JPET Fast Forward. Published on February 9, 2007 as DOI: 10.1124/jpet.106.115840

JPET #115840

Effect of YM178, a Novel Selective β_3-Adrenoceptor Agonist, on Bladder Function

Toshiyuki Takasu, Masashi Ukai, Shuichi Sato, Tetsuo Matsui, Itsuro Nagase, Tatsuya Maruyama, Masao Sasamata, Keiji Miyata , Hisashi Uchida and Osamu Yamaguchi

Institute for Drug Discovery Research, Astellas Pharma Inc., Ibaraki, Japan (T.T., M.U., S.S., T.M., I.N., T.M., M.S., K.M.); and Department of Urology, Fukushima Medical University, Fukushima, Japan (H.U., O.M.)

JPET #115840

Running title: Beta$_3$ adrenoceptor and the lower urinary tract

Corresponding author: Toshiyuki Takasu

Pharmacology Research Laboratories, Astellas Pharma Inc.

21 Miyukigaoka, Tsukuba, Ibaraki 305-8585 Japan

Phone: 81-29-863-6650

FAX: 81-29-852-5391

E-mail: toshiyuki.takasu@jp.astellas.com

Number of text pages: 15

Number of tables: 3

Number of figures: 5

Number of references: 28

Number of words in the Abstract: 205

Number of words in the Introduction: 338

Number of words in the Discussion: 1251

Abbreviations list: β-AR, β-adrenoceptor; OAB, overactive bladder; YM178, (R)-2-(2-aminothiazol-4-yl)-4'-{2-[(2-hydroxy-2-phenylethyl)amino]ethyl} acetanilide; cAMP, cyclic AMP; AC, adenylate cyclase; CHO, Chinese Hamster Ovary; EC_{50}, half-maximal effective concentration; CCh, carbachol; mRNA, messenger RNA; BSA, bovine serum albumin ; IBMX, isobutylmethyl-xanthine; HBSS, Hank's Balanced Salt Solution.

JPET Fast Forward. Published on February 9, 2007 as DOI: 10.1124/jpet.106.115840
This article has not been copyedited and formatted. The final version may differ from this version.

Section assignment: Gastrointestinal, Hepatic, Pulmonary, & Renal

Abstract

We evaluated the pharmacological characteristics of (R)-2-(2-aminothiazol-4-yl)-4'-{2-[(2-hydroxy-2-phenylethyl)amino]ethyl} acetanilide (YM178). YM178 increased cyclic AMP accumulation in Chinese Hamster Ovary (CHO) cells expressing human β_3-adrenoceptor (β_3-AR). The half-maximal effective concentration (EC_{50}) value was 22.4 nM. EC_{50} values of YM178 for human β_1- and β_2-ARs were 10,000 nM or more, respectively. The ratio of intrinsic activities of YM178 versus maximal response induced by isoproterenol (nonselective β-AR agonist) was 0.8 for human β_3-ARs, 0.1 for human β_1-ARs and 0.1 for human β_2-ARs. The relaxant effect of YM178 was evaluated in rats and humans bladder strips pre-contracted with carbachol (CCh), and compared with those of isoproterenol and CGP-12177A (β_3-AR agonist). EC_{50} values of YM178 and isoproterenol in rat bladder strips pre-contracted with 10^{-6} M CCh were 5.1 μM and 1.4 μM, respectively, while those in human bladder strips pre-contracted with 10^{-7} M CCh were 0.78 μM and 0.28 μM, respectively. In *in vivo* study, YM178 at a dose of 3 mg/kg i.v. decreased the frequency of rhythmic bladder contraction induced by intravesical filling with saline without suppressing its amplitude in anesthetized rats. These findings suggest the suitability of YM178 as a therapeutic drug for the treatment of symptoms of overactive bladder (OAB) such as urinary frequency, urgency and urge incontinence.

Introduction

The β_3-adrenoceptor (β_3-AR) is one of three β-AR subtypes, termed β_1- to β_3-AR. β_3-AR is a G-protein-coupled receptor identified by genomic cloning of human cells in the late 1980s (Emorine et al., 1989) which is sparsely distributed in humans. Functional β_3-AR-mediated responses have been observed in human brown and white fat cells, where they mediate lipolysis; and in gall bladder, stomach, small intestine, prostate, colon (Berkowitz et al., 1995), bladder, where they evoke relaxation (Fujimura et al., 1999). Although more than 15 years have passed since β_3-AR was identified, the therapeutic potential of β_3-AR agonists in humans remains unclear. BRL37344, CL316,243, and CGP-12177A are representative β_3-AR agonists that were optimized using rodent β-ARs (Arch et al., 1984; Dolan et al., 1994; Langin et al., 1991). These compounds have lower potency for human β_3-ARs than for rodent receptors, however, and act as only partial agonists in humans (Igawa et al., 1999, 2001). Subsequent recognition of important pharmacological differences between rodent and human β_3-ARs has led to the development of novel β_3-AR agonists that are potent and highly selective towards human β_3-AR (Hu and Jennings, 2003).

Although β-ARs play an important role in bladder relaxation in mammals, considerable functional interspecies differences among β-AR subtypes have been identified. In human bladder smooth muscle, β_3-AR messenger RNA (mRNA) expression is predominant, with this subtype accounting for 97% of total β-AR mRNA (Yamaguchi, 2002; Nomiya and Yamaguchi, 2003). In accordance with expression levels, human bladder relaxation is mainly induced through β_3-AR and not β_1- or β_2-ARs (Yamazaki et al., 1998; Takeda et al.,

1999; Igawa et al., 1998, 1999). Recently, it was shown that β_3-AR agonists can improve bladder overactivity in rat experimental models (Kaidoh et al., 2002; Woods et al., 2001), suggesting the usefulness of β_3-AR agonists in the treatment of overactive bladder (OAB). (R)-2-(2-aminothiazol-4-yl)-4'-{2-[(2-hydroxy-2-phenylethyl) amino]ethyl} acetanilide (YM178, Fig 1) was synthesized by Astellas Pharma Inc. Here, we report for the first time the pharmacological profile of YM178 and its effects on bladder smooth muscle, as investigated *in vitro* and *in vivo*.

Materials and methods

Materials

YM178 was synthesized at Astellas Pharma Inc. (Tokyo Japan). (-)-Isoproterenol, CL316,243, BRL37344, (±)-CGP-12177A, oxybutynin chloride, isobutylmethyl-xanthine (IBMX), and bovine serum albumin (BSA) were obtained from Sigma Chemical Co (St Louis, MO, USA). YM178, isoproterenol, CL316,243, BRL37344, and CGP-12177A were dissolved in 100% dimethyl sulfoxide and diluted with assay buffer. Chinese hamster ovary (CHO) cells expressing human β_1- and β_2- ARs were purchased from Dr. Lefkowitz at Duke University Medical Center (Durham NC, USA). CHO cells were from the American Type Culture Collection (Rockville, MD, USA). Lipofectin, G418 sulfate, Ham's F-12 medium, penicillin/streptomycin 100 units/100 µg/ml, and Hank's Balanced Salt Solution (HBSS) were from Invitrogen Co. (Carlsbad, CA, USA). Fetal bovine serum was from Bioserum (Parkville, VIC, Australia). Trypsin-EDTA was from the Research Institute for Microbial Diseases, Osaka University (Osaka, Japan). HEPES sodium salt was from Wako (Osaka, Japan). [^{125}I] cyclic AMP (cAMP) assay system was from Yamasa Shouyu Co., Ltd. (Chiba, Japan).

Cell culture

CHO cells expressing human β_3-AR were constructed by transfecting cDNA of human β_3-AR into CHO cells by the Lipofectin method. Stable transfectants were selected with 600 µg/ml G418 sulfate. CHO cells expressing each type of human β-AR were cultured at 37 °C

in a humidified atmosphere with 5% CO_2 in Ham's F-12 medium, supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin, 0.5 mg/ml G418 sulfate and 10% v/v fetal bovine serum. The cells were sub-cultured weekly, with the supernatant aspirated and trypsin-EDTA added for 10 min to detach the cells from the culture dish, followed by the addition of fresh medium and transfer of the cells to new Petri dishes.

cAMP accumulation

10^5 cells were seeded in each well of a 24-well culture plate and sub-cultured. Three days later, the medium was exchanged with 250 µl/well of HBSS containing 0.1 mM IBMX, pH 7.4. The cells were incubated with each compound (isoproterenol, YM178, BRL37344 and CL316,243 at final concentrations of 10^{-10} to 10^{-4} M) for 10 min at 37 °C, after which incubation was stopped by the addition of 250 µl of 0.2 M HCl. cAMP concentration in the reaction mixture was measured by radioimmunoassay using an [^{125}I] cAMP assay system utilizing a γ-counter (ALOKA, Tokyo, Japan). Fifty microliters of reaction mixture was incubated with 50 µl succinyl agent for 10 min at room temperature, after which the reaction was stopped by the addition of 400 µl buffer solution. Fifty microliters of succinylated sample was incubated with 50 µl [^{125}I] cAMP and 50 µl anti cAMP antibody for 24 h at 4 °C. At the end of the incubation period, 250 µl charcoal suspension was added and centrifuged for 10 min at 2800 x *g* at 4 °C. Two hundred and fifty microliters of supernatant was transferred into a tube and counted for 1 min using a γ-counter. The intrinsic activity (I.A.) relative to

isoproterenol for each β-adrenoceptor agonist was calculated using the maximal response of each compound.

Animals

Male (350 - 400 g) and female (225-290 g) Wistar rats were purchased from Charles River Japan, Inc. (Kanagawa, Japan) and for *in vitro* and *in vivo* study, respectively.

Relaxant activity in isolated rat bladder smooth muscle

After anesthesis with diethyl ether, rats were sacrificed and the whole bladder was removed. Bladder strips (approximately 3 × 10 mm) were prepared and suspended under a loading tension of 1 g in Krebs-Henseleit solution (NaCl, 118.4 mM; KCl, 4.7 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 2.5 mM; $NaHCO_3$, 25.0 mM; glucose, 11.1 mM), and oxygenated with a gas mixture of 95% oxygen and 5% carbon dioxide at 37 °C. Contractile response was measured with an isotonic transducer (TB-611T; Nihon Kohden, Tokyo, Japan) and registered on an ink-writing recorder (SR6211, SR6221; Graphtec, Tokyo, Japan). After stabilization for 30 to 60 min, 10^{-6} M carbachol (CCh) was added to induce repeated contractile responses at 30- to 60-min intervals. After the response to CCh had almost equalized, the strips were then washed and the contractile response to 10^{-6} M CCh was recorded again. After the contractile response had stabilized, a test compound or vehicle was added cumulatively at approximately 10-min intervals in the concentration range of 10^{-9}

JPET Fast Forward. Published on February 9, 2007 as DOI: 10.1124/jpet.106.115840
This article has not been copyedited and formatted. The final version may differ from this version.

to 10^{-4} M, and the relaxant effect was recorded. At the end of each experiment, 10^{-4} M papaverine was added to obtain the maximal relaxant response, which was considered a 100% response and used to calculate the percentage relaxation for each compound (n = 5).

Relaxant activity in isolated human bladder smooth muscle

This study was conducted at Fukushima Medical University in six male patients (mean age of 69.5 ± 1.8 years; range 64 to 75) with normal bladders undergoing radical cystectomy for malignancy. Bladder muscle strips from the anterior portion of the bladder dome obtained from these patients at surgery were placed immediately in pre-oxygenated Krebs-Henseleit solution at 4 °C and transported to the laboratory. Each strip (approximately 3 × 10 mm) was prepared and suspended under a loading tension of 1 g in Krebs-Henseleit solution oxygenated with a gas mixture of 95% oxygen and 5% carbon dioxide at 37 °C. Contractile response was measured with an isotonic transducer (TB-621T; Nihon Kohden) and registered on an ink-writing recorder. After stabilization for 60 min, 10^{-7} M CCh was added at 60-min intervals to induce a repeated contractile response. After the responsive to CCh had almost equalized, each strip was washed and the contractile response to 10^{-7} M CCh was recorded again. After the contractile response had stabilized, test compound or vehicle was added cumulatively at approximately 10-min intervals in the concentration range of 10^{-9} to 10^{-4} M, and the relaxant effect was recorded. At the end of each experiment, 10^{-4} M papaverine was added to obtain the maximal relaxant response,

which was considered a 100% response and used to calculate the percentage relaxation for each compound (n= 4 - 6).

Rhythmic isovolumetric reflex bladder contraction

Rats were anesthetized with urethane (1 g/kg i.p.) and a flank incision was made. Both ureters were tied and cut at the side of the kidney. A midline abdominal incision was made and a polyethylene cannula (PE-50) was inserted into the bladder through the urethra and ligated around the urethra. Urine in the bladder was removed through the cannula by gently pressing on the abdomen. The bladder cannula was connected to a pressure transducer (TP-400T; Nihon Kohden). At least 10 min after the operation, physiological saline at room-temperature was infused into the bladder through the cannula at 2.4 ml/hr, and the saline infusion was terminated after initiation of spontaneous rhythmic bladder contractions. At least 30 min after the rhythmic bladder contraction stabilized, drug was intravenously administered at escalating doses in a volume of 1 ml/kg through a cannula (PE-50) inserted into the left femoral artery. Rats were excluded if the rhythmic bladder contraction did not stabilize or was repeatedly stopped by saline administration. The frequency and amplitude of the rhythmic bladder contractions were evaluated for 10 min (minute 5 to 15 after dosing). For each parameter, the saline administration value was taken as the pretreatment value.

YM178 was dissolved in saline containing 10% dimethylacetamide and 5% cremophor® EL (Nacalai Tesque, Kyoto, Japan) and oxybutynin chloride was dissolved in saline. A

saline solution containing 10% dimethylacetamide and 5% cremophor® EL was used as a vehicle control. Subsequent dilutions of all drugs and vehicle were prepared in saline. The free form doses of 0.03, 0.1, 0.3, 1 and 3 mg/kg for YM178 and 0.0272, 0.0907, 0.272, 0.907 and 2.72 mg/kg for oxybutynin were used in this study.

Statistical analysis

Results are expressed as the mean ± S.E.M. or mean with 95% confidence intervals. EC_{50} values were calculated by non-linear regression analysis. Statistical analysis was performed using the student's *t*-test. Statistical significance was defined as a *P* value less than 0.05. All data analyses were performed using SAS statistical software (SAS Institute; Cary, NC, USA).

Ethical considerations

The animal experiments were performed in compliance with the International Guiding Principles for Biomedical Research Involving Animals. The protocol for this study was approved by the Animal Ethics Committee of Astellas Pharma Inc. The human bladder muscle study was approved by the Ethics Committee of Fukushima Medical University.

Results

cAMP accumulation in CHO cells expressing human β-ARs

YM178 concentration-dependently increased the accumulation of cAMP in CHO cells expressing human β_3-ARs (Fig. 2C), with an EC_{50} value and I.A. of 22.4 nM and 0.8, respectively (Table 1). BRL37344 and CL316,243 also concentration-dependently increased the accumulation of cAMP in these cells (Fig. 2C), with EC_{50} values of 457 nM and 4,430 nM and I.A. of 0.6 and 0.5, respectively (Table 1). YM178 and CL316,243 had little agonistic effect on β_1- and β_2-ARs (Table 1, Fig. 2A, 2B). In contrast, BRL37344 activated β_1- and β_2-ARs, with EC_{50} values of 12,900 nM and 360 nM and I.A. of 0.5 and 0.7, respectively (Table 1). YM178 did not induce cAMP elevation in untransfected CHO cells (data not shown).

Relaxant effects of YM178, isoproterenol, and CGP-12177A in rat bladder strips pre-contracted with CCh

Both YM178 and isoproterenol concentration-dependently relaxed rat bladder smooth muscle strips pre-contracted with 10^{-6} M CCh with EC_{50} values of 5.1 μM and 1.4 μM, respectively (Table 2, Fig. 3). Compared by EC_{50} value, YM178 was approximately one-third as potent as isoproterenol. The maximal relaxant effects of YM178 and isoproterenol were 94.0 ± 1.0% and 78.0 ± 1.5%, respectively, that of CCh, indicating that YM178 acts a full agonist in the rat bladder (Table 2). In contrast, CGP-12177A relaxed this

contraction by only 19.4 ± 1.2% at the highest concentration of 10^{-4} M, so the EC_{50} value for this compound could not be determined (Table 2).

Relaxant effects of YM178, isoproterenol, and CGP-12177A in human bladder strips pre-contracted with CCh

Both YM178 and isoproterenol concentration-dependently relaxed human bladder smooth muscle strips pre-contracted with 10^{-7} M CCh with EC_{50} values of 0.78 μM and 0.28 μM, respectively (Table 3, Fig 4). The maximal relaxant effects of YM178 and isoproterenol were 89.4 ± 2.3% and 85.6 ± 2.7%, respectively (Table 3). In contrast, CGP-12177A relaxed this contraction by only 48.2 ± 7.2% at the highest concentration of 10^{-4} M, indicating an EC_{50} value for this agonist of 10^{-4} M or more (Table 3).

Rhythmic isovolumetric reflex bladder contraction

YM178 produced a dose-dependent decrease in the frequency of rhythmic bladder contraction in anesthetized rats (Fig. 5A). In contrast, it did not decrease the amplitude of rhythmic bladder contraction at up to 3 mg/kg i.v. (Fig. 5B). On the contrary, oxybutynin significantly increased the frequency of rhythmic bladder contraction and decreased its amplitude at doses of 0.272 mg/kg i.v. or more (Fig. 5A, B).

Discussion

We investigated the pharmacological properties of YM178 using biochemical/pharmacological techniques. YM178 showed highly selective agonist activity for human β_3-AR over β_1- or β_2-AR. The agonistic potency of YM178 for human β_3-ARs was 20 and 200 times greater than those of BRL37344 and CL316,243, respectively. In addition, the intrinsic activity of YM178 for human β_3-ARs was higher than those of BRL37344 and CL316,243. To date, β_3-AR has been cloned in many species, including humans (Emorine et al., 1989), rats (Granneman et al., 1991) and mice (Nahmias et al., 1991), and subtle species-dependent differences in pharmacological response have been identified. In particular, BRL37344 and CL316,243 are less potent and efficacious in stimulating human receptors than rodent receptors (Liggett 1992; Dolan et al., 1994). The EC_{50} values of BRL37344 and CL316,243 in stimulating human β_3-ARs were larger and less selectivity of BRL37344 for β_3-ARs versus β_2-ARs in the present study compared with previous studies (Wilson et al., 1996; Dolan et al., 1994). Given that EC_{50} value and I.A. can vary according to experimental conditions such as receptor expression level (Wilson et al., 1996), this may have been due to the difference in receptor density in each cell type. Although we unfortunately did not measure the receptor expression levels of human β_3-AR-expressing cells, we nevertheless considered it possible to use these cells to rank the order of potency of β_3-AR agonists, and obtained a ranking (EC_{50}) of YM178 > isoproterenol > BRL37344 > CL 316,243. These data suggest that YM178 is different to earlier β_3-AR agonists, namely in having full and selective agonistic activity for human β_3-ARs. In the present study using rat

JPET Fast Forward. Published on February 9, 2007 as DOI: 10.1124/jpet.106.115840
This article has not been copyedited and formatted. The final version may differ from this version.

and human bladder muscle, YM178 showed a similar high potency and I.A. to isoproterenol. Although a difference in bladder muscle β-AR subtype expression between humans and rats has been identified (Fujimura et al., 1999; Yamaguchi, 2002), YM178 showed full agonistic activity in bladder strips of both species. In contrast, CGP-12177A showed only a slight relaxing effect even at the highest concentration in both species. CGP-12177A is known to be a partial agonist for β_3-ARs (Langin et al, 1991), and our present results are consistent with previous reports (Yamazaki et al., 1998; Igawa et al., 1999). In addition, the earlier β_3-AR agonists BRL37344A and CL316,243 are reported to show strong relaxing effects (like isoproterenol) in rat bladder strips (Longhurst and Levendusky, 1999; Yamazaki et al., 1998), but not in human bladder strips (Igawa et al., 2001). We used a different concentration of CCh in rats and humans for pre-contraction of bladder strips, which may have contributed to the difference in EC_{50} values between them. Further, there was a difference in EC_{50} values for YM178 between CHO cells expressing β_3-ARs and in human bladder, with values for human bladder being higher. We consider that this is attributable to a difference in experimental conditions, as follows. First, receptor expression level in bladder tissue may differ from that in CHO cells expressing β_3-ARs. Wilson et al. (1996) demonstrated differences in EC_{50} values and I.A. for isoproterenol, BRL37344 and CGP12177 in three different β_3-ARs expression levels in CHO cells. Second, this discrepancy was caused by restricted drug diffusion into structured tissues, which hinders equilibrium conditions. Third, the EC_{50} value for CHO cells expressing human β_3-ARs was calculated as cAMP accumulation, whereas that for human bladder was calculated as relaxant activity under

carbachol pre-contracted conditions. Longhurst and Levendusky (1999) reported that isoproterenol is about 100-fold less potent against carbachol- versus KCl-induced contraction of the rat bladder. Further, Frazier et al. (2005) demonstrated that isoproterenol is about 10-fold less potent against KCl pre-contraction versus passive tension of the rat bladder. The difference in EC_{50} values for YM178 in CHO cells expressing β_3-ARs and in human bladder is therefore considered to be mainly attributable to the difference in experimental conditions. Although we did not examine the effect of β_3-AR antagonists in the present study, Igawa et al. (1999) has reported that the relaxant effect of isoproterenol in human bladder is mediated via β_3-AR using a β_3-AR antagonist. Further, Nomiya et al. (2003) has demonstrated that β_3-AR mRNA is expressed predominantly in human bladder. Moreover, we confirmed here that YM178 showed full agonistic activity for human β_3-ARs, but not for human β_1- and β_2-ARs, and had little affinity for any other receptors or channels at a concentration of 10^{-6} M. We therefore consider that the relaxant effect of YM178 in human bladder was mediated via β_3-ARs.

We also compared the effects of YM178 and oxybutynin on rhythmic bladder contraction induced by saline bladder filling in anesthetized rats. The results showed that YM178 did not affect the amplitude of rhythmic bladder contractions at doses at which it reduced contraction frequency. Similarly, CL316,243 suppressed mechanically- or chemically-induced bladder overactivity and improved urine storage function without affecting voiding function (Woods et al., 2001; Takeda et al., 2002; Kaidoh et al., 2002). Together, these results suggest that the activation of β_3-ARs increases bladder capacity

without influencing bladder contraction or residual urine volume during the voiding phase in commonly used animal models of bladder overactivity. This characteristic distinguishes it from oxybutynin, an anti-muscarinic agent which significantly decreases the amplitude of rhythmic bladder contraction caused by blockade of muscarinic M_3 receptors in bladder smooth muscle.

It is well known that the mammalian bladder is under dual autonomic nervous system control. Specifically, sympathetic nerves play an important role in the urine storage phase. Norepinephrin induces bladder relaxation and improves compliance via β-ARs. Given that these receptors play an important role in relaxation and improvement of compliance of the mammalian bladder, it has been suggested that the activation of bladder β-ARs might be of therapeutic relevance to the treatment of OAB conditions (Yamaguchi, 2002). β_3-AR is the main subtype in human bladder muscle (Fujimura et al., 1999; Yamaguchi, 2002), while in rats not only β_3-ARs but also β_2-ARs contribute to bladder relaxation (Yamazaki et al., 1998). To date, clinical treatment of OAB has involved the use of anti-muscarinic agents, but disadvantages such as insufficient efficacy, anti-muscarinic agent resistance in patients and adverse events including dysuria and dry mouth (Yarker et al., 1995) have lead to calls for the development of more potent and better tolerated drugs.

With regard to why β_3-ARs do not affect voiding function, the following mechanism may be considered. ACh released from parasympathetic nerves during the voiding phase activates postjunctional muscarinic M_2 receptors and inhibits adenylate cyclase (AC) activity

mediated by β-ARs. At the same time, ACh also stimulates muscarinic M_3 receptors and activates the phosphatidylinositol (PI)-Ca^{2+} recruitment system (Igawa, 2000). In rats, Hegde et al (1997) demonstrated that muscarinic M_2 receptors oppose beta-adrenoceptor mediated bladder relaxation both in vitro and in vivo. A recent report (Furuno et al., 2006) supports this premise, showing an enhanced effect of isoproterenol on bladder relaxation in M_2 receptor knockout mice. In addition, such muscarinic M_2 receptor-mediated inhibition of AC has also been demonstrated in cultured human bladder cells (Daniels et al., 1999). Thus, under the administration of YM178, the relaxation of bladder smooth muscle in the voiding phase may be canceled by muscarinic M_2-receptor activation, and YM178 may therefore not affect muscarinic M_3 receptor-mediated bladder contraction. This may in turn suggest that YM178 has little risk of causing the urinary retention noted with anti-muscarinic agents.

In conclusion, our study shows that YM178 has good selectivity and agonist potency for human β_3-ARs. YM178 does not directly inhibit voiding bladder contractions, and may therefore represent a promising choice for the treatment of overactive bladder with or without lower urinary tract symptoms such as those seen with benign prostatic hypertrophy.

References

Arch JRS, Ainsworth AT, Cawthorne MA, Piercy V, Sennitt MV, Thody VE, Wilson C, and Wilson S (1984) Atypical β-adrenoceptors on brown adipocytes as a target for anti-obesity drugs. *Nature (Lond)* **309**:163-165.

Berkowitz DE, Nardone NA, Smiley RM, Price DT, Kreutter DK, Fremeau RT, and Schwinn DA (1995) Distribution of β3-adrenoceptor mRNA in human tissues. *Eur J Pharmacol* **289**:223-228.

Daniels DV, Meloy TD, Loury DN, Watson N, Hegde SS, Ford APDW, and Eglen RM (1999) Functional pharmacological characterization of the muscarinic cholinoceptor-mediated inhibition of adenylyl cyclase in primary cultured human bladder detrusor smooth muscle cells. *Life Sci* **64**:590.

Dolan JA, Muenkel HA, Burns MG, Pellegrino SM, Fraser CM, Pietri F, Strosberg AD, Largis EE, Dutia MD, Bloom JD, Bass AS, Tanikella TK, Cobuzzi A, Lai FM, and Claus TH (1994) Beta-3 adrenoceptor selectivity of the dioxolane dicarboxylate phenethanolamines. *J Pharmacol Exp Ther* **269**:1000-1006.

Emorine LJ, Marullo S, Briend-Sutren MM, Patey G, Tate K, Delavier-Klutchko C, and Strosberg AD (1989) Molecular characterization of the human β_3-adrenergic receptor. *Science*

(Wash DC) **245**:1118-1121.

Frazier EP, Mathy MJ, Peters SLM and Michel MC (2005) Does cyclic AMP mediate rat urinary bladder relaxation by isoproterenol? *J Pharmacol Exp Ther* **313**:260-267.

Fujimura T, Tamura T, Tsutsumi T, Yamamoto T, Nakamura K, Koibuchi Y, Kobayashi M, and Yamaguchi O (1999) Expression and possible functional role of the β3-adrenoceptor in human and rat detrusor muscle. *J Urol* **161**:680-685.

Furuno T, Kakizaki H, Tanaka H, Mitsui T, Kitta T, Matsui M, and Nonomura K (2006) Enhanced inhibitory effects of beta-adrenoceptor agonist on cholinergic micturition contractions in muscarinic M_2 receptor knockout mice. *Neurourol Urodyn* **25**:590-591.

Granneman J G, Lahners KN, and Chaudhry A (1991) Molecular cloning and expression of the rat β_3-adrenergic receptor. *Mol Pharmacol* **40**:895-899.

Hegde SS, Choppin A, Bonhaus D, Briaud S, Loeb M, Moy TM, Loury D, and Eglen RM (1997) Functional role of M_2 and M_3 muscarinic receptors in the urinary bladder of rats in vitro and in vivo. *Br J Pharmacol* **120**:1409-1418.

Hu B, and Jennings LL (2003) Orally bioavailable beta 3-adrenergic receptor agonists as

potential therapeutic agents for obesity and type-II diabetes. *Prog Med Chem* **41**:167-194.

Igawa Y, Yamazaki Y, Takeda H, Akahane M, Ajisawa Y, Yoneyama T, and Nishizawa O (1998) Possible β3-adrenoceptor-mediated relaxation of the human detrusor. *Acta Physiol Scand* **164**:117-118.

Igawa Y, Yamazaki Y, Takeda H, Hayakawa K, Akahane M, Ajisawa Y, Yoneyama T, Nishizawa O, and Andersson KE (1999) Functional and molecular biological evidence for a possible β3-adrenoceptor in the human detrusor muscle. *Br J Pharmacol* **126**:819-825.

Igawa Y (2000) Discussion: Functional role of M_1, M_2, and M_3 muscarinic receptors in overactive bladder. *Urology* **55 (5A)**:47-49.

Igawa Y, Yamazaki Y, Takeda H, Kaidoh K, Akahane M, Ajisawa Y, Yoneyama T, Nishizawa O, and Andersson KE (2001) Relaxant effects of isoproterenol and selective β3-adrenoceptor agonists on normal, low compliant and hyperreflexic human bladders. *J Urol* **165**:240-244.

Kaidoh K, Igawa Y, Takeda H, Takeda Y, Takeda H, Yamazaki Y, Akahane S, Miyata H, Ajisawa Y, Nishizawa O, and Andersson KE (2002) Effects of selective β2 and

β3-adrenoceptor agonists on detrusor hyperreflexia in conscious cerebral infarcted rats. *J Urol* **168**:1247-1252.

Langin D, Portillo MP, Saulnier-Blache JS, and Lafontan M (1991) Coexistence of three β-adrenoceptor subtypes in white fat cells of various mammalian species. *Er J Pharmacol* **199**:291-301.

Liggett SB (1992) Functional properties of the rat and human beta 3-adrenergic receptors: differential agonist activation of recombinant receptors in Chinese hamster ovary cells. *Mol Pharmacol* **42**:634-637.

Longhurst PA, and Levendusky M (1999) Pharmacological characterization of β-adrenoceptors mediating relaxation of the rat urinary bladder in vitro. *Br J Pharmacol* **127**:1744-1750.

Nahmias C, Blin N, Elalouf J-M, Mattei MG, Strosberg AD, and Emorine LJ (1991) Molecular characterization of the mouse β_3-adrenergic receptor: Relationship with the atypical receptor of adipocytes. *EMBO (Eur Mol Biol Organ) J* **10**:3721-3727.

Nomiya M, and Yamaguchi O (2003) A quantitative analysis of mRNA expression of α1 and β-adrenoceptor subtypes and their functional roles in human normal and obstructed bladders. *J Urol* **170**:649-653.

Takeda M, Obara K, Mizusawa T, Tomita Y, Arai K, Tsutui T, Hatano A, Takahashi K, and Nomura S (1999) Evidence for β_3-adrenoceptor subtypes in relation of the human urinary bladder detrusor: Analysis by molecular biological and pharmacological methods. *J Pharmacol Exp Ther* **288**:1367-1373.

Takeda H, Yamazaki Y, Igawa Y, Kaidoh K, Akahane S, Miyata H, Nishizawa O, Akahane M, and Andersson KE (2002) Effects of β_3-adrenoceptor stimulation on prostaglandin E_2-induced bladder hyperactivity and on the cardiovascular system in conscious rats. *Neurourol Urodyn* **21**:558-565.

Wilson S., Chambers JK, Park JE, Ladurner A, Cronk DW, Chapman CG, Kallender H, Browne MJ, Murphy GJ, and Young PW (1996) Agonist potency at the cloned human beta-3 adrenoceptor depends on receptor expression level and nature of assay. *J Pharmacol Exp Ther* **279**:214-221.

Woods M, Carson N, Norton N W, Sheldon JH, and Adgentieri TM (2001) Efficacy of the

β3-adrenergic receptor agonist CL-316243 on experimental bladder hyperreflexia and detrusor instability in the rat. *J Urol* **166**:1142-1147.

Yamaguchi O (2002) β3-adrenoceptors in human detrusor muscle. *Urology* **59 (Suppl 5A)**:25-29.

Yamazaki Y, Takeda H, Akahane M, Igawa Y, Nishizawa O, and Ajisawa Y (1998) Species differences in the distribution of β-adrenoceptor subtypes in bladder smooth muscle. *Br J Pharmacol* **124**:593-599.

Yarker YE, Goa KL, and Fitton A (1995) Oxybuthynin. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic use in detrusor instability. *Drugs Aging* **6**:243-250.

Legends for Figures

Fig. 1. Chemical structure of YM178.

Fig. 2. cAMP accumulation in CHO cells expressing human β_1-AR (A), β_2-AR (B), and β_3-AR (C). Data are expressed as percent relative to maximal accumulation of cAMP induced by 10^{-4} M isoproterenol. Each point represents the mean ± S.E. of 3 to 6 preparations.

Fig. 3. Relaxing effect of isoproterenol, YM178 and CGP-12177A in rat bladder strips pre-contracted with carbachol. Each strip was pre-contracted with 10^{-6} M carbachol. Data are expressed as a percentage of maximal relaxation induced by 10^{-4} M papaverine. Each point represents the mean ± S.E. of 5 preparations.

Fig. 4. Relaxing effect of isoproterenol, YM178 and CGP-12177A in human bladder strips pre-contracted with carbachol. Each bladder strip was pre-contracted with 10^{-7} M carbachol. Data are expressed as a percentage of maximal relaxation induced by 10^{-4} M papaverine. Each point represents the mean ± S.E. of 4 to 6 preparations.

Fig. 5. Effect of YM178 and oxybutynin on the frequency (A) and amplitude (B) of rhythmic bladder contraction in anesthetized rats. The frequency and amplitude of contractions were evaluated for 10 min (5 to 15 minutes after dosing). For each parameter, the saline

administration value was taken as the pretreatment value. Each bar represents the mean ± S.E. of five rats unless indicated otherwise. Numbers in parentheses indicate sample size. * $P<0.05$, ** $P<0.01$, significant difference from the corresponding vehicle group (Student's *t*-test with actual measurement values).

JPET Fast Forward. Published on February 9, 2007 as DOI: 10.1124/jpet.106.115840
This article has not been copyedited and formatted. The final version may differ from this version.

TABLE 1

Effect of β-AR agonists on cAMP accumulation in CHO cells expressing human β-ARs

Values represent the mean, 95% confidence interval and I.A. of 3 to 6 preparations.

Compound	β-Adrenergic Activity (EC_{50} nM) [95% CL[a)]] (I.A.[*])			Selectivity	
	β_1-AR	β_2-AR	β_3-AR	β_1/β_3	β_2/β_3
Isoproterenol	11.1 [6.1 - 18.0] (1)	1.8 [0.9 - 3.0] (1)	56.5 [27.7 - 107] (1)	0.20	0.03
YM178	> 10,000 [_b] (0.1)	> 10,000 [_b] (0.1)	22.4 [12.6 - 36.3] (0.8)	> 446	> 446
BRL37344	12,900 [10,000 - 18,900] (0.5)	360 [189 - 771] (0.7)	457 [386 - 544] (0.6)	28.2	0.79
CL316,243	> 10,000 [_b] (0)	> 10,000 [_b] (0.1)	4,430 [3,250 - 6,040] (0.5)	> 2.3	> 2.3

[*] I.A., value 1 corresponds to the I.A. equivalent of isoproterenol.

CL[a)], confidence limits.

_b, could not be calculated because of low I.A.

JPET Fast Forward. Published on February 9, 2007 as DOI: 10.1124/jpet.106.115840
This article has not been copyedited and formatted. The final version may differ from this version.

TABLE 2.

Relative potencies of β-AR agonists in relaxing carbachol-precontracted rat bladder strips

Each strip was pre-contracted with 10^{-6} M carbachol. EC_{50} values represent the mean and 95% confidence limit. Maximum relaxation values represent the mean ± S.E. Values in brackets indicate the efficacy ratio to YM178.

Agonist	EC_{50} (μM)	95% CL[a)] (μM)	Maximum Relaxation (%)
YM178	5.1	3.1 - 8.4	94.0 ± 1.0
n = 5	[1]		
Isoproterenol	1.4	0.83 - 2.3	78.0 ± 1.5
n = 5	[3.7]		
CGP-12177A	> 100	ND	19.4 ± 1.2
n = 5	[< 1/19]		

CL[a)], confidence limits. ND, not detected.

JPET Fast Forward. Published on February 9, 2007 as DOI: 10.1124/jpet.106.115840
This article has not been copyedited and formatted. The final version may differ from this version.

TABLE 3.

Relative potencies of β-AR agonists in relaxing carbachol-precontracted human bladder strips

Each strip was pre-contracted with 10^{-7} M carbachol. EC_{50} values represent the mean and 95% confidence limit. Maximum relaxation values represents the mean ± S.E. Values in brackets indicate the efficacy ratio to YM178.

Agonist	EC_{50} (μM)	95% CL[a)] (μM)	Maximum Relaxation (%)
YM178	0.78	0.32 - 1.9	89.4 ± 2.3
n = 6	[1]		
Isoproterenol	0.28	0.051 - 1.5	85.6 ± 2.7
n = 4	[3]		
CGP-12177A	> 100	ND	48.2 ± 7.2
n = 5	[< 1/130]		

CL[a)], confidence limits. ND, not detected.

JPET Fast Forward. Published on February 9, 2007 as DOI: 10.1124/jpet.106.115840

Fig. 1

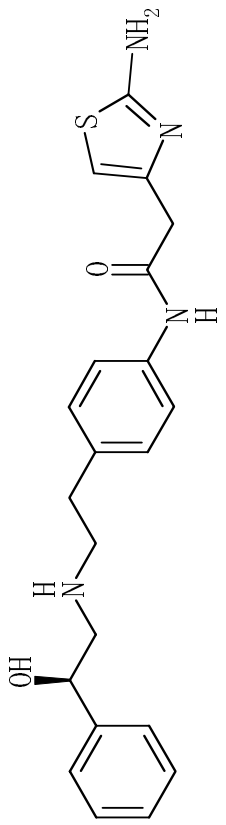

Fig. 2

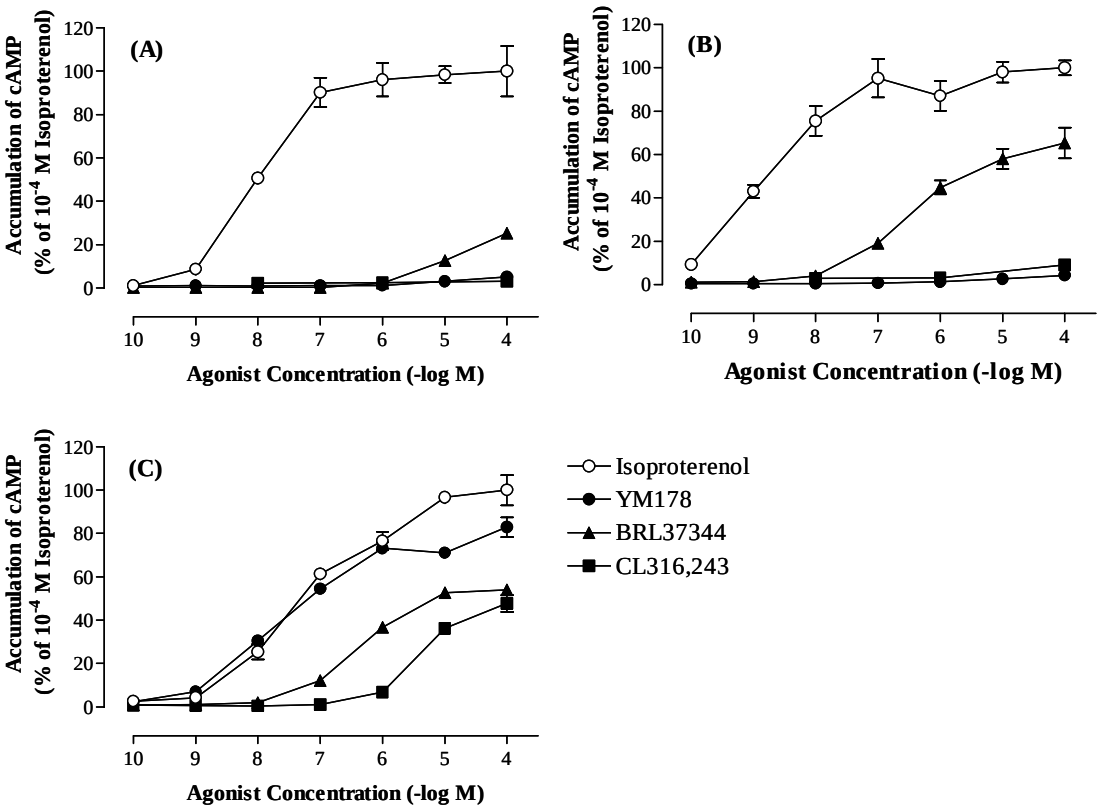

Fig. 3

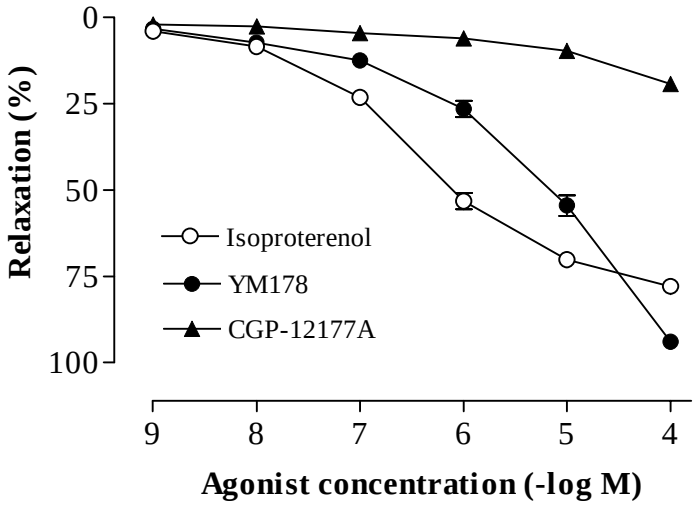

Fig. 4

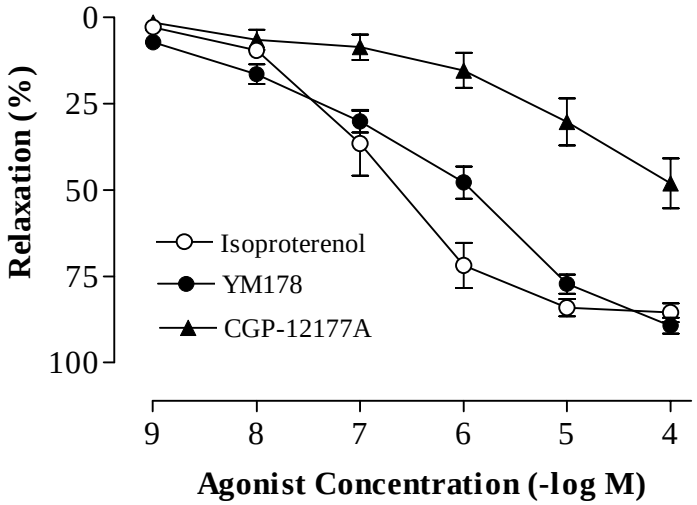

Fig. 5