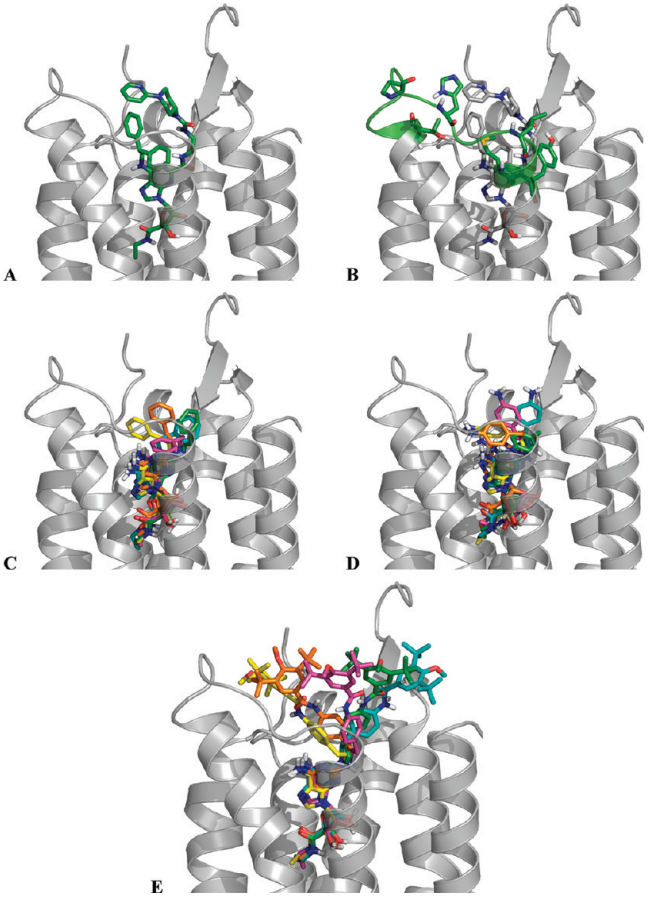

Figure 2. Crystal structure of the adenosine A_{2A} receptor in complex with agonist UK-432,097 (A) and showing ECL3 residues highlighted in green (B). Docked poses for compounds **8a** (C) and **8b** (D) and **15b** (E), displaying range of poses observed in docking.

using the GOLD modeling software. In all of the top scoring docked poses, the adenosine-5′-*N*-ethylcarboxamide (NECA) component of each ligand docked in a similar way to that of UK-432,097, with rms deviations of less than 1 Å to the corresponding segment of UK-432,097. The NECA portion of the docked ligands makes hydrogen bonding interactions to residues Thr 3.36, His 6.52, Asn 6.55, Ser 7.42, and His 7.43, which is consistent with the binding mode of UK-432,097 in the $A_{2A}R$ crystal structure.[11] The binding mode of adenosine and the agonist NECA have subsequently been determined in two recent crystal structures (PDB ID: 2YDO and 2YDV),[31] further supporting this binding mode.

Because of the open nature of the solvent exposed area of the binding site, the 2-substituents of **8a**, **8b**, and **15b** can dock in a number of diverse orientations. These binding modes are also influenced by the presence of water molecules. Interactions of the 2-substituents with residues in extracellular loop 3 (ECL3) and the extracellular side of transmembrane helix 7 (TM7, Figure 2B) may explain selectivity observed between the different subtypes. ECL3 is adjacent to the binding site and shows a large degree of variability between adenosine receptor subtypes, demonstrated by multiple sequence alignment (data not shown). Analysis of the $A_{2A}R$ crystal structure[31] in conjunction with recent biophysical mapping of the $A_{2A}R$ binding site[32] has demonstrated that the 2-substituents may interact with residues on the extracellular side of TM6 and TM7, such as Tyr 7.36. Additionally, due to the variable nature of residues in ECL3, interactions with this loop may also confer subtype selectivity.

On the basis of our docking studies, the 2-substituent of **15b** displays a range of conformations (Figure 2E), either interacting with Thr 6.58, His 264, Leu 7.32, Met 7.35, Tyr 7.36, and Ile 7.39 (TM6, TM7 and ECL3, ligands shown in yellow and orange) or with Ser 1.32, Ile 2.64, or Ser 2.65 (TM1 and TM2, ligands shown in green and blue), or protruding into the bulk solvent with only limited interactions with the receptor (ligand shown in magenta). The *para*-amino substitution in **8b** had increased selectivity over **8a**, which is likely to be a result of the ability to form additional hydrogen bonding interactions with residues on the extracellular side of TM7 (Figure 2C,D).

Simulated Ischemia Assays. Compounds **15b** and **15c** were selected for further characterization in a cell culture hypoxia model developed in house and reported previously using the rat atrial cardiomyoblast cell line H9c2.[24,33−36] These compounds showed good $A_{2A}R$ potency (with EC_{50} values of 14 and 6.6 nM), good selectivity for the $A_{2A}R$ over other receptor subtypes, and both contained antioxidant functionality. Compound **15a** was also included in this study as a close analogue of **15b**, which lacks the antioxidant functionality. In this assay, cells were exposed to media mimicking hypoxic interstitial fluid, containing lactic acid to decrease the pH and deoxy-glucose, and were then placed in an oxygen-free environment (100% N_2) for 12 h. After this time, around 40% of cells were dead, as evidenced by DAPI exclusion. This level of cell death was normalized to 100%, and this provided the baseline for the assay. Superoxide production was measured using dihydroethidium staining as previously reported, at 20 μM for 45 min (see Figure 3). Compounds **15b** and **15c** were shown to be protective in this model at submicromolar concentrations (see Figure 3). In this assay, **15a** was largely inactive, demonstrating that the protective effects seen in this cardiomyoblast model were due to the antioxidant hindered phenol group. This observation was consistent with our previous study, which found that antioxidants alone exhibit protective effects in this simulated ischemia assay.[35]

■ CONCLUSIONS

This study has identified a number of highly potent and selective $A_{2A}R$ agonists, some of which possess antioxidant functionality. Pleasingly, the incorporation of this antioxidant functionality maintained high $A_{2A}R$ potency and promoted improved selectivity for the $A_{2A}R$ over all other receptor subtypes. A molecular modeling study, which utilized the

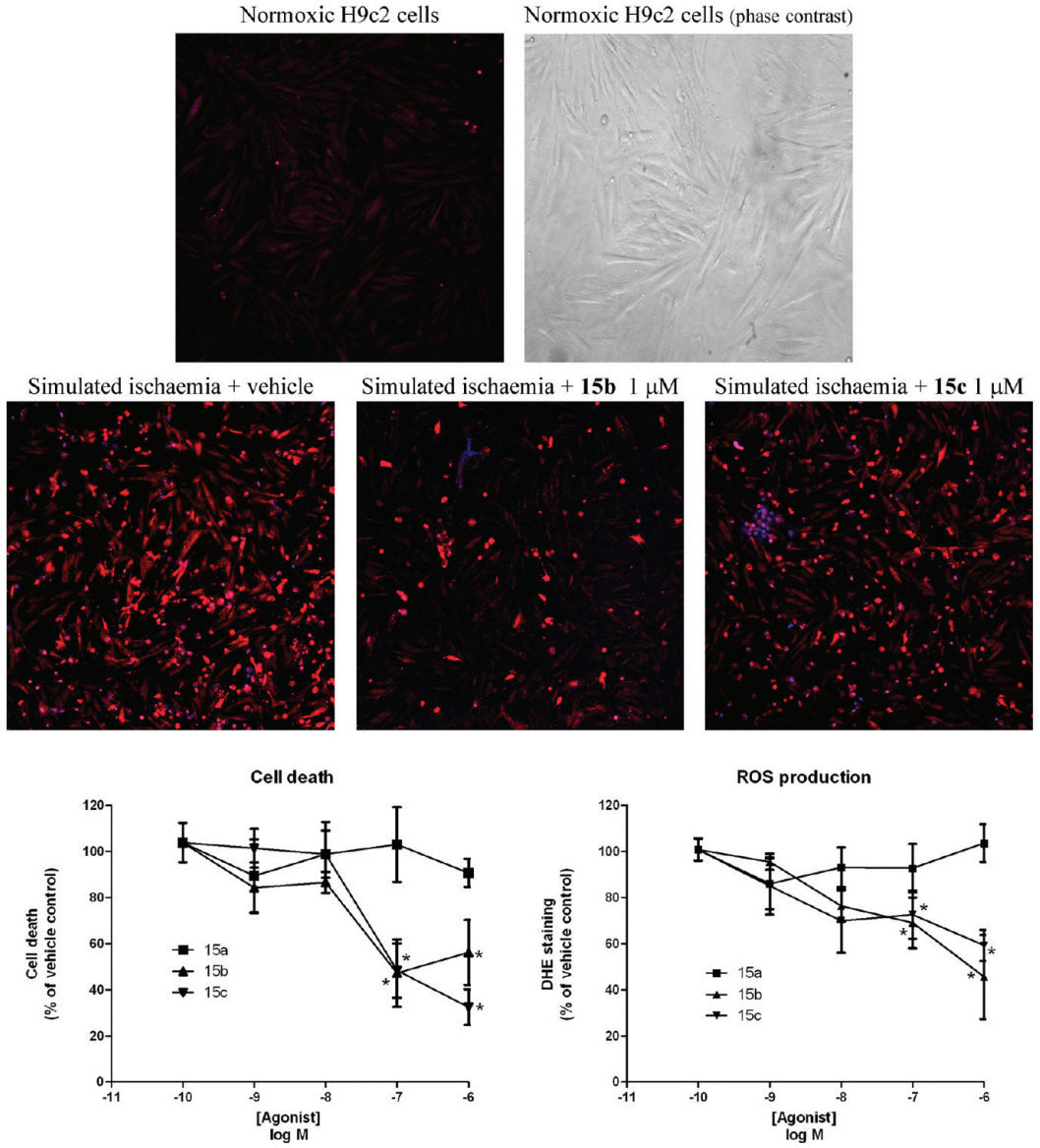

Figure 3. Compounds **15b** and **15c** reduce ROS activity and cell death due to simulated ischemia. Top panel: confocal microscopy images showing dihydroethidium staining for superoxide (red) and DAPI (blue) staining for dead cells. Bottom panel: quantitation of cell death and DHE fluorescence intensity per cell after treatment. Cells were exposed to 95% N_2 5% CO_2 in HEPES buffer with lactic acid for 12 h followed by 1 h incubation in HEPES glucose buffer. Both **15b** and **15c** were protective and reduced ROS production at 0.1 and 1 μM, while **15a** had no significant effect. *indicates $p < 0.05$; ANOVA $n = 3$ experiments conducted in quadruplicate.

recently reported X-ray crystal structure, was conducted to rationalize the binding mode of these agonists. The study identified key ligand−receptor interactions that may account for the high level of $A_{2A}R$ selectivity observed for these compounds. Interestingly, this modeling also suggests that the appended antioxidant group projects beyond the receptor, thereby placing it in a favorable position to interact with reactive oxygen species present in the cytosol. The cardioprotective effects of two of the most potent and selective $A_{2A}R$ agonists (compounds **15b** and **15c**) were further investigated in an ischemia model using rat atrial cardiomyocytes. Both of these compounds showed strong protective effects in this assay relative to the control. Compound **15a**, a closely related analogue of **15b** lacking antioxidant functionality, was also evaluated in this assay and showed no significant protective effects. This suggests that the antioxidant group is the mediator of the protective effects observed for **15b**. Two of the best established mechanisms of cardioprotection are: (i) $A_{2A}R$ activation and (ii) inactivation of damaging free radicals in the heart using small molecule antioxidants. We believe that the combination of these two modes of action within a single molecule will provide additive or synergistic benefit. $A_{2A}R$ activation is well-known to reduce the inflammatory drive to remodel the heart, and we have shown that the antioxidant functionality of our lead compounds prevents cell death in a cardiac cell line after simulated ischemia. Compounds **15b** and **15c** are therefore promising candidates for development as cardioprotective therapeutic agents. These two compounds will now be evaluated in an in vivo ischemia-reperfusion model in comparison to an $A_{2A}R$ agonist or antioxidant given alone.

EXPERIMENTAL SECTION

General Synthetic Procedures. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. All

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microwave reactions took place in a Biotage Initiator microwave synthesizer. All NMR spectra were recorded on a Bruker Avance DPX 300 MHz or Bruker Avance III 400 MHz Ultrashield Plus spectrometer, and ^{1}H and ^{13}C NMR spectra were recorded at either 300.13 or 400.13 MHz and 75.4 or 100.62 MHz, respectively. Thin layer chromatography was conducted on 0.2 mm plates using Merck silica gel 60 F_{254}. Column chromatography was achieved using Merck silica gel 60 (article size 0.063−0.200 μm, 70−230 mesh). High resolution mass spectra were obtained on a Waters LCT Premier XE (TOF) mass spectrometer fitted with an ESI ion source, coupled to a 2795 Alliance separations module. LCMS were run to verify reaction outcome and purity using an Agilent 6100 Series Single Quad coupled to an Agilent 1200 Series HPLC. All compounds were of >95% purity. The following buffers were used: buffer A 99.9% H_2O, 0.1% formic acid and buffer B 99.9% CH_3CN, 0.1% formic acid. The following gradient was used with a flow rate of 0.5 mL/min and total run time of 12 min: 0−4 min 95% buffer A and 5% buffer B, 4−7 min 0% buffer A and 100% buffer B, 7−12 min 95% buffer A and 5% buffer B. Mass spectra were acquired in positive and negative ion mode with a scan range of 0−1000 *m*/*z* at 5 V. 2-Fluoro-O^6-(benzotriazol-1-yl)-2′,3′-*O*-isopropylideneinosine-5′-*N*-methylcarboxamide (**1b**),[30] 2-fluoro-O^6-(benzotriazol-1-yl)-2′,3′-*O*-isopropylideneinosine-5′-*N*-ethylcarboxamide (**1a**),[29] and 2-fluoro-2′,3′-*O*-isopropylideneadenosine-5′-*N*-ethylcarboxamide (**2**)[29] were synthesized as previously published.

2-Fluoro-N^6-(2,2-diphenylethyl)-2′,3′-*O*-isopropylideneadenosine-5′-*N*-ethylcarboxamide (3). *General Procedure for N^6-Substitution.* To a stirred solution of **1a** (1.0 g, 2.07 mmol) and DIPEA (1 mL) in MeCN (15 mL) at 0 °C was added 2,2-diphenylethylamine (65 mg, 3.31 mmol). The solution was allowed to rise to room temperature, and stirring was continued for 16 h. The solvent was removed in vacuo, and the residue partitioned between H_2O and CH_2Cl_2. The aqueous phase was extracted ×3 with CH_2Cl_2, the organic phase washed with H_2O and brine, and dried over $MgSO_4$. The solution was filtered, the solvent removed in vacuo, and the crude mixture loaded onto silica gel and purified by column chromatography using a gradient of 1:1 petroleum spirits/EtOAc to EtOAc as the eluent to yield a pale-yellow solid **3** (825 mg, 73%), R_f = 0.41 (1:5 petroleum spirits/EtOAc), mp 85−91 °C. ^{1}H NMR (300 MHz, CD_3OD) δ: 0.69 (t, J = 7.2 Hz, 3H), 1.42 (s, 3H), 1.59 (s, 3H), 2.84−2.95 (m, 2H), 4.09−4.28 (m, 2H), 4.50 (t, J = 8.1 Hz, 1H), 4.62 (s, 1H), 5.42 (d, J = 6.3 Hz, 1H), 5.56 (d, J = 6.3 Hz, 1H), 6.27 (s, 1H), 7.20−7.24 (m, 2H), 7.28−7.36 (m, 8H), 8.07 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: 13.0, 24.1, 25.8 33.4, 44.8, 50.3, 83.4, 83.7, 87.0, 90.9, 113.6, 117.5, 126.3, 127.9, 128.3, 140.6, 142.2, 149.34 (d, J = 19.7 Hz), 156.10 (d, J = 19.7 Hz), 159.10 (d, J = 208.0 Hz). ESMS calcd for $C_{29}H_{32}N_6O_4F^+$ [M + H], 547.6; found, 548.1.

2-Fluoroadenosine-5′-*N*-ethylcarboxamide (4). *General Procedure for Isopropylidene Deprotection.* To a stirred solution of **2** (250 mg, 0.68 mmol) in MeCN (4 mL) was added 1 M HCl (20 mL). The flask was fitted with a reflux condenser, and the solution was heated to 50 °C for 6 h. The solution was allowed to cool and the pH adjusted to 8 with satd $NaHCO_3$ solution, which produced a white precipitate. The solution was extracted with EtOAc (×3), the organic phase washed with H_2O and brine, dried over $MgSO_4$, filtered, and the crude material dried onto SiO_2 and purified via column chromatography using a gradient of EtOAc to 9:1 EtOAc/MeOH to yield a white solid (**4**) (96 mg, 43%), R_f = 0.26 (9:1 EtOAc/MeOH), mp 233−240 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.03 (t, J = 4.8 Hz, 3H), 3.12−3.20 (m, 2H), 4.14 (br s, 1H), 4.29 (s, 1H), 4.53 (dd, J = 2.8, 4.8 Hz, 1H), 5.59 (d, J = 4.0 Hz, 1H), 5.72 (d, J = 2.8 Hz, 1H), 5.87 (d, J = 4.8 Hz, 1H), 7.81−8.04 (m, 2H), 8.39 (s, 1H), 8.45 (m, 1H). ^{13}C NMR(100 MHz, DMSOd_6) δ: 14.6, 33.2, 72.2, 73.0, 84.4, 87.6, 117.9, 150.3 (d, J = 19.8 Hz), 157.7 (d, J = 20.9 Hz), 158.4 (d, J = 203.3 Hz), 170.0. HRMS calcd for $C_{12}H_{16}N_6O_4F^+$ [M + H], 327.1212; found, 327.1203.

2-Fluoro-N^6-(2,2-diphenylethyl)adenosine-5′-*N*-ethylcarboxamide (5). *General Procedure for Isopropylidene Deprotection.* Compound **3** (175 mg, 0.32 mmol), MeCN (3 mL), 1 M HCl (12 mL), 50 °C, 2 h, white solid (**5**) (45 mg, 28%), R_f = 0.19 (1:5 petroleum spirits/EtOAc), mp 131−134 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 1.20 (t, J = 7.2 Hz, 3H), 3.33−3.45 (m, J = 7.2 Hz, 2H), 4.22 (dd, J = 8.2, 1.0 Hz, 2H), 4.32 (dd, J = 4.8, 1.0 Hz, 1H), 4.46 (d, J = 4.8 Hz, 1H), 4.51 (t, J = 7.8 Hz, 1H), 4.66 (dd, J = 7.8, 4.8 Hz, 1H), 5.93 (d, J = 7.8 Hz, 1H), 7.17−7.23 (m, 2H), 7.26−7.38 (m, 10H), 8.12 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: 13.7, 33.5, 44.8, 50.3, 71.9, 73.5, 85.0, 89.1, 118.4, 126.3, 127.9, 128.2, 140.9, 142.2, 149.1 (d, J = 19.2 Hz), 156.3 (d, J = 20.3 Hz), 159.0 (d, J = 207.2 Hz), 170.5. HRMS calcd for $C_{26}H_{27}N_6O_4F^+$ [M + H], 507.2151; found, 507.2161.

2-(2-Phenyl)ethylamino-2′,3′-*O*-isopropylideneadenosine-5′-*N*-ethylcarboxamide[14] (6a). *General Procedure for 2-Substitution.* Compound **2** (120 mg, 0.33 mmol), 2-phenylethylamine (83 mg, 0.69 mmol), and DIPEA (250 μL) in EtOH (2.5 mL) was stirred in a sealed reaction vessel at 70 °C. After 7 days, the crude mixture was evaporated onto silica gel and purified by column chromatography using EtOAc as the eluent to give an off-white glassy solid (**6a**) (121 mg, 79%), R_f = 0.40 (9:1 EtOAc/MeOH), mp 185−187 °C. ^{1}H NMR (300 MHz, CD_3OD) δ: 0.61 (t, J = 7.2 Hz, 3H), 1.38 (s, 3H), 1.59 (s, 3H), 2.71−2.83 (m, 1H), 2.84−2.98 (m, 3H), 3.43−3.56 (m, 1H), 3.67−3.79 (m, 1H), 4.61 (s, 1H), 5.58 (d, J = 6.0 Hz, 1H), 5.63 (d, J = 6.0 Hz, 1H), 6.22 (s, 1H), 7.13−7.20 (m, 1H), 7.21−7.32 (m, 4H), 7.88 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: 12.3, 23.8, 25.2, 33.1, 35.1, 42.3, 83.6, 84.1, 87.9, 91.1, 112.8, 113.0, 125.7, 128.0, 128.5, 138.1, 139.8, 151.0, 156.1, 159.3, 170.1. ESMS calcd for $C_{23}H_{30}N_7O_4^+$ [M + H], 468.5; found, 468.4.

2-(2-(4-Aminophenyl))ethylamino-2′,3′-*O*-isopropylideneadenosine-5′-*N*-ethylcarboxamide (6b). *General Procedure for 2-Substitution.* Compound **2** (180 mg, 0.49 mmol), 4-amino-2-phenylethylamine (147 mg, 1.08 mmol), DIPEA (300 μL), and EtOH (5 mL), 70 °C, 3 days, off-white solid (**6b**) (128 mg, 54%), R_f = 0.23 (9:1 EtOAc/MeOH), mp 90−95 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.74 (t, J = 7.2 Hz, 3H), 1.43 (s, 3H), 1.65 (s, 3H), 2.85 (t, J = 6.6 Hz, 2H), 2.89−3.09 (m, 2H), 3.48−3.61 (m, 1H), 3.62−3.73 (m, 1H), 4.71 (s, 1H), 4.99−5.05 (m, 1H), 5.49 (br s, 2H), 5.57 (d, J = 6.0 Hz, 1H), 5.66 (d, J = 6.0 Hz, 1H), 6.07 (br s, 2H), 6.69 (d, J = 7.2 Hz, 2H), 7.08 (d, J = 7.2 Hz, 2H), 7.55 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 25.0, 26.7, 33.8, 34.7, 43.0, 83.3, 84.0, 87.7, 91.3, 113.6, 114.1, 115.1, 129.3, 129.6, 137.0, 144.9, 151.3, 156.0, 159.5, 168.9. ESMS calcd for $C_{23}H_{31}N_8O_4^+$ [M + H], 483.5; found, 483.3.

2-(2-(4-Bromophenyl))ethylamino-2′,3′-*O*-isopropylideneadenosine-5′-*N*-ethylcarboxamide (6c). *General Procedure for 2-Substitution.* Compound **2** (140 mg, 0.38 mmol), 4-bromo-2-phenylethylamine (169 mg, 0.84 mmol), DIPEA (300 μL), and EtOH (3 mL), 70 °C, 6 days, off-white solid (**6c**) (199 mg, 95%), R_f = 0.43 (9:1 EtOAc/MeOH), mp 113−118 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.69 (t, J = 7.5 Hz, 3H), 1.40 (s, 3H), 1.63 (s, 3H), 2.80−3.02 (m, 4H), 3.48−3.61 (m, 1H), 3.63−3.77 (m, 1H), 4.71 (s, 1H), 5.21−5.25 (m, 1H), 5.55 (d, J = 4.8 Hz, 1H), 5.68 (s, 1H), 5.76 (br s, 2H), 6.04 (br s, 1H), 6.08 (s, 1H), 7.16 (d, J = 7.5 Hz, 2H), 7.43 (d, J = 7.5 Hz, 2H), 7.54 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 14.0, 25.4, 27.0, 34.8, 36.2, 43.6, 79.5, 85.0, 85.5, 89.3, 92.5, 114.4, 114.5, 120.8, 131.9, 132.4, 139.6, 140.5, 152.4, 157.4, 160.8, 171.6. ESMS calcd for $C_{23}H_{29}N_7O_4Br^+$ [M + H], 546.5; found, 546.2.

2-(2-Phenyl)ethylamino-N^6-(2,2-diphenylethyl)-2′,3′-isopropylideneadenosine-5′-*N*-ethyl carboxamide (7a). *General Procedure for 2-Substitution.* Compound **3** (200 mg, 0.37 mmol), 2-phenylethylamine (98 mg, 0.81 mmol), DIPEA (300 μL), and EtOH (3 mL), 70 °C, 4 days, white solid foam (**7a**) (147 mg, 63%), R_f = 0.47 (1:5 petroleum spirits/MeOH), mp 58−64 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.68 (t, J = 7.2 Hz,

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3H), 1.37 (s, 3H), 1.58 (s, 3H), 2.82−2.90 (m, 1H), 2.94−3.03 (m, 3H), 3.56−3.65 (br m, 1H), 3.68−3.76 (br m, 1H), 4.14−4.28 (br m, 2H), 4.32−4.39 (br m, 1H), 4.64 (s, 1H), 4.93 (br s, 1H), 5.46 (br s, 1H), 5.60−5.64 (m, 1H), 5.97 (s, 1H), 6.04 (br s, 1H), 7.18−7.34 (m, 16H), 7.39 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.3, 25.1, 26.8, 33.8, 36.1, 43.1, 45.0, 50.7, 83.4, 84.1, 87.7, 91.3, 113.6, 114.4, 126.3, 126.8, 128.2, 128.6, 128.8, 128.8, 128.9, 136.3, 139.6, 142.2, 154.9, 159.5, 169.1. ESMS calcd for $C_{37}H_{42}N_7O_4^+$ [M + H], 648.8; found, 648.4.

2-(2-(4-Aminophenyl))ethylamino-*N*^{6}-(2,2-diphenylethyl)-adenosine-2′,3′-isopropylidene-5′-*N*-ethylcarboxamide (7b). *General Procedure for 2-Substitution.* Compound **3** (200 mg, 0.37 mmol), 2-(4-aminophenyl)ethylamine (110 mg, 0.81 mmol), DIPEA (300 μL), and EtOH (3 mL), 70 °C, pale-yellow solid (**7b**) (195 mg, 80%), R_f = 0.35 (1:5 petroleum spirits/EtOAc), mp 54−61 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.75 (t, J = 7.2 Hz, 3H), 1.44 (s, 3H), 1.65 (s, 3H), 2.88−3.10 (m, 4H), 3.53−3.63 (m, 1H), 3.65−3.76 (m, 1H), 4.26 (br s, 2H), 4.42 (t, J = 7.2 Hz, 1H), 4.70 (s, 1H), 5.01 (br s, 1H), 5.54 (br s, 1H), 5.65 (d, J = 5.7 Hz, 1H), 5.74 (br s, 1H), 6.02 (s, 1H), 6.16 (br s, 1H), 6.67 (d, J = 7.5 Hz, 2H), 7.07 (d, J = 7.5 Hz, 2H), 7.22−7.39 (m, 10H), 7.42 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0, 25.1, 26.8, 33.8, 35.1, 43.4, 44.9, 50.7, 83.3, 84.0, 87.6, 91.4, 113.7, 114.4, 115.4, 126.8, 128.2, 128.7, 129.5, 129.7, 136.2, 142.2, 144.7, 150.5, 154.9, 159.6, 169.0.

2-(2-(4-Bromophenyl))ethylamino-*N*^{6}-(2,2-diphenylethyl)-2′,3′-isopropylideneadenosine-5′-*N*-ethylcarboxamide (7c). *General Procedure for 2-Substitution.* Compound **3** (181 mg, 0.33 mmol), 2-(4-bromophenyl)ethylamine (140 mg, 0.70 mmol), DIPEA (300 μL), and EtOH (3 mL), 70 °C, 7 days, white solid (**7c**) (142 mg, 59%), R_f = 0.55 (1:5 petroleum spirits/EtOAc), mp 62−65 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.73 (t, J = 7.2 Hz, 3H), 1.43 (s, 3H), 1.65 (s, 3H), 2.95 (t, J = 6.6 Hz, 2H), 2.87−3.08 (m, 2H), 3.59−3.78 (m, 2H), 4.26 (br s, 2H), 4.41 (t, J = 7.2 Hz, 1H), 4.71 (s, 1H), 5.00 (br s, 1H), 5.52 (br s, 1H), 5.68 (d, 1H), 6.04 (s, 1H), 7.15 (d, J = 7.5 Hz, 2H), 7.25−7.40 (m, 10H), 7.44 (d, J = 7.5 Hz, 2H), 7.45 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: 14.0, 25.1, 26.8, 33.8, 35.5, 42.9, 44.9, 50.7, 83.5, 84.0, 87.8, 91.4, 113.6, 114.5, 120.1, 126.9, 128.1, 128.8, 130.6, 131.6, 136.3, 138.6, 142.1, 154.9, 159.4, 169.1. ESMS calcd for $C_{37}H_{41}N_7O_4Br^+$ [M + H], 726.7; found, 726.3.

2-(2-Phenyl)ethylaminoadenosine-5′-*N*-ethylcarboxamide[14] (8a). *General Procedure for Isopropylidene Deprotection.* Compound **6a** (120 mg, 0.26 mmol), MeCN (2 mL), and 1 M HCl (20 mL), 50 °C, 12 h, pale-yellow solid (**8a**) (88 mg, 82%), R_f = 0.28 (9:1 EtOAc/MeOH), mp 110−114 °C (lit. 115−118 °C dec[14]). ^{1}H NMR (300 MHz, CD_3OD) δ: 1.06 (t, J = 7.2 Hz, 3H), 2.90 (t, J = 7.2 Hz, 2H), 3.07−3.18 (m, 1H), 3.20−3.34 (m, 1H), 3.46−3.68 (m, 2H), 4.40 (d, J = 2.7 Hz, 1H), 4.48−4.52 (m, 1H), 5.00−5.05 (m, 1H), 5.93 (d, J = 6.3 Hz, 1H), 7.12−7.19 (m, 1H), 7.20−7.30 (m, 5H), 7.99 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: 13.4, 33.8, 35.5, 42.9, 72.0, 73.3, 84.0, 88.6, 113.4, 125.8, 128.1, 128.5, 137.7, 139.7, 151.7, 156.1, 159.7, 170.6. HRMS calcd for $C_{20}H_{26}N_7O_4^+$ [M + H], 428.2041; found, 428.2058.

2-(2-(4-Aminophenyl))ethylaminoadenosine-5′-*N*-ethylcarboxamide (8b). *General Procedure for Isopropylidene Deprotection.* Compound **6b** (128 mg, 0.27 mmol) and 1 M HCl (15 mL), 50 °C, 4 h, tan solid (**8b**) (46 mg, 39%), R_f = 0.1 (9:1 EtOAc/MeOH), mp 95−104 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 1.05 (t, J = 7.2 Hz, 3H), 2.77 (t, J = 7.2 Hz, 2H), 3.08−3.18 (m, 1H), 3.24−3.33 (m, 1H), 3.42−3.51 (m, 1H), 3.55−3.64 (m, 1H), 4.42 (d, J = 2.8 Hz, 1H), 4.48−4.53 (m, 1H), 5.00−5.05 (m, 1H), 5.94 (d, J = 6.3 Hz, 1H), 6.70 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 7.98 (s, 1H). ^{13}C NMR (400 MHz, CD_3OD) δ: 14.7, 35.2, 36.1, 44.6, 73.2, 74.7, 85.5, 90.1, 114.7, 117.1, 130.5, 130.8, 139.2, 146.6, 153.1, 157.5, 161.2, 172.0. HRMS calcd for $C_{20}H_{27}N_8O_4^+$ [M + H], 443.2150; found, 443.2162.

2-(2-(4-Bromophenyl))ethylaminoadenosine-5′-*N*-ethylcarboxamide (8c). *General Procedure for Isopropylidene Deprotection.* Compound **6c** (180 mg, 0.33 mmol), MeCN (2 mL), and 1 M HCl (10 mL), 50 °C, 6 h, white solid (**8c**) (70 mg, 42%), R_f = 0.21 (9:1 EtOAc/MeOH), mp 80−85 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 1.04 (t, J = 7.2 Hz, 3H), 2.88 (t, J = 7.2 Hz, 2H), 3.09−3.19 (m, 1H), 3.23−3.33 (m, 1H), 3.47−3.56 (m, 1H), 3.59−3.68 (m, 1H), 4.42 (d, J = 2.9 Hz, 1H), 4.48−4.53 (m, 1H), 5.01 (dd, J = 5.2, 6.6 Hz, 1H), 5.96 (d, J = 6.6 Hz, 1H), 7.19 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.4 Hz, 2H), 8.01 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: 14.7, 35.2, 36.3, 44.0, 73.4, 74.7, 85.5, 90.0, 114.8, 120.8, 132.0, 132.5, 139.1, 140.5, 153.1, 157.5, 161.1, 172.0. HRMS calcd for $C_{20}H_{25}N_7O_4Br^+$ [M + H], 506.1146; found, 506.1165.

2-(2-Phenyl)ethylamino-*N*^{6}-(2,2-diphenylethyl)adenosine-5′-*N*-ethylcarboxamide (9a). *General Procedure for Isopropylidene Deprotection.* Compound **7a** (113 mg, 0.18 mmol), MeCN (5 mL), and 1 M HCl (12 mL), 50 °C, 4 h, white solid (**9a**) (23 mg, 21%), R_f = 0.07 (1:5 petroleum spirits/EtOAc), mp 111−115 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.91 (t, J = 7.2 Hz, 3H), 2.79 (t, J = 7.2 Hz, 2H), 2.89−3.02 (m, 1H), 3.14−3.26 (m, 1H), 3.43−3.64 (m, 2H), 4.02−4.23 (m, 2H), 4.30 (t, J = 7.8 Hz, 1H), 4.39−4.43 (m, 1H), 4.54 (br s, 1H), 4.67−4.72 (m, 1H), 4.87 (br s, 1H), 5.67 (d, J = 6.0 Hz, 1H), 5.78 (br s, 1H), 7.05−7.24 (m, 16H), 7.35 (br s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.6, 34.1, 36.0, 43.4, 44.9, 50.7, 72.3, 73.3, 84.2, 89.6, 114.7, 126.4, 126.8, 128.1, 128.6, 128.7, 136.3, 139.3, 142.1, 154.9, 159.3, 170.0. HRMS calcd for $C_{34}H_{38}N_7O_4^+$ [M + H], 608.2980; found, 608.3002.

2-(2-(4-Aminophenyl))ethylamino-*N*^{6}-(2,2-diphenylethyl)-adenosine-5′-*N*-ethylcarboxamide (9b). *General Procedure for Isopropylidene Deprotection.* Compound **7b** (190 mg, 0.29 mmol), MeCN (3 mL), and 1 M HCl (15 mL), 50 °C, 4 h, light-tan foam (**9b**) (164 mg, 92%), R_f = 0.05 (1:5 petroleum spirits/EtOAc), m: 90−96 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (t, J = 7.2 Hz, 3H), 2.80 (br t, J = 6.9 Hz, 2H), 3.00−3.12 (m, 1H), 3.28−3.42 (m, 1H), 3.50−3.66 (br m, 2H), 4.11−4.36 (br m, 2H), 4.44 (t, J = 7.5 Hz, 1H), 4.54 (s, 1H), 4.62 (br s, 1H), 4.83−4.86 (m, 1H), 4.96−5.00 (m, 1H), 5.75 (d, J = 6.3 Hz, 1H), 5.99 (br s, 1H), 6.61 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 8.4 Hz, 2H), 7.20−7.38 (m, 10H, Ar-***H***), 7.41 (s, 1H, NC***H***N). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.7, 34.2, 35.1, 43.6, 45.0, 50.7, 71.8, 73.2, 84.3, 89.4, 114.6, 115.6, 126.8, 128.2, 128.7, 129.2, 129.6, 136.7, 142.2, 144.7, 150.5, 154.9, 159.5, 170.1. HRMS calcd for $C_{34}H_{39}N_8O_4^+$ [M + H], 623.3089; found, 623.3105.

2-(2-(4-Bromophenyl))ethylamino-*N*^{6}-(2,2-diphenylethyl)-adenosine-5′-*N*-ethylcarboxamide (9c). *General Procedure for Isopropylidene Deprotection.* Compound **7c** (142 mg, 0.20 mmol), MeCN (3 mL), and 1 M HCl (10 mL), 50 °C, 2.5 h, light-tan glassy solid (**9c**) (43 mg, 32%), R_f = 0.16 (1:5 petroleum spirits/EtOAc), mp 97−101 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 1.06 (t, J = 7.2 Hz, 3H), 2.88 (t, J = 7.3 Hz, 2H), 3.10−3.20 (m, 1H), 3.27−3.37 (m, 1H), 3.51−3.70 (m, 2H), 4.21 (br d, J = 6.9 Hz, 2H), 4.42 (d, J = 2.6 Hz, 1H), 4.44−4.52 (m, 2H), 4.95 (dd, J = 5.2, 6.3 Hz, 1H), 5.90 (d, J = 6.6 Hz, 1H), 7.06 (d, J = 8.3 Hz, 2H), 7.17−7.22 (m, 2H), 7.24−7.35 (m, 10H), 7.83 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: 13.5, 33.8, 73.4, 78.1, 84.2, 88.5, 113.6, 119.4, 126.2, 127.9, 128.3, 130.4, 131.1, 136.9, 139.1, 142.7, 150.9, 154.6, 159.7, 170.7. HRMS calcd for $C_{34}H_{37}N_7O_4Br^+$ [M + H], 686.2085; found, 686.2090.

***N*-*tert*-Butoxycarbonyl-2-(4-aminophenyl)ethylamine (11).** To a stirred solution of 2-(4-aminophenyl)ethylamine (1.20 g, 8.82 mmol) in CH_2Cl_2 (120 mL) cooled to −5 °C was added *tert*-butoxycarbonyl anhydride (1.73 g, 8.09 mmol). The solution was stirred for 45 min at 0 °C and then poured quickly into a 500 mL separatory funnel charged with 80 mL ice/water. The organic phase was washed ×3 with ice/water, twice with satd NaCl solution, dried over $MgSO_4$, filtered, and solvent removed in vacuo. The product was purified via flash column chromatography (5:1 petroleum spirits/EtOAc) to give an orange crystalline solid (**11**) (1.51 g, 72%), R_f =

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0.46 (1:1 petroleum spirits/EtOAc), mp 55−60 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.35 (s, 9H), 2.58 (t, J = 6.9 Hz, 2H), 3.21−3.25 (m, 2H), 3.53 (br s, 2H), 4.58 (br s, 1H), 6.53 (d, J = 8.2 Hz, 2H), 6.88 (d, J = 8.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 28.4, 35.2, 42.0, 79.0, 115.3, 128.8, 129.6, 144.9, 156.0. ESMS calcd for $C_{13}H_{21}N_2O_2^+$ [M + H], 237.3; found, 237.4

2-(4-*N*-(3,5-di-*tert*-Butylphenylcarboxy)aminophenyl)-ethylamine (12a). *General Procedure for Amide Coupling.* Compound **11** (250 mg, 1.06 mmol), 3,5-di-*tert*-butylbenzoic acid (274 mg, 1.17 mmol), EDCI (230 mg, 1.20 mmol), HOBt (160 mg, 1.20 mmol), and DIPEA (250 μL) were combined in DMF (2.5 mL) and stirred at room temperature for 16 h. The solution was poured into 50 mL of H_2O and the resulting precipitate filtered, rinsed with H_2O, and dried in vacuo to give *N*-*tert*-butoxycarbonyl-2-(4-*N*-(3,5-di-*tert*-butylphenylcarboxy)-aminophenyl)ethylamine as an off-white solid (424 mg, 88%), R_f = 0.79 (1:1 petroleum spirits/EtOAc), mp 177−185 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.34 (s, 18H), 1.38 (s, 9H), 2.67 (t, J = 7.5 Hz, 2H), 3.04−3.16 (m, 2H), 6.89−6.95 (m, 1H), 7.17 (d, J = 8.1 Hz, 2H), 7.60 (s, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.73 (s, 2H), 10.10 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.4, 28.5, 31.3, 31.4, 35.1, 77.3, 120.6, 121.2, 126.1, 129.4, 134.8, 135.2, 136.5, 151.6, 155.9, 166.9. ESMS calcd for $C_{28}H_{41}N_2O_3^+$ [M + H], 453.6; found, 453.6.

N-*tert*-Butoxycarbonyl-2-(4-*N*-(3,5-di-*tert*-butylphenylcarboxy)-aminophenyl)ethylamine (424 mg, 0.94 mmol) was dissolved in CH_2Cl_2 (8.0 mL), and TFA (3.3 mL) was added slowly. The solution was stirred for 3 h and followed by TLC. Upon completion, the pH of the solution was adjusted to 8 via the dropwise addition of satd $NaHCO_3$ solution, then to pH = 10 with 4 M NaOH solution. The resulting precipitate was removed via filtration, and the filtrate extracted ×3 with EtOAc. The precipitate was added to the organic phase, washed with water and satd NaCl, dried over $MgSO_4$, filtered, and the solvent removed to give a tan solid (**12a**) (386 mg, 98%), R_f = 0.00 (EtOAc), mp 175−180 °C (decomp). ^{1}H NMR (300 MHz, CD_3OD) δ: 1.41 (s, 18H), 2.81 (t, J = 7.2 Hz, 2H), 2.91 (t, J = 7.2 Hz, 2H), 7.26 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 8.4 Hz, 2H), 7.69 (s, 1H), 7.80 (s, 2H), 10.09 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 31.6, 31.7, 33.5, 35.2, 121.4, 122.1, 123.2, 125.8, 129.3, 133.1, 135.0, 138.3, 151.0. ESMS calcd for $C_{23}H_{33}N_2O^+$ [M + H], 353.5; found, 353.3.

2-(4-*N*-(3,5-di-*tert*-Butyl-4-hydroxyphenylcarboxy)-aminophenyl)ethylamine (12b). *General Procedure for Amide Coupling.* Compound **11** (300 mg, 1.27 mmol), 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (362 mg, 1.45 mmol), EDCI (270 mg, 1.41 mmol), HOBt (1.41 mmol), DIPEA (300 μL), and DMF (3 mL), yellow−orange solid (475 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.28 (s, 18H), 1.36 (s, 9H), 2.70 (t, J = 6.8 Hz, 2H), 3.22−3.34 (m, 2H), 4.51 (br s, 1H), 7.10 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 7.53 (t, J = 1.8 Hz, 1H), 7.60 (s, 2H), 7.85 (s, 1H).

N-*tert*-Butoxycarbonyl-2-(4-*N*-(3,5-di-*tert*-butyl-4-hydroxyphenylcarboxy)aminophenyl) ethylamine (475 mg, 1.02 mmol), CH_2Cl_2 (9.0 mL), and TFA (1.6 mL), red−orange glassy solid (**12b**) (389 mg, 81%). ^{1}H NMR (100 MHz, CD_3OD) δ: 1.38 (s, 18H), 2.86 (t, J = 7.2 Hz, 2H), 3.09 (t, J = 7.2 Hz, 2H), 7.17 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.68 (s, 2H), 7.81 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 27.7, 30.6, 34.1, 35.7, 42.0, 123.2, 126.0, 126.8, 130.1, 133.9, 138.0, 138.7, 139.3. ESMS calcd for $C_{23}H_{33}N_2O_2^+$ [M + H], 369.5; found, 369.3.

2-(2-(4-*N*-(3,5-di-*tert*-Butylphenylcarboxy)aminophenyl))-ethylamino-2′,3′-isopropylidene adenosine-5′-*N*-ethylcarboxamide (13a). *General Procedure for 2-Substitution.* Compound **2** (120 mg, 0.33 mmol), **12a** (242 mg, 0.69 mmol), DIPEA (250 μL), and EtOH (2.5 mL), 70 °C, 5 days, light-yellow solid foam (**13a**) (108 mg, 47%), R_f = 0.48 (9:1 EtOAc/MeOH), mp 102−108 °C. ^{1}H NMR (300 MHz, CD_3OD) δ: 0.63 (t, J = 7.2 Hz, 3H), 1.41(s, 18H), 1.41 (s, 3H), 1.58 (s, 3H), 2.74−2.85 (m, 1H), 2.87−3.00 (m, 3H), 3.47−3.59 (m, 1H), 3.70−3.81 (m, 1H), 4.63 (s, 1H), 5.55−5.61 (m, 1H), 5.73−5.79 (m, 1H), 6.25 (s, 1H), 7.33 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 8.4 Hz, 2H), 7.69 (s, 1H), 7.81 (s, 2H), 7.87 (s, 1H).

2-(2-(4-*N*-(3,5-di-*tert*-Butyl-4-hydroxyphenylcarboxy)-aminophenyl))ethylamino-2′,3′-isopropylideneadenosine-5′-*N*-ethylcarboxamide (13b). *General Procedure for 2-Substitution.* Compound **2** (120 mg, 0.33 mmol), **12b** (254 mg, 0.69 mmol), DIPEA (200 μL), and EtOH (2 mL), 3 days, 60 °C, pale yellow−orange solid (**13b**) (151 mg, 64%), R_f = 0.27 (9:1 EtOAc/MeOH), mp 163−167 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.73 (t, J = 7.2 Hz, 3H), 1.43 (s, 3H), 1.52 (s, 18H), 1.64 (s, 3H), 2.87−3.08 (m, 4H), 3.50−3.64 (m, 1H), 3.66−3.78 (m, 1H), 4.71 (s, 1H), 5.09−5.15 (m, 1H), 5.52−5.73 (m, 4H), 6.08 (br s, 2H), 7.27 (d, J = 7.8 Hz, 2H), 7.53 (s, 1H), 7.61 (d, J = 7.8 Hz, 2H), 7.75 (s, 2H), 8.03 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0, 25.1, 26.8, 30.2, 33.8, 34.5, 35.1, 42.8, 83.4, 84.0, 87.8, 91.4, 113.7, 114.2, 120.7, 124.4, 126.2, 129.3, 135.4, 136.2, 136.7, 137.1, 151.3, 155.8, 157.1, 159.4, 166.6, 168.9. ESMS calcd for $C_{38}H_{51}N_8O_6^+$ [M + H], 715.9; found, 715.3.

2-(2-(4-*N*-(4-(1,2-di-Thiolane)pentanecarboxy)-aminophenyl))ethylamino-2′,3′-isopropylideneadenosine-5′-*N*-ethylcarboxamide (13c). *General Procedure for Amide Coupling.* Compound **6b** (59 mg, 0.12 mmol), EDCI (28 mg, 0.19 mmol), HOBt (19.8 mg, 0.15 mmol), 4-(1,2-dithiolane)-pentanoic acid (30 mg, 0.15 mmol), DIPEA (350 μL), and DMF (3.5 mL). Crude product evaporated onto SiO_2, the solvent removed and the product purified via column chromatography using 85:15 EtOAc/MeOH as eluent to afford a pale-yellow solid foam (**13c**) (50 mg, 61%), R_f = 0.41 (9:1 EtOAc/MeOH), mp 100−105 °C. ^{1}H NMR (300 MHz, CD_3OD): 0.61 (t, J = 7.2 Hz, 3H), 1.38 (s, 3H), 1.43−1.60 (m, 2H), 1.57 (s, 3H), 1.62−1.80 (m, 4H), 1.82−1.96 (m, 1H), 2.39 (t, J = 6.6 Hz, 2H), 2.38−2.51 (m, 1H), 2.70−2.85 (m, 1H), 2.86−2.96 (m, 3H), 3.01−3.22 (m, 2H), 3.40−3.52 (m, 1H), 3.53−3.63 (m, 1H), 3.64−3.78 (m, 1H), 4.63 (s, 1H), 5.59 (d, J = 5.2 Hz, 1H), 5.75 (d, J = 5.2 Hz, 1H), 6.24 (s, 1H), 7.25 (d, J = 7.5 Hz, 2H), 7.50 (d, J = 7.5 Hz, 2H), 7.86 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): 12.6, 24.0, 25.3, 25.6, 28.6, 33.4, 34.4, 34.9, 36.4, 38.0, 39.9, 42.5, 56.2, 83.6, 84.1, 87.9, 91.1, 113.1, 120.0, 128.8, 135.6, 136.6, 138.1, 151.0, 156.0, 159.4, 170.2, 172.8. ESMS calcd for $C_{31}H_{43}N_8O_5S_2^+$ [M + H], 671.9; found, 671.3.

2-(2-(4-*N*-(3,5-di-*tert*-Butyl-4-hydroxyphenylcarboxy)-aminophenyl))ethylamino-N^6-(2,2-diphenylethyl)-2′,3′-isopropylideneadenosine-5′-*N*-ethylcarboxamide (14b). *General Procedure for 2-Substitution.* Compound **3** (250 mg, 0.46 mmol), **12b** (370 mg, 1.01 mmol), DIPEA (350 μL), and EtOH (3 mL), 70 °C, tan solid (**14b**) (186 mg, 45%), R_f = 0.61 (1:5 petroleum spirits/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.62 (t, J = 7.2 Hz, 3H), 1.31 (s, 3H), 1.42 (s, 18H), 1.51 (s, 3H), 2.74−2.98 (m, 3H), 3.09−3.23 (m, 1H,), 3.46−3.58 (m, 1H), 3.58−3.70 (m, 1H), 4.08−4.21 (br m, 2H), 4.24−4.32 (m, 1H), 4.57 (d, J = 1.8 Hz, 1H), 4.85 (br s, 1H), 5.42 (br s, 1H), 5.53 (d, J = 1.4 Hz, 1H), 5.54 (s, 1H) 5.91 (s, 1H), 5.97 (br s, 1H), 7.10−7.29 (m, 12H), 7.33 (s, 1H), 7.47 (d, J = 8.2 Hz, 2H), 7.60 (s, 1H), 7.61 (s, 2H). ESMS calcd for $C_{52}H_{63}N_8O_6^+$ [M + H], 896.1; found, 895.9.

2-(2-(4-*N*-(3,5-di-*tert*-Butylphenylcarboxy)aminophenyl))-ethylaminoadenosine-5′-*N*-ethylcarboxamide (15a). *General Procedure for Isopropylidene Deprotection.* Compound **13a** (108 mg, 0.15 mmol) and 1 M HCl (12 mL), 60 °C, 8 h, off-white solid foam (**15a**) (74 mg, 75%), R_f = 0.36 (9:1 EtOAc/MeOH), mp 142−148 °C. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.07 (t, J = 7.2 Hz, 3H), 1.41 (s, 18H), 2.93 (t, J = 7.2 Hz, 2H), 3.08−3.20 (m, 1H), 3.22−3.34 (m, 1H), 3.50−3.61 (m, 1H), 3.62−3.73 (m, 1H), 4.41 (d, J = 3.3 Hz, 1H), 4.49−4.56 (m, 1H), 5.07−5.12 (m, 1H), 5.97 (d, J = 6.3 Hz, 1H), 7.29 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H), 7.70 (t, J = 1.5 Hz, 1H), 7.80 (d, J = 1.5 Hz, 2H), 8.00 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ: 13.3, 30.4 (6C), 33.8, 34.6, 35.0, 42.9, 72.0, 73.3, 84.0, 88.7, 113.4, 121.4, 121.6, 125.6, 128.8, 134.4, 136.1, 136.6, 137.7,

151.0, 151.7, 156.1, 159.7, 168.4, 170.6. HRMS calcd for $C_{35}H_{47}N_8O_5^+$ [M + H], 659.3664; found, 659.3676.

2-(2-(4-*N*-(3,5-di-*tert*-Butyl-4-hydroxyphenylcarboxy)-aminophenyl))ethylaminoadenosine-5′-*N*-ethylcarboxamide (15b). *General Procedure for Isopropylidene Deprotection.* Compound **13b** (150 mg, 0.21 mmol), MeCN (2 mL), and 1 M HCl (20 mL), 65 °C, 12 h, white solid (**15b**) (25 mg, 18%), R_f = 0.10 (9:1 EtOAc/MeOH), mp 230−235 °C (dec). ^{1}H NMR (300 MHz, CD_3OD) δ: 1.06 (t, J = 7.2 Hz, 3H), 2.90 (t, J = 7.2 Hz, 2H), 3.08−3.21 (m, 1H), 3.22−3.36 (m, 1H), 3.48−3.59 (m, 1H), 3.60−3.73 (m, 1H), 4.43 (s, 1H), 4.49−4.54 (m, 1H), 5.02−5.06 (m, 1H), 5.96 (d, J = 6.3 Hz, 1H), 7.26 (d, J = 7.8 Hz, 2H), 7.59 (d, J = 7.8 Hz, 2H), 7.78 (s, 2H), 8.00 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 14.8, 30.6, 35.2, 35.7, 36.4, 44.3, 73.3, 74.7, 85.5, 90.1, 114.8, 122.8, 125.9, 12 7.0, 130.2, 137.3, 138.3, 138.7, 139.1, 153.1, 157.5, 158.9, 161.2, 170.1, 172.1. HRMS calcd for $C_{35}H_{47}N_8O_6^+$ [M + H], 675.3613; found, 675.3632.

2-(2-(4-*N*-(4-(1,2-Dithiolane)pentanecarboxy)-aminophenyl))ethylaminoadenosine-5′-*N*-ethylcarboxamide (15c). *General Procedure for Isopropylidene Deprotection.* Compound **13c** (50 mg, 0.08 mmol), MeCN (4 mL), and 1 M HCl (10 mL), 55 °C, 2 h, pale-yellow solid (**15c**) (13 mg, 28%), R_f = 0.10 (9:1 EtOAc/MeOH), mp 82−85 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 1.05 (t, J = 7.2 Hz, 3H), 1.47−1.60 (m, 2H), 1.64−1.83 (m, 4H,), 1.86−1.96 (m, 1H), 2.39 (t, J = 7.2 Hz, 2H), 2.43−2.53 (m, 1H), 2.87 (t, J = 7.2 Hz, 2H), 3.07−3.23 (m, 3H), 3.24−3.33 (m, 1H), 3.48−3.58 (m, 1H), 3.58−3.69 (m, 2H), 4.42 (d, J = 2.8 Hz, 1H), 4.49−4.54 (m, 1H), 5.01 (t, J = 6.4 Hz, 1H), 5.95 (d, J = 6.4 Hz, 1H), 7.22 (d, J = 8.2 Hz, 2H), 7.49 (d, J = 8.2 Hz, 2H), 8.00 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 17.3, 29.2, 32.5, 37.7, 38.3, 38.9, 40.3, 41.9, 43.8, 46.8, 60.1, 75.8, 77.2, 88.0, 92.6, 117.3, 124.0, 132.8, 134.9, 139.5, 140.5, 141.7, 155.6, 160.0, 163.6, 174.6, 176.9. HRMS calcd for $C_{28}H_{39}N_8O_5S_2^+$ [M + H], 631.2479; found, 631.2475.

2-(2-(4-*N*-(3,5-di-*tert*-Butylphenylcarboxy)aminophenyl))-ethylamino-*N*^{6}-(2,2-diphenylethyl) adenosine-5′-*N*-ethylcarboxamide (16a). *General Procedure for Amide Coupling.* Compound **7b** (69 mg, 0.11 mmol), 3,5-di-*tert*-butylbenzoic acid (29 mg, 0.13 mmol), EDCI (24 mg, 0.13 mmol), HOBt (17 mg, 0.13 mmol), DIPEA (26 μL), and DMF (265 μL), tan glassy solid (**16a**) (39 mg, 42%), R_f = 0.19 (1:5 petroleum spirits/EtOAc), mp 124−128 °C. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.07 (t, J = 6.9 Hz, 3H), 1.40 (s, 18H), 2.92 (t, J = 6.9 Hz, 2H), 3.07−3.39 (m, 2H), 3.52−3.68 (m, 2H), 4.22 (d, J = 6.6 Hz, 2H), 4.42 (s, 1H), 4.48 (s, 1H), 4.49−4.52 (m, 1H), 4.98−5.02 (m, 1H), 5.91 (s, J = 6.3 Hz, 1H), 7.19 (d, J = 7.5 Hz, 2H), 7.12−7.20 (m, 2H), 7.21−7.44 (m, 8H), 7.59 (d, J = 7.5 Hz, 2H), 7.69 (s, 1H), 7.81 (s, 2H), 7.91 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 14.8, 31.8, 35.2, 36.0, 36.8, 44.7, 46.0, 51.9, 73.1, 74.7, 85.6, 90.0, 114.9, 122.8, 122.9, 127.1, 127.7, 129.3, 129.7, 130.2, 135.8, 137.6, 138.0, 138.4, 144.1, 152.5, 156.1, 161.2, 170.0, 172.1. HRMS calcd for $C_{49}H_{59}N_8O_5^+$ [M + H], 839.4603; found, 839.4642.

2-(2-(4-*N*-(3,5-di-*tert*-Butyl-4-hydroxyphenylcarboxy)-aminophenyl))ethylamino-*N*^{6}-(2,2-diphenylethyl)adenosine-5′-*N*-ethylcarboxamide (16b). *General Procedure for Isopropylidene Deprotection.* Compound **14b** (175 mg, 0.20 mmol), MeCN (5 mL), and 1 M HCl (15 mL), 50 °C, 6 h, white solid (**16b**) (20 mg, 12%), R_f = 0.23 (1:5 petroleum spirits/EtOAc), mp 118−124 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.93 (t, J = 7.2 Hz, 3H), 1.37 (s, 18H), 2.75 (br t, J = 6.0 Hz, 2H), 2.91−3.04 (m, 1H), 3.18−3.32 (m, 1H), 3.39−3.62 (br m, 2H), 4.13 (br s, 2H), 4.29 (t, J = 7.6 Hz, 1H), 4.39 (d, J = 2.4 Hz, 1H), 4.50 (br s, 1H), 4.69 (t, J = 5.5 Hz, 1H), 4.84−4.87 (m, 1H), 5.54 (br s, 1H), 5.63 (d, J = 6.0 Hz, 1H), 5.72 (br s, 1H), 7.03 (d, J = 8.2 Hz, 2H), 7.10−7.16 (m, 2H), 7.17−7.25 (m, 10H), 7.29 (br s, 1H), 7.38 (d, J = 8.2 Hz, 2H), 7.61 (s, 2H), 7.94 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ: 18.3, 33.9, 38.0, 38.3, 39.4, 47.1, 48.8, 54.5, 75.3, 76.8, 88.3, 93.4, 118.3, 124.9, 128.4, 129.7, 130.6, 132.0, 132.6, 133.1, 139.3, 140.1, 140.5, 140.6, 146.1, 154.3, 158.7, 161.0, 163.4, 171.4, 174.2. HRMS calcd for $C_{49}H_{59}N_8O_6^+$ [M + H], 855.4552; found, 855.4538.

2-(2-(4-*N*-(4-(1,2-Dithiolane)pentanecarboxy)-aminophenyl))ethylamino-*N*^{6}-(2,2-diphenylethyl)adenosine-5′-*N*-ethylcarboxamide (16c). *General Procedure for Amide Coupling.* Compound **7b** (160 mg, 0.26 mmol), 4-(1,2-dithiolane)pentanoic acid (79 mg, 0.38 mmol), EDCI (73 mg, 0.38 mmol), HOBt (52 mg, 0.38 mmol), DIPEA (250 μL), and DMF (2.5 mL), white solid (**16c**) (31 mg, 15%), R_f = 0.24 (1:5 petroleum spirits/EtOAc), mp 107−112 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 0.96 (t, J = 7.2 Hz, 3H), 1.37−1.52 (m, 2H), 1.55−1.72 (m, 4H), 1.75−1.86 (m, 1H), 2.28 (t, J = 7.4 Hz, 2H), 2.31−2.41 (m, 1H), 2.80 (t, J = 7.2 Hz, 2H), 2.93−3.11 (m, 3H), 3.17−3.28 (m, 1H), 3.45−3.53 (m, 2H), 3.52−3.64 (m, 1H), 4.12 (d, J = 7.1 Hz, 2H), 4.31 (d, J = 2.6 Hz, 1H), 4.32−4.36 (br m, 1H), 4.39 (t, J = 7.8 Hz, 1H), 4.82 (dd, J = 5.2, 6.4 Hz, 1H), 5.76 (d, J = 6.6 Hz, 1H), 7.04 (d, J = 8.5 Hz, 2H), 7.07−7.13 (m, 2H), 7.15−7.24 (m, 8H), 7.35 (d, J = 8.5 Hz, 2H), 7.68 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ: 17.3, 29.2, 32.5, 37.7, 38.3, 38.9, 40.3, 41.9, 43.8, 46.8, 60.1, 75.8, 77.2, 88.0, 92.6, 117.3, 124.0, 132.8, 139.5, 140.5, 141.7, 155.6, 160.0, 163.6, 174.6, 176.9. HRMS calcd for $C_{42}H_{51}N_8O_5S_2^+$ [M + H], 811.3418; found, 811.3427.

2-Fluoro-*N*^{6}-(2,2-diphenylethyl)-2′,3′-*O*-isopropylideneadenosine-5′-*N*-methylcarboxamide (17). *General Procedure for N^6-Substitution.* Compound **1b** (225 mg, 0.48 mmol), 2,2-diphenylethylamine (142 mg, 0.72 mmol), DIPEA (250 μL), and MeCN (15 mL), yellow solid (**17**) (203 mg, 80%), R_f = 0.38 (1:5 petroleum spirits/EtOAc); mp 92−95 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 1.38 (s, 3H), 1.56 (s, 3H), 2.34 (s, 3H), 4.13−4.21 (m, 2H), 4.47 (t, 1H, J = 8.0 Hz), 4.60 (d, J = 1.2 Hz, 1H), 5.34 (dd, J = 6.1, 1.5 Hz, 1H), 5.46 (d, J = 1.7 Hz, 1H), 6.20 (d, J = 1.2 Hz, 1H), 7.14−7.19 (m, 2H), 7.22−7.33 (m, 8H), 8.00 (s, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ: 25.3, 25.8, 27.1, 46.1, 51.7, 84.7, 85.0, 88.1, 92.4, 115.1, 118.9 (d, J = 4.0 Hz), 127.3 (×2), 129.3 (×4), 129.6 (×4), 142.0 (d, J = 3.0 Hz), 143.6 (d, J = 1.1 Hz), 149.8 (d, J = 20.5 Hz), 155.6 (d, J = 20.7 Hz), 160.5 (d, J = 210.0 Hz), 172.0. ESMS calcd for $C_{28}H_{30}N_6O_4F^+$ [M + H], 533.6; found, 533.8.

2-Fluoro-*N*^{6}-(2,2-diphenylethyl)adenosine-5′-*N*-methylcarboxamide (18). *General Procedure for Isopropylidene Deprotection.* Compound **17** (60 mg, 0.11 mmol), MeCN (2 mL), and 1 M HCl (10 mL), 50 °C, 2 h, white solid (**18**) (22 mg, 40%), R_f = 0.29 (89:10:1 CH_2Cl_2/MeOH/NH_4OH), mp 128−131 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 2.87 (s, 3H), 4.15−4.25 (m, 2H), 4.29 (dd, 1H), 4.46 (s, 1H), 4.49 (t, J = 8.1 Hz, 1H), 4.62 (dd, J = 7.7, 4.8 Hz, 1H), 5.91 (d, J = 7.8 Hz, 1H), 7.17−7.23 (m, 2H), 7.24−7.36 (m, 8H), 8.09 (s, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ: 25.8, 46.2, 51.7, 73.2, 78.9, 86.5, 90.6, 119.8 (d, J = 4.0 Hz), 127.2 (×2), 129.3 (×4), 129.6 (×4), 142.2 (d, J = 2.7 Hz), 143.6 (d, J = 1.1 Hz), 150.5 (d, J = 19.8 Hz), 157.8 (d, J = 20.3 Hz), 160.4 (d, J = 208.5 Hz), 172.6. HRMS calcd for $C_{25}H_{26}N_6O_4F^+$ [M + H], 493.1994; found, 493.2010.

2-(2-(4-*N*-(3,5-di-*tert*-Butyl-4-hydroxyphenylcarboxy)-aminophenyl))ethylamino-*N*^{6}-(2,2-diphenylethyl)adenosine-5′-*N*-methylcarboxamide (20). *General Procedure for 2-Substitution.* Compound **17** (188 mg, 0.35 mmol), **12b** (169 mg, 0.46 mmol), DIPEA (250 μL), and MeCN, 150 °C (MW), 2 h, yellow solid (**19**) (229 mg, 74%).

General Procedure for Isopropylidene Deprotection. Compound **19** (200 mg, 0.23 mmol), MeCN (10 mL), and 1 M HCl (15 mL), 2 h, white solid (**20**) (45 mg, 24%), R_f = 0.30 (89:10:1 CH_2Cl_2/MeOH/NH_4OH), mp 120−123 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 1.47 (s, 18H), 2.71 (s, 3H), 2.89 (t, J = 6.9 Hz, 2H), 3.53−3.60 (m, 1H), 3.65−3.72 (m, 1H), 4.20 (d, J = 6.4 Hz, 2H), 4.40 (s, 2H), 4.48 (t, J = 7.8 Hz, 1H), 4.97−4.99 (m, 1H), 5.85 (d, J = 7.0 Hz, 1H), 7.13−7.17 (m, 4H), 7.23−7.31 (m, 8H), 7.52 (d, J = 8.4 Hz, 2H), 7.76−7.78 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ: 26.3, 30.6, 35.6, 36.7, 44.6, 46.0, 51.9, 72.6, 74.7, 85.8, 90.0, 115.0, 122.8, 125.9, 127.0, 127.6,

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129.2, 129.3, 129.5, 129.6, 130.1, 137.2, 138.2, 138.6, 138.7, 144.0, 152.2, 156.0, 158.9, 161.2, 170.0, 172.8. HRMS calcd for $C_{48}H_{57}N_8O_6^+$ [M + H], 841.4396; found, 841.4397.

Cell Culture and cAMP Assay. Four stably transfected CHO cell lines, each overexpressing one of the human A_1, A_{2A}, A_{2B}, and A_3Rs, were used for cAMP functional assays. The reporter lines were a kind gift from Prof. A. Christopoulos, Monash Institute of Pharmaceutical Sciences. Cells were cultured in high glucose Dulbecco's Modified Eagle's Medium plus GlutaMAX containing 1 mM sodium pyruvate, 15 mM HEPES, 1 mg/mL hygromycin B, and 5% fetal bovine serum in a humidified atmosphere of 95% air and 5% CO_2. All cell culture reagents were purchased from Invitrogen, Australia. Cells were grown to ~80% confluence, split (0.01% trypsin), then seeded into 96-well plates, at 20000 cells/well, and grown overnight. Cyclic AMP functional assays were performed using a Perkin-Elmer Alphascreen kit according to manufacturer's instructions. Briefly, media was removed from wells and replaced with a cAMP acceptor bead solution consisting of phenol red free DMEM/F-12 media containing 15 mM HEPES and L-glutamine, supplemented with 100 μM Rolipram (Tocris Biosciences) and anti-cAMP acceptor beads at 2 units/well. This was followed by the addition of test compounds. The acceptor bead solution and compound mix were left to incubate for 30 min prior to addition of the donor bead solution, containing 0.1% BSA, 0.3% final 10% Tween-20, 5 mM HEPES, 2 units/well of streptavidin coated donor beads, and 2 units/well of biotinylated cAMP. After addition of the donor bead solution, the cells were incubated for 2 h in the dark at 37 °C and 5% CO_2 prior to being read on a Perkin-Elmer Wallac, 2101 multiplate reader. Vehicle controls consisted of 0.1% DMSO and 0.9% ethanol, final concentration.

For the A_{2A} and A_{2B} assays, cells were incubated with compounds in the range 0.1 nM through to 100 μM. The assay is essentially a competitive binding, proximity assay with endogenous cAMP and exogenously added biotinylated cAMP competing for binding to an antibody conjugated to the acceptor beads. For the A_1R and A_3R assays, cells were incubated with 10 μM forskolin (final concentration, to activate adenylate cyclase) and compounds in the range 0.1 nM through to 100 μM. Results were expressed either as an elevation of cAMP (A_{2A} and A_{2B}R assays generating EC_{50} values) or an A_1 or A_3R mediated inhibition of the forskolin-stimulated elevation of cAMP (IC_{50} values).

Cell Culture and Simulated Ischemia Assay. The H9c2(2−1) embryonic rat atrial cell line (American Type Culture Collection, ATCC, Manassas, VA, USA) was used for this study. The cell line was grown in Dulbecco's Modified Eagle's Medium containing 4 mM L-glutamate, 4.5 g/L glucose, 3.7 g/L sodium bicarbonate, 100 U/mL penicillin, and 100 mg/mL streptomycin supplemented with 10% fetal bovine serum (Invitrogen, Mount Waverley, VIC, Australia) in a 5% CO_2 incubator. Cells were used at 60−70% confluence and plated one day prior to assay at 40000 cells per well of 96-well plate. Simulated ischemia was induced using conditions developed in our lab and described previously.[24,33−36] In short, ischemia was achieved by incubating the cells in hypoxic simulated ischemia (SI) medium at pH 6.4 containing (in mM): 137 NaCl, 3.5 KCl, 0.88 $CaCl_2{\cdot}2H_2O$, 0.51 $MgSO_4{\cdot}7H_2O$, 5.55 D-glucose, 4 HEPES, 10 2-deoxy-D-glucose, and 20 DL-lactic acid (Sigma, Castle Hill, NSW, Australia) plus 2% fetal bovine serum. Cells were incubated under nitrogen (100% N_2 gas atmosphere) for 12 h at 37 °C. Fresh simulated ischemia medium was prepared for each experiment and sterile filtered prior to experimentation. Agonists were dissolved in SI buffer and added to the wells at concentrations between 0.5 and 20 μM. Each treatment was repeated in three independent assays performed in triplicate wells.

Cell Viability (DAPI) Assay and DHE Imaging of H9C2 (2−1) Cells. Detection of nonviable cells resulted from ischemia was achieved by 4′,6-diamidino-2-phenylindole (DAPI) exclusion assay. This was performed as previously except that DAPI was used rather than propidium iodide in order to allow coimaging with DHE. Twelve hours post simulated ischemia, cells were first washed with PBS and stained with 400 nM DAPI (Sigma) for 5 min, followed by PBS rinse twice prior to imaging. Images were taken using a confocal microscope (Nikon A1; Nikon Instruments, Tokyo, Japan) using 405 nm laser excitation. The SI assay was repeated in at least three different passages. PI-positive cells were quantified using ImageJ (NIH Image; National Institutes of Health, USA). The normalized dead cell percentage was calculated by dividing the number of DAPI-positive cells per well by the average number of DAPI-positive cells in the SI treated wells for that experiment. DHE staining was performed by incubating the cells at 20 μM for 45 min, prior to imaging, to determine superoxide levels within the H9c2 cells.

Statistical Analysis. The effects of compounds **15a**, **15b**, and **15c** on cardiomyoblast cell death during hypoxia were determined using a two-way analysis of variance (ANOVA), with one factor being antioxidant type and one factor being concentration. Identification of individual group-to-group differences was performed using Bonferroni posthoc analysis.

Molecular Modeling. Ballesteros−Weinstein nomenclature[37] was used to identify GPCR residues, except in loop regions, where crystal structure numbering was used. Ligands were prepared using LigPrep 2.4[38] at physiological pH. The crystal structure of the adenosine A_{2A} receptor (A_{2A}R) extracted from its complex with the agonist UK-432,097 (PDB: 3QAK) was used in docking studies. The T4-lysozyme was deleted, and the protein was prepared in Maestro 9.1[39] with the ProteinPrep wizard. Water molecules within 5 Å of the crystal structure ligand were retained (residue numbers 1204 and 1209).

Ligands were docked into the crystal structure using GOLD.[40,41] The wizard in the HERMES interface was used to generate the input files for GOLD. The centroid of the binding site was defined by the center of the ligand UK-432,097, including all atoms within 12 Å of the centroid. The "goldscore_p450_csd" template was used with the following modifications. Each ligand was subjected to 200 genetic algorithm runs, generating 200 poses per ligand. The genetic algorithm search options were set to "very flexible". Early termination of docking was allowed if the two top scoring poses were within 2.0 Å of each other, and the option to generate diverse solutions was selected, clustering to an rmsd of 2.0 Å.

The resulting docked poses were clustered in Maestro, based on their similarity to the adenosine-5-*N*-ethylcarboxamide (NECA) portion of UK-432,097 in the A_{2A}R crystal structure.

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Notes

The authors declare no competing financial interest.

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