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Keywords: Phenylazide / Benzophenone / Phenylidiazirine / Phenethylamine / Adenosine receptors / 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 receptor 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.[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 trifluoromethylidiazirinyl 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),[5] are G protein-coupled receptors. In the brain, A2A receptors are expressed at high densities in the striatum. Descriptions of the A3),[5] are G protein-coupled receptors. In the brain, A2A receptors have been cloned and categorized into four subtypes (A1, A2A, A2B, and A3),[5] 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, phenylazide and benzophenone. The effect of substitution with photoreactive components was determined using an adenosine receptor assay.

3-4-[2-[6-Amino-9-[2(R, 3R, 4S, 5S)-5-ethylcarbamoyl]-3,4-dihydroxy-oxolan-2-yl]purin-2-yl]amine]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 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]

Recently been published. However, crystal structure studies with agonists are not yet complete.

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 (I, 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, benzenones 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 (A2AR), 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)ethylamime (2) was selected as starting material because its Boc protected derivative (3) was common precursor for both phenylazide and trifluoromethylphenyldiazirine. 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 system[11] did not consume the haloarene derivative (4) in a moderate yield (Scheme 2a).

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 low 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 under the high-temperature conditions. The ethyl moiety was observed after three days. After the work-up, the remaining starting materials were not consumed completely, but no more significant reaction was observed after three days. After the work-up, the remaining starting materials were not consumed completely, but no more significant reaction was observed.

The benzophenone derivative was synthesized from the corresponding N-acetyl phenylethylamine derivatives (10).[4] 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)[14] was treated with benzoic anhydride in trifluoromethanesulfonic acid (TfOH) at room temperature. The Friedel-Crafts benzylation was improved using TfOH as catalyst and solvent[15]. Following by alkaline deprotection of trifluoroaeryl group afforded 12 in good yield (up to 82% for two steps) (Scheme 2c).

Retro-synthesis 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'-ketadenosine 15. The original methods, which heated the reaction mixtures over 130 °C in ethanol, were applied for the condensations.[9b] Detailed studies revealed that the trifluoromethylphenyl diazirinyl 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 diazirane 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. 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-Bromophenyl ethylamine (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'-

For trifluoromethylphenyldiazirine photophore, the compound 3 was subjected to trifluoroacetylation with CF3COEt in the presence of tert-BuLi and KH to produce 6. Trifluoroacetyl moiety was converted to trifluoromethyl diazirinyl 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 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)[14] was treated with benzoic anhydride in trifluoromethanesulfonic acid (TfOH) at room temperature. The Friedel-Crafts benzylation was improved using TfOH as catalyst and solvent[15]. Following by alkaline deprotection of trifluoromethyl group afforded 12 in good yield (up to 82% for two steps) (Scheme 2c).

Retro-synthesis 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]
dimethylethlenediamine 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).

The synthesized photoreactive CGS 21680 derivatives were subjected to competitive binding assays[17] with an agonist or antagonist at the purified A2AR.[10] Competitive inhibition assays with agonist ([3H]-NECA) revealed that the affinities of both derivatives (19 and 20) were in the order of less than 1 µM, which is sufficient to elucidate the biological function of the A2AR. Inhibition assays with an A2AR-specific antagonist ([3H]-ZM24138) suggested that the synthetic photoreactive compounds had enough activities for functional analysis of A2ARs. 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 A2AR. Further functional analysis of A2AR 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 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 product was purified by column chromatography (CH2Cl2-hexane, 1:4 to CH2Cl2) 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), 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 (CH2Cl2-hexane, 1:3 to CH2Cl2) 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 C13H18N4O2Na 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 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, NH2) ppm. 13C-NMR (68 MHz, CDCl3): δ = 137.9, 136.6, 131.0, 119.0, 43.4, 39.3 ppm. HR-ESIMS: calcd for C12H17N3O2Na 285.1327; found 285.1350.
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 = 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, (CH₂)₃) ppm. ¹³C-NMR (68 MHz, CDCl₃): δ = 157.4, 147.9, 130.3, 128.1, 125.8, 116.6 (q, JCF = 291.1 Hz), 79.3, 41.1, 36.4, 28.2 ppm. ¹⁹F-NMR (470 MHz, CDCl₃): δ = -71.34 ppm. HR-ESIMS: calcd for C₁₁H₁₂F₂N₂O₂Na 352.1249; found 352.1254.

2-(4-(3-(Trifluoromethyl)-3H-diazirin-3-yl)phenethyl)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 MgSO₄, and evaporated to yield 9 (105 mg, 95%) as yellow oil. ¹H NMR (270 MHz, CDCl₃): 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, CH₂N), 2.74 (t, J = 6.8 Hz, 2 H, PhCH₂) ppm. ¹³C-NMR (68 MHz, CDCl₃): δ = 141.7, 129.2, 126.9, 126.5, 122.1 (q, JCF = 273.2 Hz) 43.0, 39.4, 28.2 (q, JCF = 40.4 Hz) ppm. ¹⁹F-NMR (470 MHz, CDCl₃): δ = -65.32 ppm. HR-ESIMS: calcd for C₁₁H₁₂F₂N₂O₂Na 352.1254; found 352.1254.

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and N,V'-dimethylenediamine (5.5 μl, 0.051 mmol) in EtOH (1.4 mL) and H2O (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 MgSO4, 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. 1H NMR (270 MHz, CD2OD): 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), 3.73-3.82 (m, 1 H, NCH2CH2Ph), 3.57-3.42 (m, 1 H, NCH2CH2Ph), 3.00-2.70 (m, 4 H, CH2Ph & CH2CH2), 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, CD3OD): δ = 171.6, 160.8, 157.4, 152.4, 139.9, 132.2, 138.3, 134.1, 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: calculated for C27H30N7O5 509.2373; found 509.2357. HR-ESIMS: calculated for C27H30N7O5 509.2373; found 509.2357.

(2S,3R,5S)-2-(4-Amino-2-(4-benzoylphenethylamino)-9H-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. NaHCO3, and extracted with AcOEt. The organic layer was washed with brine, dried over MgSO4 and evaporated to yield 20 (20.6 mg, 89%) as white amorphous solid. 1H NMR (270 MHz, CD2OD): 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), 4.51-4.44 (m, 1 H, 3’-H), 4.1 (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, CH2CH2), 2.7 (t, J = 7.6 Hz, 3 H, CH2CH3) ppm. 13C-NMR (68 MHz, CD2OD): δ = 198.5, 172.1, 146.8, 139.5, 139.0, 136.7, 133.7, 131.4, 131.0, 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 C24H29N7O7 532.2308; found 532.2334.

**Ligand binding assays for Adenosine A2A receptor (A2A-R):** The synthetic compounds 17 and 18 were subjected to ligand binding assays to purified human A2A-R, which was expressed in *Pichia pastoris,* using radioligands of the antagonist [H3]-ZM241385 and the agonist [H7]-NECA as described previously.19 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): H1- and H3-C- NMR spectra.

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**References**


