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

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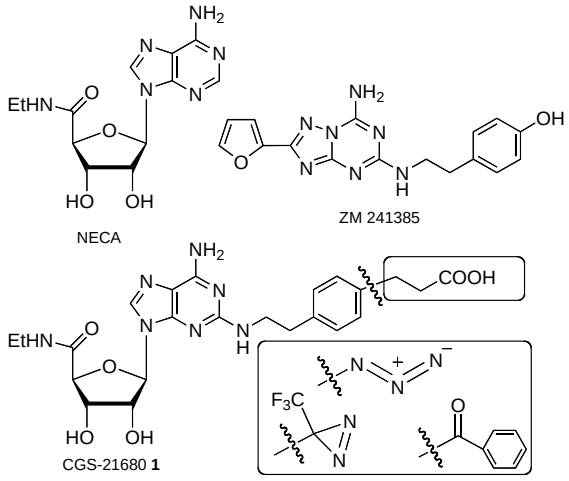
Comprehensive synthesis of photoreactive 2-phenylethylamine derivatives, which are well known as a mother skeleton for many bioactive compounds, to elucidate the biological functional analysis. The preparation promoted to make adenosine receptors ligands, which have many functional roles in biology and have been extensively studied for their many roles in maintaining homeostasis. Adenosine is one of the commonest biochemical compounds, but photoaffinity labeling methodologies have not yet been used to study adenosine receptors. Synthetic methods for producing photoreactive adenosine derivatives active at adenosine receptors were established for several photophores, phenylazide and benzophenone. The effect of substitution with photoreactive components was determined using an adenosine receptor assay.

Introduction

2-Phenylethylamine (2-PEA) skeletons are contributed for many biological active natural products, especially as neurotransmitters or neuromodulators in central nervous systems.[1] Biological functional analysis of 2-PEAs are given much attention in the pharmaceutical fields. Photoaffinity labeling is a method used in the study of the interactions of low molecular weight bioactive compounds with biomolecules.[2] It is suitable for the analysis of biological interactions because it is based on the affinity of the bioactive compound for biomolecules. But few photoreactive modifications of 2-PEA with azide[3] and benzophenone[4] have been reported previously. It has not been reported yet for trifluoromethyldiazirinyl derivative of 2-PEA.

2-PEA was also utilized as a partial structure for 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 nonselective adenosine receptor agonists (namely adenosine and *N*-ethyladenosine-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, crystal structure studies with 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, which binds with the constitutively active receptors, stabilize them, and thus reduce the activity[8], for A_{2A} receptors[5], and has hypotensive activity in vivo. CGS-21680 and ZM241385 have 2-PEA substituents at the 2-position of adenine. Broad acceptability has been observed for substitutions at the *p*-position of the 2-PEA moiety.[9] The chemical substitutions at the *p*-position of 2-PEA may be utilized for the introduction of photophores to the ligand skeleton. Appropriate selection of photophores for photoaffinity labeling is critical to obtain satisfactory results, but there is no universal choice for the best selection of photophore.[2e]



Scheme 1. Structures of adenosine receptor ligands and designs of photoreactive CGS-21680 derivatives. NECA (a nonselective agonist), ZM241385 (an A_{2A} selective antagonist), and CGS-21680 (**1**, an A_{2A} selective inverse agonist). Carboxyethyl group of CGS-21680 was substituted by photoreactive groups in this study.

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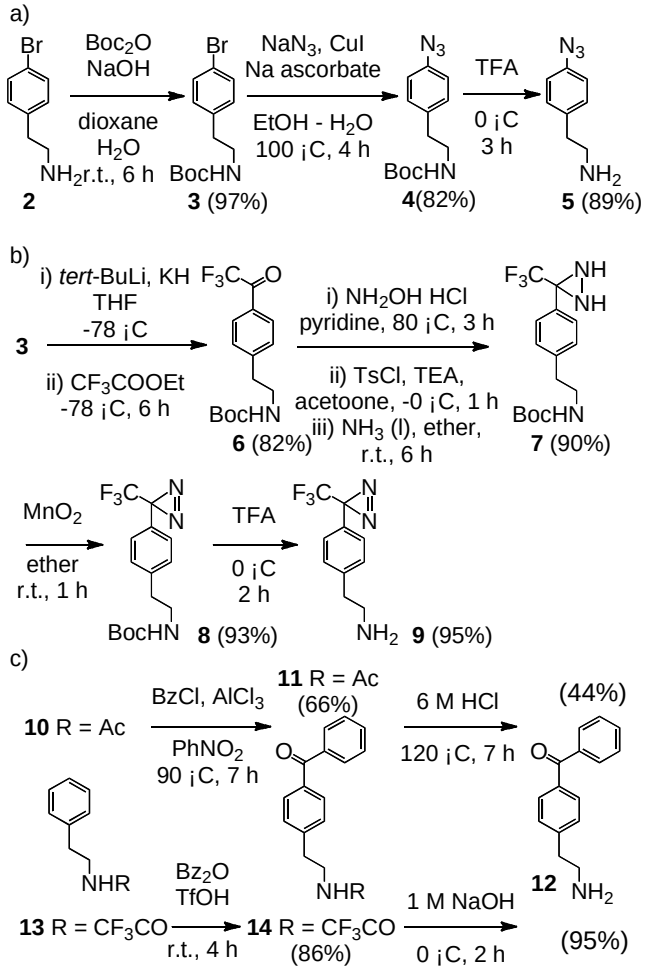
In this study, synthesis of photoreactive 2-PEA derivatives with various photophores, phenylazides, benzophenones and trifluoromethylphenyldiazirines and their introduction to NECA skeleton as photoreactive CGS-21680 derivatives (Scheme 1) are reported, as well as the results of assays for determining their biological activities on the purified human adenosine A_{2A} receptor ($A_{2A}R$), expressed in *Pichia pastoris*.[10]

Results and Discussion

Our synthetic methodologies are based on the constructions of photophores on 2-PEA derivatives. 2-(4-Bromophenyl)ethylamine (**2**) was selected as starting material because its Boc protected derivative (**3**) was common precursor for both phenylazide and trifluoromethyldiazirine. The compound 3 was subjected to substitution of bromide with the azide moiety (**4**) in a Cu(I)-catalyzed reaction. Yields were influenced by the selsction of ligands. Proline-NaOH system[11] did not consume the haloarene completely. On the other hand, *N*, *N*'-dimethylethylenediamine-sodium ascorbate system[12] afforded the desired product **4** effectively. Acidic treatment to remove the Boc group produced the phenylazide derivative (**5**) in a moderate yield (Scheme 2a). The overall yields for the preparation of **5** is identical to a sole previous report, which started from 2-(4-aminophenyl)ethylamine with diazotization-azidation.[5]



Scheme 2. Synthesis of photoreactive phenylethylamine derivatives. a) phenylazide, b) trifluoromethylphenyldiazirine, and c) benzophenone.

For trifluoromethylphenyldiazirine photophore, the compound **3** was subjected to trifluoroacetylation with CF_3COOEt in the presence of *tert*-BuLi and KH to produce **6**. Trifluoroacetyl moiety was converted to trifluoromethyldiazirinyl moiety **8** according to a general method.[13] Deprotection with TFA afforded the desired product **9** with a moderate yield (Scheme 2b).

The benzophenone derivative was synthesized from the corresponding *N*-acetyl phenylethylamine derivatives (**10**).[4] Friedel-Crafts benzoylation with aluminum chloride at 90 °C for 7 h (**11**) was used, followed by deprotection of the acetyl moiety under acidic conditions to produce the benzophenone derivatives (**12**) in low yield (less than 30% for 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 using TfOH as catalyst and solvent.[15] Following by alkaline deprotection of trifluoroacetyl group afforded **12** in good yield (up to 82% for two steps) (Scheme 2c).

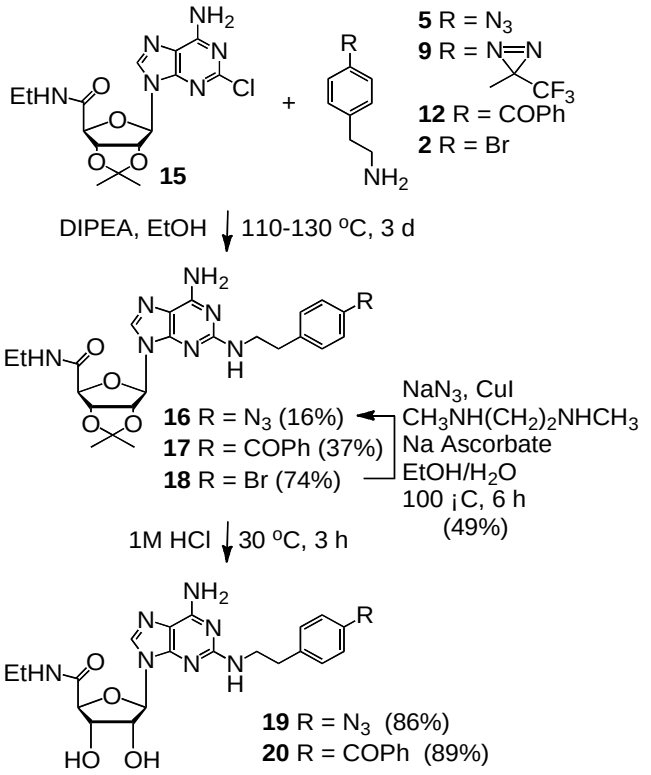
Retrosynthesis of the photoreactive CGS-21680 skeleton was designed to employ condensation reactions involving 2-chloro *N*-ethyladenosine-5'-uronamide derivatives and photoreactive phenylethylamine derivatives in order to construct 2-position-substituted adenosine derivatives.[9a, 16]

The photoreactive 2-PEA derivatives were subjected to condensation with the adenosine derivative, 2-Cl-5'-ethyl carboxamide-2', 3'-ketal adenosine **15**. The original methods, which heated the reaction mixtures over 130 °C in ethanol, were applied for the condensations.[9a] Detailed studies revealed that the trifluoromethylphenyl diazirinyl compound **9** was not tolerated under the high-temperature conditions. The ethyl moiety was always observed in ^{1}H-NMR with identical integrations. ^{19}F-NMR of 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 trifluoromethyldiazirine moiety was broken down during the reaction. Several precursors of the diazirine precursor (de-Boc **6** and **7**) were subjected to condensation but no desired reactions were observed.

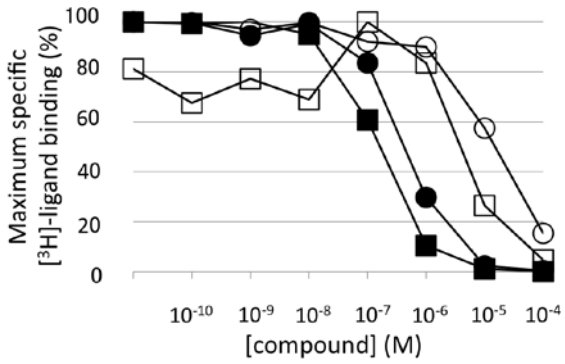
No decomposition of the other photophores (phenyl azide (**5**) and benzophenone (**12**)) was observed during the condensations. The reactions were very slow (3 d) and care was taken regarding evaporation of the solvent during the course of the reaction. Detailed studies revealed that two equivalents of the phenylethylamine derivatives in the presence of a large excess (20 equivalents) of diisopropylethyl amine were required to maintain the nucleophilicity of the phenylethylamine. A temperature of 110 °C was enough to promote the reaction to produce 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 condensation temperature of **12** and **15**. The reaction required several days to complete. The starting materials **15** and photophore **12** were not consumed completely, but no more significant reaction was observed after three days. After the work-up, the remaining starting materials **12** and **15** could be re-subjected to the reaction in the same conditions to afford the products at the same yield. No improvements were observed by using the equivalent tertiary amine.

The azide derivative **16** was prepared in another way. *p*-Bromophenylethylamine (**2**) was condensed with adenosine derivative **15** to afford **18**, 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 afford photoreactive adenosine derivatives **19** and **20** (Scheme 3).



Scheme 3. Synthesis of photoreactive CGS-21680 derivatives.



Scheme 4. Competitive inhibition assay for the 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**).

The synthesized photoreactive CGS 21680 derivatives were subjected to competitive binding assays[17] with an agonist or antagonist at the purified $A_{2A}R$.[10] Competitive inhibition assays with agonist ([^{3}H]-NECA) revealed that the affinities of both synthetic compounds (**19** and **20**) were in the order of less than 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 enough activities for functional analysis of $A_{2A}Rs$. The modifications did not cause a significant decrease in the affinity of the derivatives (Scheme 4).

Conclusions

We have developed comprehensive synthesis of 2-PEA derivatives containing three photophores for photoaffinity labeling. These derivatives were subjected to the condensations with 2-Cl adenosine derivatives to elucidate functional analysis of adenosine receptors. Preliminary experiments for the photoreactive ligand binding assays indicated they have enough activities to elucidate 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

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

***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 dioxan (25 mL) and H_2O (25 mL), and cooled to 0 °C. Di-*tert*-butyl dicarbonate (2.28 g, 10.532 mmol) in dioxan (12 mL) was added dropwise to the reaction. The reaction was stirred at room temperature for 6 hours, and evaporated. The crude compound was purified by column chromatography (CH_2Cl_2/hexane, 1/4 to CH_2Cl_2) to yield **3** (2.05 g, 97%) as 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 hours at 100 °C. The reaction mixture was poured into ice water, the organic compound was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The crude product was purified by column chromatography (CH_2Cl_2/hexane, 1:3 to CH_2Cl_2) to yield **4** (291 mg, 82%) as 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 (brs, 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. HR-ESIMS: 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 was stirred for 3 hours at the same temperature. The reaction was basified with 1 M NaOH and stirred for 10 minutes, extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to yield (**5**) (62.0 mg, 89%) as 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 (brs, 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. HR-ESIMS: 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 (276 mg, 30 % suspension in mineral oil, 2.04 mmol) in THF (10 mL) at 0 °C under N_2. The reaction was cooled to -78 °C and a solution of *tert*-BuLi (2.6 mL of 1.7 M pentane solution, 416 mmol) was added dropwise over a period of 10 minutes. Ethyl trifluoroacetate (1.2 mL, 10.3 mmol) was added and the mixture was stirred for 6 hours at the same temperature, then sat. ammonium chloride (5 mL) was added to the reaction. The reaction mixture was extracted with diethyl ether. The organic layer was washed with brine,

dried over $MgSO_4$, and evaporated. The crude product was purified by column chromatography (AcOEt /hexane, 1:3) to yield **6** (531 mg, 82 %) as 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 (brs, 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, $^2J_{CF}$ = 34.6 Hz), 155.8, 147.9, 130.3, 129.5, 128.1, 116.6 (q, $^1J_{CF}$ = 291.1 Hz), 79.3, 41.1, 36.4, 28.2 ppm. ^{19}F-NMR (470 MHz, $CDCl_3$): δ = -71.34 ppm. HR-ESIMS: 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 hours. After pyridine was removed on rotary evaporator, the residue was dissolved in diethyl ether and washed with 1 M HCl, brine, dried over $MgSO_4$, and evaporated. The crude product was purified by column chromatography (CH_2Cl_2 to AcOEt/hexane, 1:2) to yield *tert*-butyl 4-(2,2,2-trifluoro-1-(hydroxyimino)ethyl)phenethyl-carbamate (506 mg, 90 %) as 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 (brs, 1 H, NH), 3.40 (brs, 2 H, CH_2N), 2.82 (brs, 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, $^2J_{CF}$ = 32.2 Hz), 141.4, 141.0, 128.9 (2C), 128.8, 128.5, 124.6, 120.9 (q, $^1J_{CF}$ = 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 to the reaction at the same temperature, and stirred for 1 hour. After evaporation, the crude residue was purified by column chromatography (CH_2Cl_2 to AcOEt/Hex 1/3) to yield *tert-butyl* 4-(2,2,2-trifluoro-1-(tosyloxyimino)ethyl)phenethylcarbamate (220 mg, 84 %) as colorless amorphous solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.90 (d, J = 8.2 Hz, 2H, Ar-H), 7.43-7.22 (m, 6H, Ar-H), 4.62 (brs, 1H), 3.43-3.33 (m, 2H, CH_2N), 2.88-2.80 (m, 2H, $PhCH_2$), 2.48-2.46 (m, 3H, $PhCH_3$), 1.43-1.42 (m, 9H, *t*Bu) ppm. *tert*-Butyl 4-(2,2,2-trifluoro-1-(tosyloxyimino)ethyl)phenethyl-carbamate (181 mg, 0.372 mmol) was dissolved in diethyl ether (5 mL). In shield tube, liquid ammonia was added at -78 °C and the ether solution was added. The reaction mixture was warmed to rt then stirred for 6 h at same temperature. After excess ammonium gas was removed in draft chamber, the residual solution was concentrated. The crude residue was purified by column chromatography (AcOEt/hexane, 1:2) to yield **7** (108 mg, 88 %) as 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 (brs, 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, $^1J_{CF}$ = 278.2 Hz), 79.4, 57.8 (q, $^2J_{CF}$ = 35.8 Hz), 41.5, 36.0, 28.3 ppm. ^{19}F-NMR (470 MHz, $CDCl_3$): δ = -75.54 ppm. HR-ESIMS: 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):** The 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 hour, then filtrated insoluble material. The filtrate was concentrated, and the residue was purified by column chromatography (CH_2Cl_2) to yield compound **8** (197 mg, 93%) as colorless amorphous solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.23 (2d, J = 8.2 Hz, 2 H, Ar-H), 7.13 (d, J = 8.2 Hz, 2 H, Ar-H), 4.52 (brs, 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, $^1J_{CF}$ = 273.8 Hz), 79.2, 41.5, 35.9, 28.2 (q, $^2J_{CF}$ = 40.2 Hz), 28.2 ppm. ^{19}F-NMR (470 MHz, $CDCl_3$): δ = -65.31 ppm. HR-ESIMS: 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 the compound **8** (159 mg, 0.482 mmol) at 0 °C. The reaction mixture was stirred for 2 hours at the same temperature, and TFA was removed on rotary evaporator. The residue was dissolved in MeOH (1 mL) and 1 M NaOH (1 mL), and stirred for 1 hour at room temperature. The reaction mixture was extracted with AcOEt (30 mL), and washed with brine, dried over $MgSO_4$, and evaporated to yield **9** (105 mg, 95%) as 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 (brs, 2 H, NH_2) ppm. ^{13}C-NMR (68 MHz, $CDCl_3$): δ = 141.7, 129.2, 126.9, 126.5, 122.1 (q, $^1J_{CF}$ = 273.2 Hz), 43.0, 39.4, 28.2 (q, $^2J_{CF}$ = 40.4 Hz) ppm. ^{19}F-NMR (470 MHz, $CDCl_3$): δ = -65.32 ppm. HR-ESIMS: calcd for $C_{10}H_{11}F_3N_3$ 230.0905; found 230.0889.

***N*-(4-Benzoylphenethyl)acetamide (11):** Benzoyl chloride (0.862g, 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 hours, poured into concentrated hydrochloric acid and concentrated. The residue was extracted with ether. The organic phase was washed with 10 % sodium hydroxide and with water, dried over sodium sulfate, filtrated and concentrated. The residue was subjected to silica gel chromatography ($CHCl_3$/MeOH, 20:1) to yield **11** (1.08 g, 66%) as pale yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.83-7.28 (m, 9 H, Ar-H), 5.48 (brs, 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. HR-ESIMS: 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 ℃. The reaction mixture was warmed to room temperature and stirred for 4 h. The reaction was quenched with ice water/AcOEt. The organic layer was washed brine, and evaporated. The crude product was purified by column chromatography (AcOEt/hexane, 1:4) to yield **14** (382 mg, 86%) as colorless oil.

^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.75-7.17 (10H, m, 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, $^2J_{CF}$ = 37.1 Hz), 142.87, 137.34, 135.92, 132.45, 130.45, 129.79, 128.56, 128.23, 115.73 (q, $^1J_{CF}$ = 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): (From **11**) The compound **11** (0.524g, 1.96 mmol) was dissolved in 6 M HCl (3.2 mL) and heated at 120 °C for 7 h. The solution was extracted with AcOEt twice. The aqueous layer was basified with NaOH and extracted with AcOEt three times. The organic layer was concentrated to afford **12** (0.194 mg, 44%) as yellow oil. (From **14**) Aqueous solution of NaOH (1 M, 4 mL) were added to 14 (264 mg, 0.821 mmol) in methanol (1 mL) at 0 ℃, and the reaction mixture was stirred for 2 h at the same temperature. The compound was extracted with AcOEt, and washed with brine. The organic layer was evaporated to yield **12** (177 mg, 95 %) as colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.83-7.29 (m, 9 H, Ar-H), 3.04 (brs, 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. HR-ESIMS: 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): (From **13**) The reaction 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 days. The solvent was removed, and the crude product was purified by column chromatography (AcOEt to AcOEt/MeOH, 10:1) to yield **16** (9.9 mg, 16%) as white amorphous solid. (From **18**) The 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 hours. The reaction mixture was poured into ice water (15 mL) and extracted with AcOEt (30 mL). The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The crude product was purified by column chromatography (AcOEt to AcOEt/MeOH, 10:1) to yield **16** (34.4 mg, 49 %) as 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, NC*H*$_2$CH$_2$Ph), 3.57-3.42 (m, 1 H, NC*H*$_2$CH$_2$Ph), 3.00-2.70 (m, 4 H, CH$_2$Ph & C*H*$_2$CH$_3$), 1.58 (s, 3 H, CH$_3$), 1.38 (s, 3 H, CH$_3$), 0.61 (t, *J* = 7.4 Hz, 3 H, CH$_2$C*H*$_3$) 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. HR-ESIMS: 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): The reaction 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 days. The solvent was removed, and the crude product was purified by column chromatography (AcOEt to AcOEt/MeOH, 10:1) to yield **15** (34.1 mg, 37%) as 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, NC*H*$_2$ CH$_2$Ph), 3.62-3.48 (m, 1 H, NC*H*$_2$ CH$_2$Ph), 3.02 (t, *J* = 7.3 Hz, 2 H, C*H*$_2$CH$_3$), 2.94-2.68 (m, 2 H, NCH$_2$C*H*$_2$Ph), 1.54 (s, 3 H, CH$_3$), 1.34 (s, 3 H, CH$_3$), 0.59 (t, *J* = 7.3 Hz, 3 H, CH$_2$C*H*$_3$) 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. HR-ESIMS: 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): The reaction 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 days. The solvent was removed, and the crude product was purified by column chromatography (AcOEt to AcOEt/MeOH, 10:1) to yield **18** (74.1 mg, 74%) as 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, NC*H*$_2$ CH$_2$Ph), 3.58-3.48 (m, 1 H, NC*H*$_2$CH$_2$Ph), 2.88 (m, 4 H, CH$_2$Ph & C*H*$_2$CH$_3$), 1.61 (s, 3 H, CH$_3$), 1.40 (s, 3 H, CH$_3$), 0.64 (t, *J* = 7.3 Hz, 3 H, CH$_2$C*H*$_3$) 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. HR-ESIMS: 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 1 M HCl (10 mL) and MeCN (2 mL) was stirred for 3 hours at 40 °C. The solution was cooled to 0 °C, basified with sat. $NaHCO_3$, and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to yield **19** (26.5 mg, 86%) as colorless amorphous solid. ^{1}H 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, NC*H*$_2$ CH$_2$Ph), 3.29-3.05 (m, 2 H, CH$_2$Ph), 2.86 (t, *J* = 7.1 Hz, 2 H, C*H*$_2$CH$_3$), 1.03 (t, *J* = 7.1 Hz, 3 H, CH$_2$C*H*$_3$) 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. HR-ESIMS: 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 1 M HCl (10 mL) and MeCN (3 mL) was stirred for 3 hours at 40 °C. The solution was cooled to 0 °C, basified with sat. $NaHCO_3$, and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to yield **20** (20.6 mg, 89%) as white amorphous solid. ^{1}H 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, NC*H*$_2$ CH$_2$Ph), 3.27-3.10 (m, 2 H, CH$_2$Ph), 3.00 (t, *J* = 7.6 Hz, 2 H, C*H*$_2$CH$_3$), 1.04 (t, *J* = 7.6 Hz, 3 H, CH$_2$C*H*$_3$) 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. HR-ESIMS: 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): The synthetic compounds **17** and **18** were subjected to ligand binding assays to purified human A_{2A}AR, which was expressed in *Pichia pastoris*,[7] using radioligands of the antagonist [^{3}H]-ZM241385 and the agonist [^{3}H]-NECA as described previously.[10] Briefly, GF/F glass filters were presoaked with 0.3% polyethyleneamine. The binding experiments were carried out as single point binding measurements in duplicate using 20 nM radioligand in 20 mM Hepes (pH 7.0) containing 100 mM NaCl at 25 °C for 30 min. The incubation was terminated by 2 mL of 20 mM Tris-HCl pH 7.4), and the mixture was rapidly filtered through the GF/F filters. The filters were then washed three times with 5 mL of the above buffer.

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

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