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Synthesis of Photoreactive 2-Phenethylamine Derivatives – Synthesis of Adenosine Derivatives Enabling Functional Analysis of Adenosine Receptors by Photoaffinity Labeling

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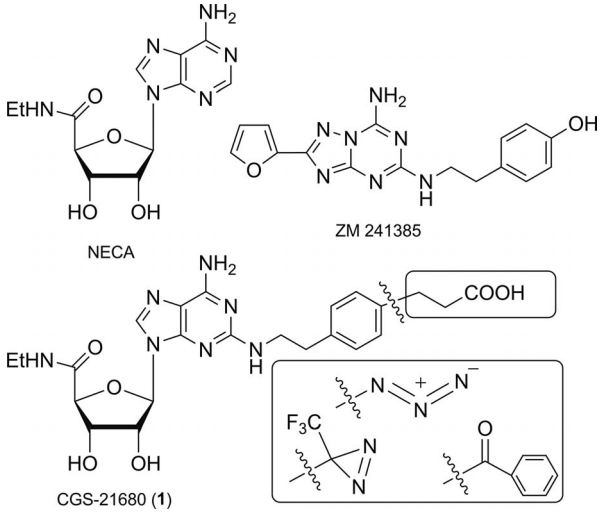
2-Phenylethylamine is well known as a substructure of many biologically active compounds, and the synthesis of its photoreactive derivatives to allow the analysis of biological functions is reported. This allowed us to synthesise ligands for adenosine receptors, which have many functional roles in biology and have been extensively studied for their many roles in maintaining homeostasis. Adenosine is one of the most common biochemical compounds, but photoaffinity labeling has not yet been used to study adenosine receptors. Synthetic methods for producing photoreactive adenosine derivatives that can be used with adenosine receptors were established for the photophores phenyl azide and benzophenone. The effect of the introduction of the photoreactive components was determined using an adenosine receptor assay.

Introduction

2-Phenylethylamine (2-PEA) skeletons are components of many biologically active natural products, especially neurotransmitters or neuromodulators in the central nervous system.[1] Analysis of the biological functions of 2-PEAs has been the subject of much research in the pharmaceutical field. Photoaffinity labeling is a method used to study the interactions of low-molecular-weight biologically active compounds with biomolecules.[2] It is suitable for the analysis of biological interactions because it is based on the affinity of the biologically active compound for the biomolecule. But only a few photoreactive modifications of 2-PEA with azide[3] or benzophenone[4] have been reported previously, and the (trifluoromethyl)diazirinyl derivative of 2-PEA has not yet been reported.

2-PEA has also been used as a substructure of adenosine-receptor ligands (Scheme 1). Adenosine receptors, which have been cloned and categorized into four subtypes (A_1, A_{2A}, A_{2B}, and A_3),[5] are G-protein-coupled receptors. In the brain, A_{2A} receptors are expressed at high densities in the striatum. Descriptions of the crystal structures of human A_{2A} receptors in complexes with unselective adenosine-receptor agonists (namely adenosine and *N*-eth-



Scheme 1. Structures of adenosine-receptor ligands and designs of photoreactive CGS-21680 derivatives. NECA (an unselective agonist), ZM241385 (an A_{2A} selective antagonist), and CGS-21680 (**1**, an A_{2A}-selective inverse agonist). The carboxyethyl group of CGS-21680 was exchanged for photoreactive groups in this study.

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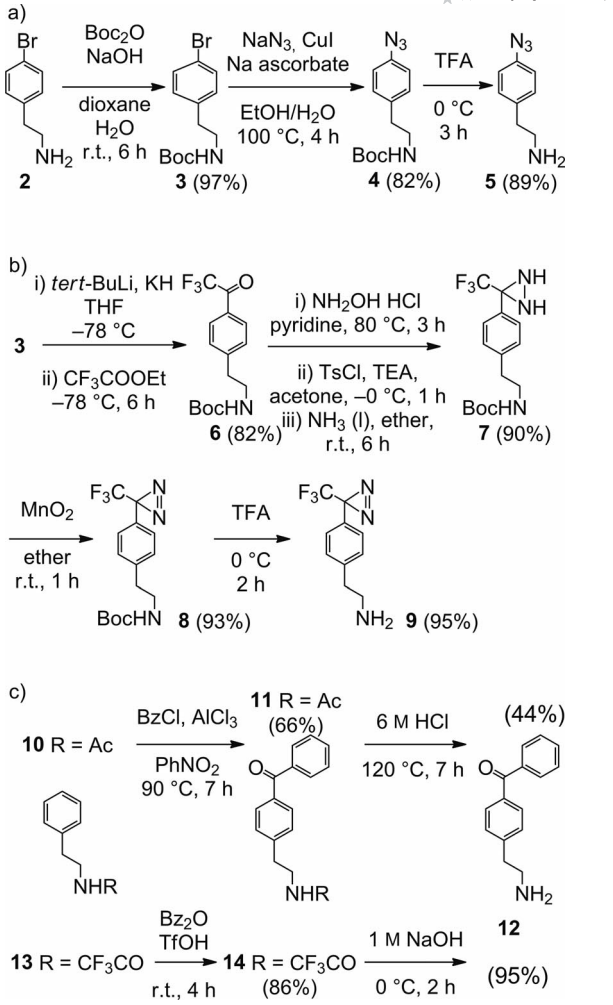
yladenosine-5′-uronamide (NECA),[6]), and also with an A_{2A}-selective antagonist (4-(2-[7-amino-2-(2-furyl)-[1,2,4]-triazolo-[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM241385)[7]), have recently been published. However, the crystal-structure studies with the agonists are not yet complete.

3-{4-[2-({6-Amino-9-[(2*R*,3*R*,4*S*,5*S*)-5-(ethylcarbamoyl)-3,4-dihydroxy-oxolan-2-yl]purin-2-yl}amino)ethyl]phenyl}-propanoic acid (CGS-21680, **1**) is a specific inverse agonist for A_{2A} receptors,[5] and has hypotensive activity in vivo. Inverse agonists bind to the receptors and stabilize them, and thus reduce their activity.[8] CGS-21680 and ZM241385 have 2-PEA substituents at the 2-position of adenine. Broad acceptability has been observed for modifications at the *p*-position of the 2-PEA moiety.[9] Modification at the *p*-position of 2-PEA may be used for the introduction of photophores into the ligand skeleton. For satisfactory results to be obtained in photoaffinity labeling, the right choice of photophore is critical, but there is no universal rule about the best choice of photophore.[2e]

In this paper, the synthesis of photoreactive 2-PEA derivatives with various photophores, phenyl azides, benzophenones, and (trifluoromethyl)phenyldiazirines, and their introduction into the NECA skeleton to give photoreactive CGS-21680 derivatives (Scheme 1) are reported. We also report the results of assays for determining the biological activities of these compounds on the purified human adenosine A_{2A} receptor (A_{2A}R), which had been expressed in *Pichia pastoris*.[10]



Scheme 2. Synthesis of photoreactive phenylethylamine derivatives. a) phenyl azide, b) (trifluoromethyl)phenyldiazirine, and c) benzophenone.

Results and Discussion

Our synthetic methodology was based on the initial installation of the photophores into 2-PEA derivatives. 2-(4-Bromophenyl)ethylamine (**2**) was selected as the starting material because its Boc-protected derivative (i.e., **3**; Boc = *tert*-butoxycarbonyl) could be a common precursor for both the phenyl azide and the (trifluoromethyl)diazirine. Compound **3** was subjected to substitution of bromide by azide (to give **4**) in a Cu^I-catalyzed reaction. The yields were influenced by the choice of ligands. A proline / NaOH system[11] resulted in the incomplete consumption of the haloarene. On the other hand, a *N*,*N*′-dimethylethylenediamine/sodium ascorbate system[12] gave the desired product (i.e., **4**) effectively. Acidic treatment to remove the Boc group produced phenyl azide derivative **5** in moderate yield (Scheme 2, a). The overall yield for the preparation of **5** was identical to that from a sole previous report, in which the synthesis started from 2-(4-aminophenyl)ethylamine and used diazotization–azidation.[5]

For the (trifluoromethyl)phenyldiazirine photophore, compound **3** was subjected to trifluoroacetylation with CF_3COOEt in the presence of *t*BuLi and KH to produce **6**. The trifluoroacetyl moiety was converted into a (trifluoromethyl)diazirinyl moiety (in **8**) following a general method.[13] Deprotection with trifluoroacetic acid (TFA) gave the desired product (i.e., **9**) in moderate yield (Scheme 2, b).

The benzophenone derivative was synthesized from the corresponding *N*-acetylphenylethylamine derivative (i.e., **10**).[4] Friedel–Crafts benzoylation with aluminum chloride at 90 °C for 7 h gave **11**, and this was followed by deprotection of the acetyl moiety under acidic conditions to produce benzophenone derivative **12** in low yield (less than 30% over two steps). *N*-Trifluoroacetyl phenylalanine (**13**)[14] was treated with benzoic anhydride in trifluoromethanesulfonic acid (TfOH) at room temperature. The Friedel–Crafts benzoylation was improved by using TfOH as catalyst and solvent.[15] Subsequent alkaline deprotection of the trifluoroacetyl group gave **12** in good yield (up to 82% for two steps) (Scheme 2, c).

The synthesis of the photoreactive CGS-21680 skeleton was designed such that condensation reactions between 2-chloro-*N*-ethyladenosine-5′-uronamide derivatives and photoreactive phenylethylamines would be used to construct adenosine derivatives modified at the 2-position.[9a,16]

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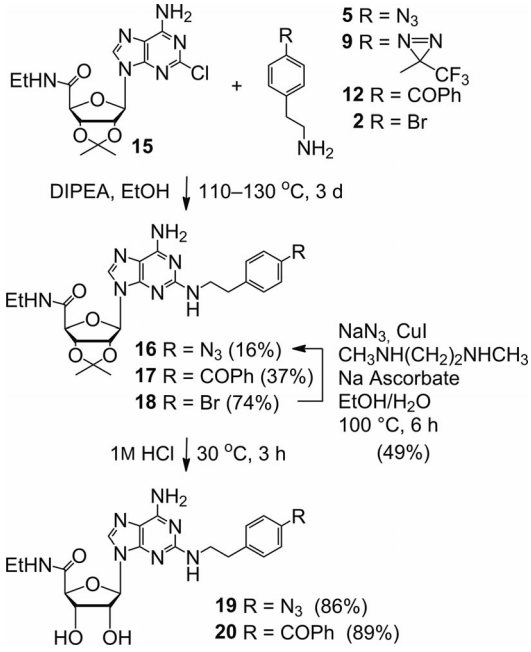
The photoreactive 2-PEA derivatives were subjected to condensation with 2-chloroadenosine derivative **15**. The original method, in which the reaction mixtures were heated to 130 °C in ethanol, was used for the condensations.[9a] Detailed studies revealed that (trifluoromethyl)phenyl diazirinyl derivative **9** did not tolerate the high-temperature conditions. The ethyl moiety was always observed in the ^{1}H NMR spectra of the products with identical integrations. In the ^{19}F NMR spectra, the trifluoromethyl group in the diazirine was observed at –66 ppm, and the peak was shifted to –80 ppm after the condensations. These results show that the (trifluoromethyl)diazirine moiety was broken down during the reaction. Precursors of the diazirine precursor (i.e., Boc-deprotected **6** and **7**) were also subjected to the condensation conditions, but the desired reactions were not observed.

On the other hand we can establish the condensation conditions for other photophores (i.e., phenylazides **5** and benzophenone **12**) without decomposition. The reactions were very slow (3 d), and care was taken regarding evaporation of the solvent during the course of the reaction. Detailed studies revealed that 2 equiv. of the phenylethylamine derivatives and a large excess (20 equiv.) of diisopropylethylamine were required to maintain the nucleophilicity of the phenylethylamines. A temperature of 110 °C was enough to promote the reaction of compounds **5** and **15**. It was observed that compound **5** decomposed during the reaction at 130 °C, which was reported in the original paper. However, no difference was observed between 110 and 130 °C for the reaction of **12** and **15**. The reaction required several days to go to completion. The starting materials (i.e., **15** and **12**) were not consumed completely, but no significant further reaction was observed after three days. After work-up, the remaining starting materials **12** and **15** could be re-subjected to the reaction conditions to give the products in the same yield. No improvements were observed when the equivalent tertiary amine was used.

Azide derivative **16** was prepared in another way: *p*-bromophenylethylamine (**2**) was condensed with adenosine derivative **15** to give **18**, and this was followed by azidation with sodium azide in the presence of catalytic amounts of Cu^I, *N*,*N*′-dimethylethylenediamine, and sodium ascorbate at 100 °C for 6 h.[12] The azidation reactions went smoothly and effectively. Compounds **16** and **17** were subjected to ketal hydrolysis under acidic conditions at 30 °C to give photoreactive adenosine derivatives **19** and **20** (Scheme 3).

The synthesized photoreactive CGS 21680 derivatives were subjected to competitive binding assays[17] with purified A_{2A}R with an agonist or antagonist.[10] Competitive inhibition assays with agonist ([^{3}H]-NECA) revealed that the affinities of both of the synthetic compounds (i.e., **19** and **20**) were of the order of <1 μM, which is sufficient to elucidate the biological function of the A_{2A}R. Inhibition assays with an A_{2A}R-specific antagonist ([^{3}H]-ZM24138) suggested that the synthetic photoreactive compounds had sufficient activity for functional analysis of A_{2A}Rs. The modifications did not cause a significant decrease in the affinity of the derivatives (Figure 1).



Scheme 3. Synthesis of photoreactive CGS-21680 derivatives. DIPEA = diisopropylethylamine.

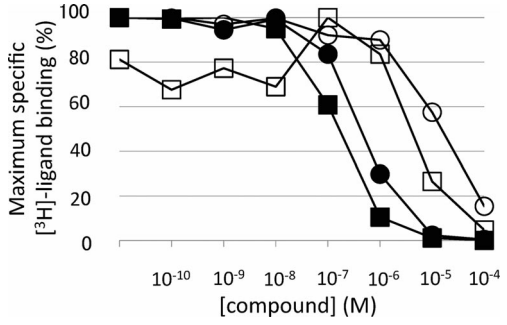


Figure 1. Competitive inhibition assay for photoreactive CGS-21680 derivatives **19** and **20** against [^{3}H]-NECA (closed circle for **19** and closed square for **20**) or [^{3}H]-ZM24138 (open circle for **19** and open square for **20**).

Conclusions

We have developed a comprehensive synthesis of 2-PEA derivatives containing three photophores for photoaffinity labeling. These derivatives were coupled with 2-Cl adenosine derivatives to elucidate functional analysis of adenosine receptors. Preliminary binding assays for the photoreactive ligands indicated that they have enough activity to warrant further analysis by photoaffinity labeling of the A_{2A}R. Further functional analysis of A_{2A}R with these synthetic photoreactive reagents is underway.

Experimental Section

General Remarks: NMR spectra were measured with JEOL EX-280 or Bruker AMX500 spectrometers. All solvents were of reagent grade and were distilled using the appropriate methods. ESI-TOF-MS data were obtained with a Waters UPLC ESI-TOF mass spectrometer.

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***tert*-Butyl 4-Bromophenethylcarbamate (3):** 2-(4-Bromophenyl)-ethylamine (1.41 g, 7.02 mmol) and NaOH (418 mg, 10.5 mmol) were dissolved in dioxane (25 mL) and H_2O (25 mL), and the mixture was cooled to 0 °C. Di-*tert*-butyl dicarbonate (2.28 g, 10.53 mmol) in dioxane (12 mL) was added dropwise to the reaction. The reaction was stirred at room temperature for 6 h, and then the solvents were evaporated. The crude compound was purified by column chromatography (CH_2Cl_2/hexane, 1:4 to CH_2Cl_2) to give **3** (2.05 g, 97%) as a colorless amorphous solid. Analytical data were identical to those reported in the literature.[18]

***tert*-Butyl 4-Azidophenethylcarbamate (4):** *tert*-Butyl 4-bromophenethylcarbamate (**3**, 405 mg, 1.35 mmol), NaN_3 (180 mg, 2.70 mmol), sodium ascorbate (13.2 mg, 0.067 mmol), CuI (26 mg, 0.135 mmol), and *N*,*N*′-diethylethylenediamine (22 μL, 0.202 mmol) in EtOH (1.4 mL) and H_2O (0.6 mL) were stirred for 4 h at 100 °C. The reaction mixture was poured into ice/water, and the organic compound was extracted with EtOAc. The organic extract was washed with brine and dried with $MgSO_4$, and the solvents were evaporated. The crude product was purified by column chromatography (CH_2Cl_2/hexane, 1:3 to CH_2Cl_2) to give **4** (291 mg, 82%) as a colorless amorphous mass. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.17 (d, J = 8.2 Hz, 2 H, Ar-H), 6.95 (d, J = 8.2 Hz, 2 H, Ar-H), 4.70 (br. s, 1 H, NH), 3.34 (q, J = 6.9 Hz, 2 H, CH_2N), 2.76 (t, J = 6.9 Hz, 2 H, $PhCH_2$), 1.43 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 155.7, 138.1, 135.7, 130.0, 119.0, 79.1, 41.7, 35.5, 28.3 ppm. HRMS (ESI): calcd. for $C_{13}H_{18}N_4O_2Na$ 285.1327; found 285.1350.

2-(4-Azidophenyl)ethanamine (5): TFA (200 μL) was added to *tert*-butyl 4-azidophenethylcarbamate (**4**, 113 mg, 0.43 mmol) at 0 °C, and the reaction mixture was stirred for 3 h at the same temperature. The mixture was basified with NaOH (1 M) and stirred for 10 min, then it was extracted with EtOAc. The organic phase was washed with brine, dried with $MgSO_4$, and evaporated to give **5** (62.0 mg, 89%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.19 (d, J = 8.2 Hz, 2 H, Ar-H), 6.97 (d, J = 8.2 Hz, 2 H, Ar-H), 2.95 (t, J = 6.8 Hz, 2 H, CH_2N), 2.73 (t, J = 6.8 Hz, 2 H, $PhCH_2$), 1.39 (br. s, 2 H, NH_2) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 137.9, 136.6, 130.1, 119.0, 43.4, 39.3 ppm. HRMS (ESI): calcd. for $C_8H_{11}N_4$ 163.0984; found 163.0988.

***tert*-Butyl 4-(2,2,2-Trifluoroacetyl)phenethylcarbamate (6):** *tert*-Butyl 4-bromophenethylcarbamate (**3**, 612 mg, 2.04 mmol) in THF (5 mL) was added dropwise to a suspension of potassium hydride (30% suspension in mineral oil; 276 mg, 2.04 mmol) in THF (10 mL) at 0 °C under N_2. The reaction was cooled to –78 °C, and *t*BuLi (1.7 M in pentane; 2.6 mL 416 mmol) was added dropwise over a period of 10 min. Ethyl trifluoroacetate (1.2 mL, 10.3 mmol) was added, and the mixture was stirred for 6 h at the same temperature, then ammonium chloride (satd. aq.; 5 mL) was added to the reaction mixture. The mixture was extracted with diethyl ether. The organic phase was washed with brine and dried with $MgSO_4$, and the solvents were evaporated. The crude product was purified by column chromatography (EtOAc/hexane, 1:3) to give **6** (531 mg, 82%) as a colorless amorphous solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.01 (d, J = 8.2 Hz, 2 H, Ar-H), 7.39 (d, J = 8.2 Hz, 2 H, Ar-H), 4.84 (br. s, 1 H, NH), 3.40 (t, J = 6.8 Hz, 2 H, CH_2N), 2.91 (t, J = 6.8 Hz, 2 H, $PhCH_2$), 1.42 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 179.9 (q, $^2J_{C,F}$ = 34.6 Hz), 155.8, 147.9, 130.3, 129.5, 128.1, 116.6 (q, $^1J_{C,F}$ = 291.1 Hz), 79.3, 41.1, 36.4, 28.2 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = –71.34 ppm. HRMS (ESI): calcd. for $C_{15}H_{19}F_3NO_3$ 318.1317; found 318.1326.

***tert*-Butyl 4-[3-(Trifluoromethyl)diaziridin-3-yl]phenethylcarbamate (7):** *tert*-Butyl 4-(2,2,2-trifluoroacetyl)phenethylcarbamate (**6**, 531 mg, 1.67 mmol) and hydroxylamine hydrochloride (346 mg, 5.02 mmol) in pyridine were stirred at 80 °C for 3 h. The pyridine was removed on a rotary evaporator, and then the residue was dissolved in diethyl ether. This solution was washed with HCl (1 M) and brine, dried with $MgSO_4$, and the solvents were evaporated. The crude product was purified by column chromatography (CH_2Cl_2 to EtOAc/hexane, 1:2) to give *tert*-butyl 4-[2,2,2-trifluoro-1-(hydroxyimino)ethyl]phenethylcarbamate (506 mg, 90%) as a colorless amorphous solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 10.55 (s, 0.4 H), 10.23 (s, 0.6 H), 7.46 (d, J = 8.2 Hz, 0.8 H, Ar-H), 7.35 (d, J = 8.2 Hz, 1.2 H, Ar-H), 7.28 (d, J = 8.2 Hz, 0.8 H, Ar-H), 7.19 (d, J = 8.2 Hz, 1.2 H, Ar-H), 4.71 (br. s, 1 H, NH), 3.40 (br. s, 2 H, CH_2N), 2.82 (br. s, 2 H, $PhCH_2$), 1.44 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 156.3, 146.6 (q, $^2J_{C,F}$ = 32.2 Hz), 141.4, 141.0, 128.9 (2 C), 128.8, 128.5, 124.6, 120.9 (q, $^1J_{C,F}$ = 274.7 Hz), 80.0, 41.5, 36.1, 28.3 ppm.

tert-Butyl 4-[2,2,2-trifluoro-1-(hydroxyimino)ethyl]phenethylcarbamate (178 mg, 0.538 mmol) was dissolved in acetone (4 mL) and cooled to 0 °C. Triethylamine (220 μL) and *p*-toluenesulfonyl chloride (205 mg, 1.07 mmol) were added, and the mixture was stirred at the same temperature for 1 h. The solvents were evaporated, and the crude residue was purified by column chromatography (CH_2Cl_2 to EtOAc/Hex, 1:3) to give *tert*-butyl 4-[2,2,2-trifluoro-1-(tosyloxyimino)ethyl]phenethylcarbamate (220 mg, 84%) as a colorless amorphous solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.90 (d, J = 8.2 Hz, 2 H, Ar-H), 7.43–7.22 (m, 6 H, Ar-H), 4.62 (br. s, 1 H), 3.43–3.33 (m, 2 H, CH_2N), 2.88–2.80 (m, 2 H, $PhCH_2$), 2.48–2.46 (m, 3 H, $PhCH_3$), 1.43–1.42 (m, 9 H, *t*Bu) ppm.

tert-Butyl 4-[2,2,2-trifluoro-1-(tosyloxyimino)ethyl]phenethylcarbamate (181 mg, 0.372 mmol) was dissolved in diethyl ether (5 mL). The ether solution was added to excess liquid ammonia at –78 °C in a pressure tube. The reaction mixture was warmed to room temp. and then it was stirred for 6 h at that temperature. The excess ammonia gas was removed in a draft chamber, and the residual solution was concentrated. The crude residue was purified by column chromatography (EtOAc/hexane, 1:2) to give **7** (108 mg, 88%) as a colorless amorphous solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.55 (d, J = 8.2 Hz, 2 H, Ar-H), 7.25 (d, J = 8.2 Hz, 2 H, Ar-H), 4.61 (br. s, 1 H, CH_2NH), 3.37 (q, J = 6.5 Hz, 2 H, CH_2N), 2.81 (m, 3 H, $PhCH_2$, CF_3CNH), 2.23 (d, J = 9.6 Hz, 1 H, CF_3CNH), 1.43 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 155.8, 141.4, 129.8, 129.2, 128.3, 123.5 (q, $^1J_{C,F}$ = 278.2 Hz), 79.4, 57.8 (q, $^2J_{C,F}$ = 35.8 Hz), 41.5, 36.0, 28.3 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = –75.54 ppm. HRMS (ESI): calcd. for $C_{15}H_{21}F_3N_3O_2$ 332.1586; found 332.1615.

***tert*-Butyl 4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]phenethylcarbamate (8):** Compound **7** (213 mg, 0.645 mmol) and activated MnO_2 (500 mg) were suspended in diethyl ether (15 mL). The reaction mixture was stirred at room temperature for 1 h, then the insoluble material was removed by filtration. The filtrate was concentrated, and the residue was purified by column chromatography (CH_2Cl_2) to give **8** (197 mg, 93%) as a colorless amorphous solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.23 (d, J = 8.2 Hz, 2 H, Ar-H), 7.13 (d, J = 8.2 Hz, 2 H, Ar-H), 4.52 (br. s, 1 H, CH_2NH), 3.36 (q, J = 6.7 Hz, 2 H, CH_2N), 2.80 (t, J = 6.7 Hz, 2 H, $PhCH_2$), 1.42 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 155.8, 141.0, 129.2, 127.1, 126.6, 122.1 (q, $^1J_{C,F}$ = 273.8 Hz), 79.2, 41.5, 35.9, 28.2 (q, $^2J_{C,F}$ = 40.2 Hz), 28.2 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = –65.31 ppm. HRMS (ESI): calcd. for $C_{15}H_{18}F_3N_3O_2Na$ 352.1249; found 352.1254.

2-{4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}ethanamine (9): TFA (250 μL) was added to compound **8** (159 mg, 0.482 mmol) at

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0 °C. The reaction mixture was stirred for 2 h at the same temperature, and then the TFA was removed on a rotary evaporator. The residue was dissolved in MeOH (1 mL) and NaOH (1 M; 1 mL), and the mixture was stirred for 1 h at room temperature. The reaction mixture was extracted with EtOAc (30 mL), and the extract was washed with brine, dried with $MgSO_4$, and evaporated to give **9** (105 mg, 95%) as a yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.22 (d, J = 8.6 Hz, 2 H, Ar-H), 7.12 (d, J = 8.6 Hz, 2 H, Ar-H), 2.94 (t, J = 6.8 Hz, 2 H, CH_2N), 2.74 (t, J = 6.8 Hz, 2 H, $PhCH_2$), 1.23 (br. s, 2 H, NH_2) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 141.7, 129.2, 126.9, 126.5, 122.1 (q, $^1J_{C,F}$ = 273.2 Hz), 43.0, 39.4, 28.2 (q, $^2J_{C,F}$ = 40.4 Hz) ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = –65.32 ppm. HRMS (ESI): calcd. for $C_{10}H_{11}F_3N_3$ 230.0905; found 230.0889.

***N*-(4-Benzoylphenethyl)acetamide (11):** Benzoyl chloride (0.862 g, 7.11 mmol) and *N*-acetyl-β-phenethylamine (**10**, 1.0 g, 6.13 mmol) were dissolved in dry nitrobenzene (3 mL), and $AlCl_3$ (1.63 g, 12.2 mmol) was added in small portions to the solution at 0 °C. The reaction mixture was heated at 90 °C for 7 h, then it was poured into HCl (conc.), and the mixture was concentrated. The residue was extracted with diethyl ether. The organic phase was washed with sodium hydroxide (10% aq.) and with water, dried with sodium sulfate, filtered and concentrated. The residue was subjected to silica gel chromatography ($CHCl_3$/MeOH, 20:1) to give **11** (1.08 g, 66%) as a pale yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.83–7.28 (m, 9 H, Ar-H), 5.48 (br. s, 1 H, NH), 3.56 (q, J = 6.6 Hz, 2 H, CH_2N), 2.92 (t, J = 6.6 Hz, 2 H, $PhCH_2$), 1.96 (s, 3 H, $COCH_3$) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 196.3, 170.1, 143.9, 137.6, 136.0, 132.4, 130.5, 129.9, 128.7, 128.3, 40.4, 35.7, 23.3 ppm. HRMS (ESI): calcd. for $C_{17}H_{18}NO_2$ 268.1338; found 268.1330.

***N*-(4-Benzoylphenethyl)-2,2,2-trifluoroacetamide (14):** TfOH (1 mL) was added to *N*-trifluoroacetyl-β-phenethylamine **13**[13] (299 mg, 1.38 mmol) and benzoic anhydride (624 mg, 2.76 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The reaction was quenched with ice/water/EtOAc. The organic phase was washed brine, and the solvents were evaporated. The crude product was purified by column chromatography (EtOAc/hexane, 1:4) to give **14** (382 mg, 86%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.75–7.17 (m, 10 H, Ar-H and NH), 3.64 (q, J = 6.7 Hz, 2 H, CH_2N), 2.98 (t, J = 7.3 Hz, 2 H, $PhCH_2$) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 196.56, 157.38 (q, $^2J_{C,F}$ = 37.1 Hz), 142.87, 137.34, 135.92, 132.45, 130.45, 129.79, 128.56, 128.23, 115.73 (q, $^1J_{C,F}$ = 288.1 Hz), 40.81, 34.89 ppm. HRMS (ESI): calcd. for $C_{17}H_{15}F_3NO_2$ 322.1055; found 322.1068.

[4-(2-Aminoethyl)phenyl](phenyl)methanone (12)

Method 1, from 11: Compound **11** (0.524 g, 1.96 mmol) was dissolved in HCl (6 M; 3.2 mL) and the mixture was heated at 120 °C for 7 h. The solution was extracted with EtOAc twice. The aqueous phase was basified with NaOH and extracted with EtOAc three times. The organic phase was concentrated to give **12** (0.194 mg, 44%) as a yellow oil.

Method 2, from 14: NaOH (1 M aq.; 4 mL) was added to **14** (264 mg, 0.821 mmol) in methanol (1 mL) at 0 °C, and the reaction mixture was stirred for 2 h at the same temperature. The compound was extracted with EtOAc, and washed with brine. The organic phase was evaporated to give **12** (177 mg, 95%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.83–7.29 (m, 9 H, Ar-H), 3.04 (br. s, 2 H, NH_2), 2.85 (t, J = 6.8 Hz, 2 H, CH_2N), 1.25 (d, J = 6.8 Hz, 2 H, $PhCH_2$) ppm. ^{13}C NMR (68 MHz, $CDCl_3$): δ = 196.4, 144.8, 137.7, 135.7, 132.3, 130.4, 129.9, 128.7, 128.2, 43.0, 39.8 ppm. HRMS (ESI): calcd. for $C_{15}H_{16}NO$ 226.1232; found 226.1215.

(3a*R*,4*S*,6*R*,6a*S*)-6-[6-Amino-2-(4-azidophenethylamino)-9*H*-purin-9-yl]-*N*-ethyl-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carboxamide (16)

Method 1, from 13: A mixture of **5** (98.1 mg, 0.604 mmol), **15** (45.9 mg, 0.119 mmol), and DIPEA (166 μL, 0.956 mmol) in EtOH (2 mL) was stirred at 110 °C for 3 d. The solvent was removed, and the crude product was purified by column chromatography (EtOAc to EtOAc/MeOH, 10:1) to give **16** (9.9 mg, 16%) as a white amorphous solid.

Method 2, from 18: Compound **18** (75.4 mg, 0.138 mmol), NaN_3 (22.4 mg, 0.345 mmol), sodium ascorbate (3.5 mg, 0.017 mmol), CuI (6.7 mg, 0.035 mmol), and *N*,*N*′-dimethylethylenediamine (5.5 μL, 0.051 mmol) in EtOH (1.4 mL) and H_2O (0.6 mL) were stirred at 100 °C for 6 h. The reaction mixture was poured into ice/water (15 mL) and extracted with EtOAc (30 mL). The organic phase was washed with brine and dried with $MgSO_4$, and the solvents were evaporated. The crude product was purified by column chromatography (EtOAc to EtOAc/MeOH, 10:1) to give **16** (34.4 mg, 49%) as a colorless amorphous solid. ^{1}H NMR (270 MHz, CD_3OD): δ = 7.88 (s, 1 H, 8-H), 7.34 (d, J = 8.2 Hz, 2 H, Ar-H), 6.99 (d, J = 8.2 Hz, 2 H, Ar-H), 6.24 (s, 1 H, 1′-H), 5.74 (d, J = 6.3 Hz, 1 H, 2′-H), 5.58 (d, J = 6.3 Hz, 1 H, 3′-H), 4.63 (s, 1 H, 4′-H), 3.78–3.62 (m, 1 H, NCH_2CH_2Ph), 3.57–3.42 (m, 1 H, NCH_2CH_2Ph), 3.00–2.70 (m, 4 H, CH_2Ph, CH_2CH_3), 1.58 (s, 3 H, CH_3), 1.38 (s, 3 H, CH_3), 0.61 (t, J = 7.4 Hz, 3 H, CH_2CH_3) ppm. ^{13}C NMR (68 MHz, CD_3OD): δ = 171.6, 160.8, 157.4, 152.4, 139.5, 139.2, 138.3, 131.4, 119.9, 114.5, 114.3, 92.5, 89.3, 85.5, 85.0, 43.8, 36.1, 34.8, 27.0, 25.4, 14.0 ppm. HRMS (ESI): calcd. for $C_{23}H_{29}N_{10}O_4$ 509.2373; found 509.2357.

(3a*R*,4*S*,6*R*,6a*S*)-6-[6-Amino-2-(4-benzoylphenethylamino)-9*H*-purin-9-yl]-*N*-ethyl-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carboxamide (17): A mixture of **12** (114 mg, 0.51 mmol), **15** (62.8 mg, 0.163 mmol), and DIPEA (284 μL, 1.63 mmol) in EtOH (2 mL) was stirred at 120 °C for 3 d. The solvent was removed, and the crude product was purified by column chromatography (EtOAc to EtOAc/MeOH, 10:1) to give **15** (34.1 mg, 37%) as a colorless amorphous solid. ^{1}H NMR (270 MHz, CD_3OD): δ = 7.84 (s, 1 H, 8-H), 7.76–7.40 (m, 9 H, Ar-H), 6.21 (s, 1 H, 1′-H), 5.72 (d, J = 5.6 Hz, 1 H, 2′-H), 5.53 (d, J = 5.6 Hz, 1 H, 3′-H), 4.60 (s, 1 H, 4′-H), 3.84–3.70 (m, 1 H, NCH_2 CH_2Ph), 3.62–3.48 (m, 1 H, NCH_2 CH_2Ph), 3.02 (t, J = 7.3 Hz, 2 H, CH_2CH_3), 2.94–2.68 (m, 2 H, NCH_2CH_2Ph), 1.54 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3), 0.59 (t, J = 7.3 Hz, 3 H, CH_2CH_3) ppm. ^{13}C NMR (68 MHz, CD_3OD): δ = 198.4, 171.6, 160.8, 157.4, 152.4, 147.1, 139.6, 139.1, 136.6, 133.6, 131.3, 130.9, 130.1, 129.5, 114.5, 114.3, 92.5, 89.3, 85.5, 85.0, 43.4, 36.9, 34.78, 27.0, 25.4, 14.0 ppm. HRMS (ESI): calcd. for $C_{30}H_{34}N_7O_5$ 572.2621; found 572.2646.

(3a*R*,4*S*,6*R*,6a*S*)-6-[6-Amino-2-(4-bromophenethylamino)-9*H*-purin-9-yl]-*N*-ethyl-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carboxamide (18): A mixture of **2** (141 μL, 0.993 mmol), **15** (70.7 mg, 0.184 mmol), and DIPEA (320 μL, 1.84 mmol) in EtOH (2 mL) was stirred at 130 °C for 3 d. The solvent was removed, and the crude product was purified by column chromatography (EtOAc to EtOAc/MeOH, 10:1) to give **18** (74.1 mg, 74%) as a colorless amorphous solid. ^{1}H NMR (270 MHz, CD_3OD): δ = 7.90 (s, 1 H, 8-H), 7.45 (d, J = 8.6 Hz, 2 H, Ar-H), 7.26 (d, J = 8.6 Hz, 2 H, Ar-H), 6.27 (s, 1 H, 1′-H), 5.76 (d, J = 6.3 Hz, 1 H, 2′-H), 5.61 (d, J = 6.3 Hz, 1 H, 3′-H), 4.66 (s, 1 H, 4′-H), 3.80–3.70 (m, 1 H, NCH_2 CH_2Ph), 3.58–3.48 (m, 1 H, NCH_2CH_2Ph), 2.88 (m, 4 H,

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CH_2Ph, CH_2CH_3), 1.61 (s, 3 H, CH_3), 1.40 (s, 3 H, CH_3), 0.64 (t, J = 7.3 Hz, 3 H, CH_2CH_3) ppm. ^{13}C NMR (68 MHz, CD_3OD): δ = 168.8, 159.3, 155.8, 151.1, 138.4, 137.0, 131.4, 130.5, 112.0, 114.1, 113.5, 91.2, 87.8, 83.8, 83.3, 42.4, 35.0, 33.7, 26.6, 24.8, 14.1 ppm. HRMS (ESI): calcd. for $C_{23}H_{29}BrN_7O_4$ 546.1464 and 548.1444; found 546.1464 and 548.1456.

(2*S*,3*R*,4*S*,5*R*)-5-[6-Amino-2-(4-azidophenethylamino)-9*H*-purin-9-yl]-*N*-ethyl-3,4-dihydroxytetrahydrofuran-2-carboxamide (19): A solution of **16** (33.8 mg, 0.066 mmol) in HCl (1 M; 10 mL) and MeCN (2 mL) was stirred for 3 h at 40 °C. The solution was cooled to 0 °C, basified with $NaHCO_3$ (satd. aq.), and extracted with EtOAc. The organic phase was washed with brine, dried with $MgSO_4$, and evaporated to give **19** (26.5 mg, 86%) as a colorless amorphous solid. ^1H NMR (270 MHz, CD_3OD): δ = 8.00 (s, 1 H, 8-H), 7.25 (d, J = 8.2 Hz, 2 H, Ar-H), 6.96 (d, J = 8.2 Hz, 2 H, Ar-H), 5.94 (d, J = 6.3 Hz, 1 H, 1′-H), 4.99 (t, J = 5.9 Hz, 1 H, 2′-H), 4.53–4.48 (m, 1 H, 3′-H), 4.41 (d, J = 2.6 Hz, 1 H, 4′-H), 3.67–3.39 (m, 2 H, NCH_2 CH_2Ph), 3.29–3.05 (m, 2 H, CH_2Ph), 2.86 (t, J = 7.1 Hz, 2 H, CH_2CH_3), 1.03 (t, J = 7.1 Hz, 3 H, CH_2CH_3) ppm. ^{13}C NMR (68 MHz, CD_3OD): δ = 172.0, 161.1, 157.5, 153.0, 139.2, 139.0, 138.2, 131.4, 120.0, 114.7, 90.0, 85.4, 74.7, 73.4, 44.2, 36.3, 35.1, 14.7 ppm. HRMS (ESI): calcd. for $C_{20}H_{25}N_{10}O_4$ 469.2060; found 469.2065.

(2*S*,3*R*,4*S*,5*R*)-5-[6-Amino-2-(4-benzoylphenethylamino)-9*H*-purin-9-yl]-*N*-ethyl-3,4-dihydroxytetrahydrofuran-2-carboxamide (20): A solution of **17** (24.9 mg, 0.0435 mmol) in HCl (1 M; 10 mL) and MeCN (3 mL) was stirred for 3 h at 40 °C. The solution was cooled to 0 °C, basified with $NaHCO_3$ (satd. aq.), and extracted with EtOAc. The organic phase was washed with brine, dried with $MgSO_4$, and evaporated to give **20** (20.6 mg, 89%) as a white amorphous solid. ^1H NMR (270 MHz, CD_3OD): δ = 8.03 (s, 1 H, 8-H), 7.74–7.38 (m, 9 H, Ar-H), 5.95 (d, J = 6.6 Hz, 1 H, 1′-H), 4.88 (t, J = 5.9 Hz, 1 H, 2′-H), 4.51–4.45 (m, 1 H, 3′-H), 4.41 (d, J = 3.0 Hz, 1 H, 4′-H), 3.79–3.53 (m, 2 H, NCH_2 CH_2Ph), 3.27–3.10 (m, 2 H, CH_2Ph), 3.00 (t, J = 7.6 Hz, 2 H, CH_2CH_3), 1.04 (t, J = 7.6 Hz, 3 H, CH_2CH_3) ppm. ^{13}C NMR (68 MHz, CD_3OD): δ = 198.5, 172.1, 146.8, 139.5, 139.0, 136.7, 133.7, 131.4, 131.0, 130.1, 129.5, 90.1, 85.4, 79.5, 74.7, 73.9, 43.8, 37.0, 35.3, 32.8, 23.7, 14.7, 14.4 ppm. HRMS (ESI): calcd. for $C_{27}H_{30}N_7O_5$ 532.2308; found 532.2324.

Ligand Binding Assays for Adenosine A_{2A} Receptor (A_{2A}AR): Compounds **17** and **18** were subjected to ligand binding assays with purified human A_{2A}AR, which had been expressed in *Pichia pastoris*,[7] using radioligands of the antagonist [^3H]-ZM241385 and the agonist [^3H]-NECA as described previously.[10] Briefly, GF/F glass filters were pre-soaked with polyethyleneamine (0.3%). The binding experiments were carried out as single-point binding measurements in duplicate using radioligand (20 nM) in Hepes (20 mM; pH 7.0) containing NaCl (100 mM) at 25 °C for 30 min. The incubation was terminated by the addition of Tris-HCl (pH 7.4; 20 mM; 2 mL), and the mixture was rapidly filtered through the GF/F filters. The filters were then washed three times with the above buffer (5 mL).

Supporting Information (see footnote on the first page of this article): ^1H and ^{13}C NMR spectra.

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