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<td><strong>Author(s)</strong></td>
<td>Murai, Yuta; Masuda, Katsuyoshi; Ogasawara, Yui; Wang, Lei; Hashidoko, Yasuyuki; Hatanaka, Yasumaru; Iwata, So; Kobayashi, Takuya; Hashimoto, Makoto</td>
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Comprehensive synthesis of photoreactive 2-phenethylamine 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-Phenethylamine (2-PEA) skeletons are contributed for many biological active natural products, especially as neurotransmitters or neuromodulators in central nervous systems.[3] 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 (A1, A2A, A2B, and A3),[3] are G protein-coupled receptors. In the brain, A2A receptors are expressed at high densities in the striatum. Descriptions of the A2A receptors were established for several photophores, 3-[4-[2-[(6-amino-9-[(2-furyl)-[1,2,4]triazolo-[2,3-a][1,3,5]triazin-5-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 A2A 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 α-position of the 2-PEA moiety.[9] The chemical substitutions at the α-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 A2A selective antagonist), and CGS-21680 (1, an A2A selective inverse agonist). Carboxyethyl group of CGS-21680 was substituted by photoreactive groups in this study.
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 A2A receptor (A2A-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 trifluoromethylaziridine. 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 selection of ligands. Proline-NaOH system11 did not consume the haloarene completely. On the other hand, N, N’-dimethylmethyleneediaminesodium ascorbate system12 afforded the desired product 4 effectively. Acidic treatment to remove the Boc group produced sodium ascorbate system [12] afforded the desired product, completely. On the other hand, N, N’-dimethylmethyleneediaminesodium ascorbate system12 afforded the desired product 4 effectively. Acidic treatment to remove the Boc group produced sodium ascorbate system [12] afforded the desired product, completely. On the other hand, N, N’-dimethylmethyleneediaminesodium ascorbate system12 afforded the desired product 4 effectively. Acidic treatment to remove the Boc group produced sodium ascorbate system [12] afforded the desired product, completely. On the other hand, N, N’-dimethylmethyleneediaminesodium ascorbate system12 afforded the desired product 4 effectively. Acidic treatment to remove the Boc group produced sodium ascorbate system [12] afforded the desired product, completely. On the other hand, N, N’-dimethylmethyleneediaminesodium ascorbate system12 afforded the desired product 4 effectively. Acidic treatment to remove the Boc group produced sodium ascorbate system [12] afforded the desired product, completely. On the other hand, N, N’-dimethylmethyleneediaminesodium ascorbate system12 afforded the desired product 4 effectively. Acidic treatment to remove the Boc group produced sodium ascorbate system [12] afforded the desired product, completely. On the other hand, N, N’-dimethylmethyleneediaminesodium ascorbate system12 afforded the desired product 4 effectively. Acidic treatment to remove the Boc group produced sodium ascorbate system [12] afforded the desired product, completely. On the other hand, N, N’-dimethylmethyleneediaminesodium ascorbate system12 afforded the desired product 4 effectively. Acidic treatment to remove the Boc group produced sodium ascorbate system [12] afforded the desired product, completely.

The overall yields for the preparation of a) phenylazide, b) trifluoromethylphenyldiazirine, and c) benzophenone. For trifluoromethylphenyldiazirine photophore, the compound 3 was subjected to trifluoroacetylation with CF3COOEt in the presence of tert-PrLi 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).14 Friedel-Crafts benzylation 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)15 was treated with benzoic anhydride in trifluoromethanesulfonic acid (TIOH) at room temperature. The Friedel-Crafts benzylation was improved using TIOH as catalyst and solvent15. 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',5'-uronamide 15. The original methods, which heated the reaction mixtures over 130 °C in ethanol, were applied for the condensations.9c Detailed studies revealed that the trifluoromethylphenyldiazirinyl compound 9 was not tolerated under the high-temperature conditions. The ethyl moiety was always observed in 1H-NMR with identical integrations.19F-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 disopropylethyl 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(1), NN'-

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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 photoactive adenosine derivatives 19 and 20 (Scheme 3).

The synthesized photoactive CGS 21680 derivatives were subjected to competitive binding assays\[17\] with an agonist or antagonist at the purified A2A R. Competitive inhibition assays with an agonist or antagonist at the purified A2A R.\[10\] Competitive inhibition assays indicated they have enough activities to elucidate further analysis by photoaffinity labeling of the A2A R. Further functional analysis of A2A R with these synthetic photoaffinity reagents is underway.

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 photoactivatable ligand binding assays indicated they have enough activities to elucidate further analysis by photoaffinity labeling of the A2A R. Further functional analysis of A2A R with these synthetic photoaffinity 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 H2O (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 (CH3Cl-hexane, 1:4 to CH3Cl) 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-azidophenethylcarbamate (3, 405 mg, 1.35 mmol), NaN3 (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 H2O (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 MgSO4, and evaporated. The crude product was purified by column chromatography (CH3Cl-hexane, 1:3 to CH3Cl) to yield 4 (291 mg, 82%) as colorless amorphous mass. 1H NMR (270 MHz, CDCl3): 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, CH2N), 2.76 (t, J = 6.9 Hz, 2 H, PhCH2), 1.43 (s, 9 H, tBu) ppm. 13C-NMR (68 MHz, CDCl3): δ = 155.7, 138.1, 135.7, 130.0, 119.0, 79.1, 41.7, 35.5, 28.3 ppm. HR-ESIMS: calcd for C14H19N4 243.1490; found 243.1489.

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 MgSO4, and evaporated to yield 5 (62.0 mg, 89%) as colorless oil. 1H NMR (270 MHz, CDCl3): 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, CH2N), 2.73 (t, J = 6.8 Hz, 2 H, PhCH2), 1.39 (brs, 2 H, NH) ppm. 13C-NMR (68 MHz, CDCl3): δ = 137.9, 136.6, 131.0, 119.0, 43.4, 39.2 ppm. HR-ESIMS: calcd for C10H10N2O2Na 258.0837; found 258.0835.
dried over MgSO₄, and evaporated. The crude product was purified by column chromatography (AcOEt/hexane, 1:3) to yield 6 (531 mg, 82 %) as colorless amorphous solid. 1H NMR (270 MHz, CDCl₃, δ): 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, CH₂N), 2.91 (t, J = 6.8 Hz, 2 H, PhCH₂), 1.42 (s, 9 H, Bu) ppm. 13C-NMR (68 MHz, CDCl₃, δ): δ = 179.9 (q, J_CF₂ = 34.6 Hz), 155.8, 147.9, 130.3, 129.8, 124.5, 120.9 (q, J_CF₂ = 32.2 Hz), 79.3, 41.1, 36.4, 28.2 ppm. 19F-NMR (470 MHz, CDCl₃, δ): δ = -71.34 ppm. HR-ESIMS: calc for C₁₅H₁₈F₃N₃O₂Na: 352.1317; found 352.1326.

2-(4-(3-(Trifluoromethyl)-3H-diazirin-3-yl)phenethylamino)-9H-purin-9-yl)-ethyl-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carboxamide (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 °C, 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 dryness. The crude product was purified by column chromatography (AcOEt/hexane, 1:3) to yield 12 (177 mg, 95 %) as colorless oil. 1H NMR (270 MHz, CDCl₃, δ): δ = 7.83-7.29 (m, 9 H, Ar-H), 3.64 (q, J = 6.7 Hz, 2 H, CH₂N), 2.98 (t, J = 7.3 Hz, 2 H, PhCH₂) ppm. 13C-NMR (68 MHz, CDCl₃, δ): δ = 156.8, 141.4, 129.8, 128.4, 123.3, 123.3 (q, J_CF₂ = 287.8 Hz), 79.4, 57.8 (q, J_CF₂ = 35.8 Hz), 41.5, 36.0, 28.3 ppm. 19F-NMR (470 MHz, CDCl₃, δ): δ = -75.54 ppm. HR-ESIMS: calc for C₁₅H₁₂F₂N₂O₂Na: 332.1586; found 332.1615.

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and N,N'-dimethylisourea (5.5 µl, 0.051 mmol) in EtOH (1.4 mL) and H₂O (0.6 mL) were stirred at 100 °C for 4 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 MgSO₄, and evaporated. The crude product was purified by column chromatography (AcOEt to AcOEt/MeOH, 0.5:1) to yield 16 (34.4 mg, 49 %) as colorless amorphous solid. ¹H NMR (270 MHz, CDOD): 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, NCH₂CH₂Ph), 3.57-3.42 (m, 1 H, NCH₂CH₂Ph), 3.00-2.70 (m, 4 H, CH₂Ph & CH₂CH₃), 1.58 (s, 3 H, CH₃), 1.38 (s, 3 H, CH₃), 0.61 (t, J = 7.4 Hz, 3 H, CH₂CH₃) ppm. ¹³C-NMR (68 MHz, CDOD): δ = 171.6, 160.8, 157.4, 152.4, 139.9, 132.1, 133.4, 119.1, 114.5, 91.3, 89.3, 85.5, 85.0, 43.8, 36.1, 34.8, 27.0, 25.4, 14.0 ppm. HR-ESIMS: calculated for C₂₇H₃₀N₇O₅ 570.2210; found 570.2210.

(3R,3R,3R,5R)-6-(4-Amino-2-(4-benzoylphenethylamino)-9H-purin-9-yl)-N-ethyl-2,2-dimethyltetrahydrofuran-3,4-diol (18): 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. NaHCO₃, and extracted with AcOEt. The organic layer was washed with brine, dried over MgSO₄, and evaporated to yield 18 (20.6 mg, 89%) as white amorphous solid. ¹H NMR (270 MHz, CDOD): 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, NCH₂CH₂Ph), 3.27-3.10 (m, 2 H, CH₂Ph), 3.00 (t, J = 7.6 Hz, 2 H, CH₂CH₃), 1.04 (t, J = 7.6 Hz, 3 H, CH₂CH₃) ppm. ¹³C-NMR (68 MHz, CDOD): δ = 198.5, 172.1, 146.8, 139.5, 139.0, 136.7, 133.7, 131.4, 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: calculated for C₂₇H₂₉NO₅ 472.2308; found 472.2324.

Ligand binding assays for Adenosine A₂A receptor (A₂AAR): The synthetic compounds 17 and 18 were subjected to ligand binding assays to purified human A₂AAR, which was expressed in Pichia pastoris, as previously described. Briefly, GF/F glass filters were preoasched with 0.3% polyethyleneglycol. 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): ¹H- and ¹³C- NMR spectra.

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