SI

Supporting Information

Discovery of Highly Potent and Selective Biphenyl Acylsulfonamide-based β_3-Adrenergic Receptor Agonists and Evaluation of Physical Properties as Potential Overactive Bladder Therapies: Part V

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SII

Biological Materials and Methods.

In vitro Experiments.

(1) Cell culture

We used stably transfected Chinese Hamster Ovary (CHO) cells expressing recombinant human β_1-, β_2-, β_3-ARs and recombinant canine β_3-AR. CHO cells were seeded 2 days before the assays in 96-well plates at a density of 1 to 1.3X10^4 cell/well.

(2) cAMP accumulation assay

CHO cells grown to confluence were washed twice with assay buffer [130 mM NaCl, 5 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$ 1.5mM $CaCl_2 \cdot 2H_2O$, 10 mM Glucose, 10 mM Glucose, 10 mM HEPES, 0.1% bovine serum albumin, pH7.4] and incubated with 180 µL of assay buffer containing 0.5 mM 3-isobutyl-methylxanthine (IBMX) at 37°C for 10 min. Test compound (20 µL) dissolved in assay buffer containing 1% DMSO was then added and cells incubated at 37°C for 15 min, the reaction was stopped by addition of 80 µL of 0.1 mol/L HCl. After 1 hr at 4°C, cells were centrifuged at 2000 rpm for 5 min at 4°C. The amount of cAMP in the supernatant was determined using a cAMP enzymeimmunoassay (EIA) kit (Amersham Biosciences). The supernatant was frozen below -80°C until the measurement of cAMP levels.

(3) Data analysis

cAMP acumulation elicited by test compounds were expressed as a percentage of the maximal response to isoproterenol. Fifty percent effective concentration (EC_{50}) values were calculated using GraphPad Prism (Ver.3.03) from the concentration response curve.

In vivo Experiments.

(1) Materials and Methods

Female beagle dogs (11.5 – 15.0 kg, Oriental Yeast Co., Ltd.) were deprived of food and water from about 40 h and 17 h before administration, respectively. A group of 5 dogs were used for the whole study. Under halothane anesthesia, a catheter was inserted into the urinary bladder through the urethra and connected to a pressure transducer to measure intravesical pressure (IVP). Carbachol (1.8µg/0.2 mL/kg, saline solution) was given intravenously (via the saphenous vein) several times at intervals of about 30 min. A polyethylene tube for intraduodenal (i.d.) administration was inserted into the duodenum at about 15 cm from the pylorus using an endoscope. When reproducible responses (increase in IVP) were obtained, test compound (10, 32 and 100 or 3.2, 10 and 32 µg /kg) was given intraduodenally. Thirty minutes after test compound administration, carbachol injection was restarted at intervals of about 30 min. Vehicle (polyethylene glycol #400, 0.2 mL/kg) was given at the same point as test compound administration. Responses to carbachol were observed for 180 min.

(2) Data analysis

Percent inhibition of IVP increased by test compound was calculated by dividing IVPa (IVP increase induced by carbachol after test compound administration). by IVPb (IVP increase induced by carbachol just before test compound administration). In the vehicle administration group, inhibitory effect was calculated in the same way. The data were analyzed using Dunnett's multiple comparison test compared to the vehicle group. ED_{50} values were calculated by least-square linear regression analysis using maximum percent inhibition at each dose. All values were expressed as mean ± S.E.

SIII

Combustion Analysis Data (C,H,N):

Compound	Calcd.	Found.
6a	**C, 59.46; H, 5.79; N, 5.55**	**C, 59.54; H, 5.87; N, 5.28**
6b	**C, 60.83; H, 6.24; N, 5.26**	**C, 60.81; H, 6.13; N, 5.11**
6c	**C, 62.07; H, 6.65; N, 4.99**	**C, 61.83; H, 6.85; N, 4.99**
6d	**C, 61.90; H, 6.34; N, 4.98**	**C, 61.91; H, 6.23; N, 4.91**
6e	**C, 62.87; H, 6.51; N, 4.89**	**C, 62.80; H, 6.57; N, 4.81**
6f	**C, 58.01; H, 6.00; N, 4.51**	**C, 57.60; H, 5.74; N, 4.10**
6g	**C, 63.02; H, 6.72; N, 4.74**	**C, 62.89; H, 6.57; N, 4.69**
6h	**C, 55.29; H, 6.36; N, 7.16**	**C, 55.11; H, 6.06; N, 7.34**
6i	**C, 55.89; H, 6.72; N, 6.52**	**C, 55.46; H, 6.62; N, 6.57**
6j	**C, 67.99; H, 6.93; N, 5.60**	**C, 67.85; H, 6.81; N, 5.45**
6k	**C, 63.71; H, 6.53; N, 5.12**	**C, 63.86; H, 6.47; N, 5.06**
6l	**C, 64.67; H, 6.69; N, 5.03**	**C, 64.36; H, 6.61; N, 4.96**
6m	**C, 64.18; H, 6.95; N, 4.83**	**C, 64.33; H, 6.89; N, 4.68**
6n	**C, 59.05; H, 6.06; N, 5.10**	**C, 58.64; H, 6.00; N, 5.03**
6o	**C, 59.05; H, 6.06; N, 5.10**	**C, 58.87; H, 5.94; N, 5.13**
6p	**C, 61.15; H, 6.33; N, 4.75**	**C, 60.60; H, 6.23; N, 4.72**
7b	**C, 67.99; H, 6.93; N, 5.60**	**C, 67.51; H, 6.49; N, 5.36**
7e	**C, 54.94; H, 6.30; N, 6.63**	**C, 54.78; H, 6.39; N, 6.63**
7h	**C, 48.50; H, 6.14; N, 8.70**	**C, 48.49; H, 6.11; N, 8.25**
7i	**C, 51.34; H, 6.33; N, 8.26**	**C, 51.30; H, 6.51; N, 7.98**
7j	**C, 53.64; H, 6.50; N, 6.95**	**C, 53.22; H, 6.40; N, 6.03**
7k	**C, 54.54; H, 6.38; N, 6.81**	**C, 54.51; H, 6.14; N, 6.50**
7o	**C, 50.45; H, 5.96; N, 6.79**	**C, 50.451; H, 6.04; N, 6.55**
8b	**C, 53.50; H, 6.20; N, 6.93**	**C, 53.50; H, 6.34; N, 6.65**
8e	**C, 55.45; H, 6.48; N, 6.47**	**C, 55.36; H, 6.24; N, 6.41**
9q	**C, 54.69; H, 6.54; N, 10.20**	**C, 54.94; H, 6.35; N, 10.09**
9b	**C, 55.64; H, 6.72; N, 9.98**	**C, 55.40; H, 6.45; N, 9.90**
9e	**C, 51.25; H, 6.53; N, 8.24**	**C, 51.28; H, 6.22; N, 8.24**
9o	**C, 51.94; H, 6.20; N, 9.32**	**C, 51.54; H, 6.00; N, 9.11**