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New series of *N*-substituted phenyl ketone oxime ethers: synthesis and bovine β_3 -adrenergic agonistic activities

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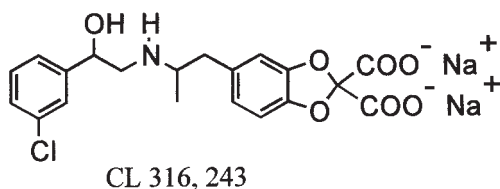
A series of ten novel phenyl ketone oxime ethers substituted on the terminal nitrogen by either 1,3 benzodioxole, alkyl, aralkyl or aryl moiety were synthesized and tested for their activity at bovine β_3 -adrenoceptors. The best compound, which was the benzodioxole dicarboxylate derivative, showed potent β_3 -adrenergic agonistic activities in Chinese hamster ovary cells expressing the bovine β_3 -adrenoceptors with K_{act} and K_i values better than compound CL 316,243 used as reference (14 ± 6 nM and 203 ± 71 nM, respectively). In this series three compounds showed an antagonistic activity. Structure-activity relationships in these ketone oxime ethers are discussed.

1. Introduction

In a previous paper, we described the synthesis of novel aryloxypropanolamines substituted on the terminal nitrogen in various ways intended to stimulate bovine β_3 -adrenoceptors which are involved in lipolysis in white adipose tissue [1]. The search for specific bovine β_3 -adrenergic agonists targeted to be used in veterinary medicine to increase overall lean body mass, led us to design a new series of compounds.

β -Adrenergic ligands are very homogeneous in their chemical structures and generally belong either to the aryl-

ethanolamine or to the aryloxypropanolamine class [2, 3]. However, it has been previously shown that insertion of a C=N–O group between the propanolamine side chain and the aromatic moiety of β -blockers did not only abolish the β -adrenoceptors activity but also, in some cases, could lead to potent and selective ligands [4–6]. Up to now, only few ligands of this type have been described, particularly in the β_3 -adrenergic field [7]. In other respects, another important feature to promote a good β_3 -adrenergic selectivity is the presence of a long and bulky amine substituent moiety linked to the nitrogen of the lateral propanolamine chain. Besides, it was also demonstrated that either mono or dicarboxyl groups bearing by the lateral aralkylamine enhance the β_3 -adrenergic selectivity by decreasing the β_1 and β_2 binding affinity [8]. In addition, Bloom et al. observed, in a series of aryloxypropanolamines that the *N*-(1,3-benzodioxole) group increased antihyperglycemia, anti-obesity properties, and β_3 -adrenergic selectivity, as exemplified by the potent and highly selective CL 316,243 compound [9–12].



Scheme 1

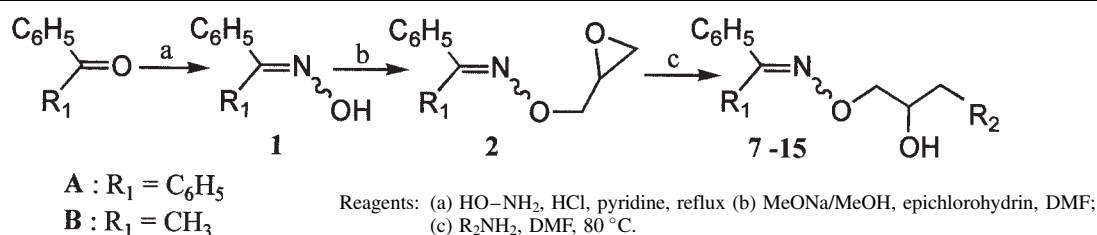


Table 1: Structure and chemical data of *N*-substituted diphenyl ketone oxime ethers 7–15

Compd. ^a	R ₁	R ₂	Yield (%) ^b	Purification ^c	M.p. (°C)
7	C ₆ H ₅		83	EtOAc	100–101
8a	C ₆ H ₅		35	EtOAc/cyclohexane(2/8)	Oil
8b	CH ₃		23	EtOAc/cyclohexane (2/8)	Red oil
9	C ₆ H ₅		65	EtOAc	86–87
10	C ₆ H ₅		72	EtOAc	180
11	C ₆ H ₅		17	H ₂ O/CH ₃ CN (2/8) ^d	Hygroscopic
12	C ₆ H ₅		32	EtOAc/cyclohexane (2/8)	Red oil
13	C ₆ H ₅		79	MeOH ^d	282
14	C ₆ H ₅	HN–CH ₃	52	EtOAc/cyclohexane (5/5)	Yellow oil
15	C ₆ H ₅	NH ₂	35	EtOAc/cyclohexane (5/5)	Yellow oil

^a All described compounds were fully characterized including spectroscopic and elemental analysis for C, H and N. ^b Yield is based on the epoxide. ^c Purification by column chromatography on silica gel using a mixture of solvents. ^d Recrystallisation

On the basis of these considerations, it appeared to be of interest to synthesize a new series of phenyl ketone oxime ethers substituted on the terminal nitrogen either by 1,3 benzodioxole, alkyl, aralkyl or aryl moiety. Furthermore, the pharmacological bovine β_3 -adrenoceptor and adenylyl cyclase activation properties of the synthesized compounds were evaluated.

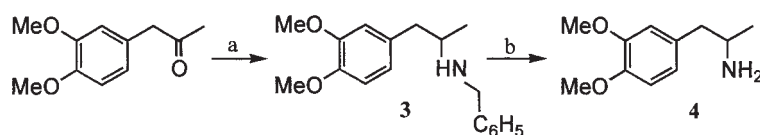
2. Investigations, results and discussion

2.1. Chemistry

The compounds prepared as indicated in Scheme 1 and are listed in Table 1. The key compounds in the preparation of this series of ketone oxime ethers were the epoxide **2** and the requisite amines. The oximes were usually

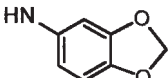
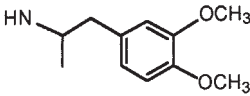
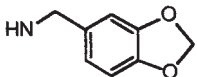
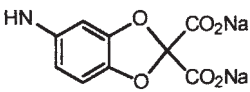
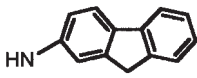
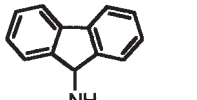
prepared by refluxing hydroxylamine hydrochloride with the suitable ketones in anhydrous pyridine for 12 h. The sodium salt of the oxime was allowed to react with epichlorohydrine in anhydrous dimethylformamide (DMF) and the crude epoxide was used without purification for the subsequent reactions with an excess of the required amine in anhydrous DMF. In other respects, 3,4-dimethoxyphenylpropanone and benzylamine were reacted with sodium cyanoborohydride in methanol, giving **3** which was hydrogenated with Pd/C (10%) in methanol leading to **4** (Scheme 2). By another way, 4-nitrocatechol (Scheme 3) was reacted with diethyldibromomalonate and anhydrous potassium carbonate in acetone, yielding 5-nitro-1,3-benzodioxole-2,2-dicarboxylic acid, diethyl ester **5** which was reduced with Pd/C (10%) in methanol providing the amine derivative **6**.

Scheme 2



Reagents: benzylamine, NaBH₃CN, MeOH; (b) H₂, 10% Pd/C, MeOH, 40 °C

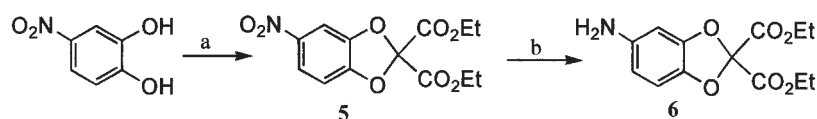
Table 2: Biological activity on the bovine β_3 -adrenoceptor of the *N*-substituted diphenyl ketone oxime ethers 7–15

Compd.	R ₁	R ₂	Agonist			Antagonist % inhib ^d
			IA ^a	K _i (nM) ^b	K _{act} ^c (nM)	
7	C ₆ H ₅		—	—	—	100 ± 9
8a	C ₆ H ₅		0.71 ± 0.01	30,000	32 ± 13	—
8b	CH ₃		nd ^e	nd ^e	nd ^e	nd ^e
9	C ₆ H ₅		—	—	—	73 ± 7
10	C ₆ H ₅		—	—	—	31 ± 6
11	C ₆ H ₅		1.12 ± 0.13	203 ± 71	14 ± 6	—
12	C ₆ H ₅		0.28 ± 0.18	—	—	—
13	C ₆ H ₅		nd ^e	nd ^e	nd ^e	nd ^e
14	C ₆ H ₅	HN-CH ₃	0.14 ± 0.13	—	—	—
15	C ₆ H ₅	NH ₂	0.87 ± 0.07	30,000	102 ± 54	—
CL 316,243			1.6 ^f	14,000 ^f	68 ^f	

All the values are means ± SEM of at least 2 or 3 independent experiments performed in duplicate on the racemics.

^a IA: intrinsic activity measured relatively to the maximal cyclase stimulation obtained for (–)-isoproterenol 10^{–4} M. ^b K_i: inhibition constant determined in the presence of ¹²⁵Iodocyanopindolol (according to Cheng and Prusoff) [13]. ^c K_{act}: activation constant defined as the concentration of ligand necessary to obtain 50% of the maximal effect. ^d % inhib: for agonistic class, the inhibition activity of each ligand at 10^{–5} M were calculated relative to their capacity to inhibit (–)-isoproterenol 10^{–8} M. ^e net determined. ^f Values obtained from CHO-K1 cells expressing human β_3 -adrenoceptors [2].

Scheme 3



Reagents: (a) Br₂C(CO₂Et)₂, K₂CO₃, acetone, (b) H₂, 10% Pd/C, MeOH

2.2. Biological results and discussion

The 10 oximes synthesized were pharmacologically examined and classified in two groups with regards to their agonistic or antagonistic activity so as to define the pharmacological properties of the ligands (Table 2). The stimulation of adenylyl cyclase was measured by the determination of cAMP accumulation in CHO-K1 cells expressing bovine β_3 -adrenoceptors.

5 out of 10 derivatives were qualified as agonists, 3 as antagonists for the bovine β_3 -adrenoceptor. For the agonist class, intrinsic activity (IA) values were calculated for each ligand relative to the maximal cAMP accumulation obtained with (–)-isoproterenol 10^{–4} M. Two ligands displayed a full agonistic activity with an IA superior to 0.8. Three ligands were further examined with respect to their binding and potency by the determination of their inhibition constant (K_i) and their adenylyl cyclase activation constant (K_{act}). These 3 ligands e.g. **8a**, **11** and **15**,

showed high affinity and/or potency for bovine β_3 -adrenoceptors. One of them displayed full and potent β_3 agonistic effects with a K_{act} value of 14 ± 6 nM and a K_i value of 203 ± 71 nM i.e. 4-fold and 70-fold higher, respectively, than the partial β -adrenergic agonist CL 316,243 chosen as reference for its potent thermogenic, antidiabetic and antiobesity properties in animal models of non-insulin-dependent diabetes mellitus and obesity [2]. It should be noted that, for CL 316,243, pharmacological data was given for CHO-K1 cells expressing human β_3 -adrenoceptors because, up to now, it has not been evaluated on cells expressing bovine β_3 -adrenoceptors.

According to the values given in Table 2, it could be observed that, in this series, the activity only depends on the lateral amino chain because all the tested compounds are *N*-substituted diphenyl ketone oxime and so are identical at the oxime moiety. These data also showed that the best structures have a lateral amino chain derived from benzodioxole (**8a**) and benzodioxole dicarboxylate (**11**) as for

CL 316,243. Particularly, compound **11** displayed the best values at once for K_i , K_{act} and intrinsic activity. Taking in account the presence of two carboxylate groups in this structure, one could expect also a good subtype selectivity, as observed for CL 316,243. As it was emphasized by Sher et al., such groups which increase the steric bulk and decrease lipophilicity at the end of the amino chain, enhance selectivity toward β_3 -adrenoceptors [8]. So, further complementary pharmacological investigations on compound **11** targeted on a selectivity study between the three β -adrenoceptor subtypes are necessary. By other respects, it can be noted that fluorenylamino chain showed a very weak activity ($IA = 0.28$) which could be related to a lack of flexibility of the lateral amino chain, a very important feature for β_3 -adrenergic activity and selectivity [3].

Three antagonists were found in this novel series showing that slight modifications of the amino lateral chain can be responsible for inversion of the β_3 -adrenergic effect. For example the simple insertion of a carbon atom between the benzodioxole structure and the nitrogen atom of the amino chain can reverse activity (**8a** is an agonist and **10** is an antagonist). In this series, it can be noted for the three antagonists (**7**, **9** and **10**) that the presence of a $(CH_2)_n$ spacer between the aromatic moiety and the amino nitrogen is a common feature.

In conclusion, compound **11** seems to be the most interesting derivative because of its highest agonistic affinity and potency together with its highest intrinsic activity on bovine β_3 -adrenoceptors. In other respects, though the therapeutic potential of the β_3 antagonists is already unknown, our study elicited, in a novel ketone oxime ether series, 3 ligands displaying an antagonistic activity which would be of interest as a tool for further conclusive functional identification of β_3 -adrenoceptors. These results confirmed ketone oxime ethers as an alternative to the aryethanolamine or aryloxypropanolamine structure in the search for specific β_3 -adrenergic ligands.

3. Experimental

3.1. Apparatus

Melting points were determined on a calibrated Kofler bench and were uncorrected. 1H NMR spectra were recorded at 200.13 MHz (Bruker AC 200 F.T.). Chemical shifts are reported in parts per million (δ ppm) downfield from an internal standard of tetramethylsilane (Me_4Si) for $CDCl_3$, D_2O and $DMSO-d_6$. IR spectra were recorded on a PYE UNICAM SP3-100 IR spectrophotometer (Philips). Elemental analysis was performed by the Analytical Department of CNRS, Vernaison, France. All starting ketones were commercially available. The compounds were purified by column chromatography on silica gel unless otherwise stated. Solvents are listed in Table 1.

3.2. Chemistry

3.2.1. *N*-Benzyl-2-(3,4-dimethoxyphenyl)-1-methylethylamine (**3**)

A mixture of 2 g (10.3 mmol) of 3,4-dimethoxyphenylacetone, 2.4 g (11 mmol) of benzylamine, 0.884 g (14 mmol) of sodium cyanoborohydride and 20 ml of methanol was stirred for 48 h at 80 °C. The mixture was cooled and evaporated. The residue was purified by flash chromatography, eluting with ethyl acetate/cyclohexane (5/5). 1H NMR ($CDCl_3$): δ ppm = 1.11 (d, 3H, $J = 6.2$ Hz), 1.75 (s, 2H), 2.60 (dd, 1H, $J = 13.2$, 2.6 Hz), 2.68 (dd, 1H, $J = 13.2$, 6.0 Hz), 2.91 (sextuplet, 1H, $J = 6.4$ Hz), 3.82 (s, 3H), 3.86 (s, 3H), 6.65–6.81 (m, 3H, Ar-H), 7.17–7.25 (m, 5H, Ar-H).

3.2.2. 2-(3,4-Dimethoxyphenyl)-1-methylethylamine (**4**)

To a solution of 1.7 g (6 mmol) of **3** in 50 ml of methanol was added 0.5 g of Pd/C (10%) and the mixture was hydrogenated for 24 h at 2.5 kg/cm². The catalyst was filtered off and the solvent removed from the filtrate to give the required amine which was chromatographed on silica gel column using AcOEt/cyclohexane (5/5) as eluent (yield = 65%). 1H NMR ($CDCl_3$): δ ppm = 1.19 (d, 3H, $J = 6.4$ Hz), 2.64 (dd, 1H, $J = 12.0$, 5.6 Hz), 2.81

(dd, 1H, $J = 12.0$, 6.4 Hz), 3.28 (sextuplet, 1H, $J = 7.2$ Hz), 3.84 (s, 6H), 6.38 (s, 2H, NH_2), 6.70–6.81 (m, 3H, Ar-H).

3.2.3. 5-Amino-1,3-benzodioxol-2,2-dicarboxylic acid, diethyl ester (**6**)

A mixture of 2 g (12.89 mmol) of 4-nitrocatechol, 4.15 g of diethyldibromomalonate, 6.77 g of anhydrous potassium carbonate and 80 ml of acetone were stirred over night with the addition of a few drops of diethyldibromomalonate. The mixture is filtered, washed with acetone and the combined filtrates were evaporated to give a yellow oil. The oil is purified by chromatography on silica gel column eluting AcOEt/cyclohexane (2/8). The pure fractions were combined and evaporated giving rise to 5 g of nitrobenzodioxole derivative **5**. Compound **5** was dissolved in 30 ml of methanol containing 1 g of Pd/C (10%) and hydrogenated for 12 h at 2.5 kg/cm². The catalyst was filtered off and the solvent removed from the filtrate to give the required amine **6** as a colorless oil (4.6 g).

3.2.4. General preparation of the oximes

A solution of the appropriate ketone (1 equiv.) and hydroxylamine hydrochloride (2 equiv.) in dry pyridine (300 ml), was refluxed under stirring for 12 h. The mixture was then poured into excess water leading, generally, to the oxime precipitation. The precipitate was collected by filtration and recrystallized. When the oxime were oily, extraction with ethyl acetate was carried out. The extract was washed several times with dilute HCl, then with H_2O , dried on $MgSO_4$ and finally evaporated to dryness.

3.2.5. General synthetic procedure for *N*-substituted diphenyl ketone oxime ethers

The preparation of **7** is illustrative of the method used for the synthesis of *N*-substituted diphenyl ketone oxime ethers **7**–**15**.

3.2.5.1. [3-(1,1-Dimethylethylphenyl)amino-2-hydroxypropyl]oximino-diphenylmethylethylene (**7**)

A solution of sodium methoxide was prepared from 0.65 g (28 mmol) of sodium and 80 ml methanol. Oxime of benzophenone (5.33 g, 28 mmol) was added to this solution over a period of 5 min. The mixture was refluxed for 1 h. The methanol was then thoroughly removed in vacuo and the dry residue taken up in 40 ml of anhydrous DMF. This solution was then added dropwise to a solution of 2.61 g (28 mmol) of epichlorohydrin in 10 ml anhydrous DMF and stirred for 2 h. The mixture was poured into 300 ml H_2O and extracted three times with 80 ml CH_2Cl_2 . The organic phase was washed twice with 100 ml H_2O , dried on $MgSO_4$ and evaporated to dryness. The yellow oil of epoxide was used without further purification. The above epoxide (0.4 g) was dissolved in DMF containing 2-phenyl-1,1-dimethylethylamine (1 equiv.) and stirred for 12 h at 80 °C. After cooling, the solvent was removed in vacuo, and the oily residue was chromatographed on silica gel eluting with a mixture of solvents as indicated in Table 1 (m.p. 100–101 °C, yield = 83%). 1H NMR ($CDCl_3$): δ ppm = 1.18 (s, 3H), 1.21 (s, 3H), 2.87 (m, 3H), 3.02 (m, 1H), 4.15 (m, 1H), 4.35 (m, 2H), 7.12–7.49 (m, 15H, Ar-H). $C_{26}H_{30}N_2O_2$

3.2.5.2. [3-(3,4-Methylenedioxy)aniline-2-hydroxypropyl] oximino-diphenylmethylethylene (**8a**)

Maleate: 1H NMR ($DMSO-d_6$): δ ppm = 2.88 (dd, 1H, $J = 12.8$, 6.6 Hz), 3.06 (dd, 1H, 12.8, 4.4 Hz), 3.92 (m, 1H), 4.10 (m, 2H), 5.81 (s, 2H), 6.0 (dd, 1H, ArH, $J = 8.0$, 2.0 Hz), 6.20 (s, 2H), 6.30 (d, 1H, ArH, $J = 1.8$ Hz), 6.61 (d, 1H, ArH, $J = 8.0$ Hz), 7.30–7.46 (m, 10H, Ar-H). $C_{23}H_{22}N_2O_4$

3.2.5.3. [3-(3,4-Methylenedioxy)aniline-2-hydroxypropyl] oximino-1-methyl-1-phenylmethylethylene (**8b**)

1H NMR ($CDCl_3$): δ ppm = 2.26 (s, 3H), 3.70 (dd, 1H, $J = 11.9$, 5.6 Hz), 3.79 (dd, 1H, $J = 11.9$, 4.0 Hz), 4.29 (d, 2H, $J = 5.0$ Hz), 6.36 (s, 2H), 6.82 (m, 3H, ArH), 7.34–7.41 (m, 3H, Ar-H), 7.57–7.62 (m, 2H, Ar-H). $C_{18}H_{20}N_2O_4$

3.2.5.4. [3-(2-(3,4-Methoxyphenyl)-1-methylethyl)amino-2-hydroxypropyl] oximino-diphenylmethylethylene (**9**)

1H NMR ($DMSO-d_6$): δ ppm = 0.90 (d, 3H, $J = 6$ Hz), 2.49 (m, 1H), 2.62 (m, 3H), 2.85 (m, 2H), 3.69 (s, 6H), 3.83 (m, 1H), 4.06 (m, 2H), 6.67 (dd, 1H, ArH, $J = 8.0$, 1.6 Hz), 6.76 (d, 1H, ArH, $J = 1.5$ Hz), 6.81 (d, 1H, ArH, $J = 8.0$ Hz), 7.27–7.45 (m, 10H, Ar-H). $C_{27}H_{32}N_2O_4$

3.2.5.5. (3-Piperonylamino-2-hydroxypropyl) oximino-diphenylmethylethylene (**10**)

1H NMR ($CDCl_3$): δ ppm = 2.64 (dd, 1H, $J = 12.2$, 6.8 Hz), 2.75 (dd, 1H, $J = 12.2$, 4.4 Hz), 3.69 (s, 2H), 4.19 (m, 1H), 4.38 (d, 2H, $J = 6.0$ Hz), 5.93 (s, 2H), 6.72–6.79 (m, 3H), 7.31–7.43 (m, 10H, Ar-H). $C_{24}H_{24}N_2O_4$

3.2.5.6. 5-[3-(1,1-Diphenyloximino)-2-hydroxypropyl]amino-1,3-benzodioxole-2,2-dicarboxylic acid, disodium salt (**11**)

The disodium salt **11** was obtained from corresponding diester by treatment with 1N NaOH at room temperature for 24 h. The solvent was evaporated and the residue was recrystallized in a mixture of H₂O-CH₃CN: 2/8 (yield = 17%, hygroscopic green solid). ¹H NMR (D₂O): δ ppm = 2.89 (m, 2H), 3.95 (m, 1H), 4.08 (m, 2H), 6.01 (dd, 1H, ArH, J = 8.0, 1.8 Hz), 6.29 (d, 1H, ArH, J = 1.7 Hz), 6.63 (d, 1H, ArH, J = 8.0 Hz), 7.29–7.49 (m, 10H, Ar-H).

C₂₅H₂₀N₂Na₂O₈

3.2.5.7. [3-(Fluorenyl-2-amino)-2-hydroxypropyl] oximino-diphenylmethylenene (**12**)

¹H NMR (CDCl₃): δ ppm = 3.85 (s, 2H), 3.99 (m, 2H), 4.24 (m, 3H), 7.27–7.44 (m, 17H, Ar-H).

C₂₉H₂₆N₂O₂

3.2.5.8. [3-(Fluorenyl-9-amino)-2-hydroxypropyl] oximino-diphenylmethylenene (**13**)

Recrystallisation with MeOH (yield = 79%, m.p. = 282 °C). ¹H NMR (DMSO-d₆): δ ppm = 3.59 (m, 2H), 4.19 (m, 3H), 5.61 (s, 1H), 6.92–7.42 (m, 18H, Ar-H).

C₂₉H₂₆N₂O₂

3.2.5.9. [3-Methylamino-2-hydroxypropyl] oximino-diphenylmethylenene (**14**)

¹H NMR (CDCl₃): δ ppm = 3.39 (s, 3H), 3.47 (m, 2H), 4.15 (m, 1H), 4.25 (m, 2H), 7.32–7.46 (m, 10H, Ar-H).

C₁₇H₂₀N₂O₂

3.2.5.10. [3-Amino-2-hydroxypropyl] oximino-diphenylmethylenene (**15**)

¹H NMR (CDCl₃): δ ppm = 3.62 (dd, 1H, J = 11.53, 5.48 Hz), 3.72 (dd, 1H, J = 11.53, 4.07 Hz), 4.05 (m, 1H), 4.26 (d, 2H, J = 5.12 Hz), 7.28–7.49 (m, 10H, Ar-H).

C₁₆H₁₈N₂O₂

3.3. Pharmacology

3.3.1. cAMP accumulation experiments

For studies of agonist activity, CHO-β₃-adrenoceptors, were grown to pre-confluence in six-well dishes to a density of approximately 0.6 × 10⁶ cells/well. After washing with 1 ml Ham's F12 medium buffered with 20 mM Hepes (pH 7.4) supplemented with 1 mM IBMX, cell monolayers were incubated for 30 min at 37 °C in the absence or in the presence of 10⁻⁴ M (–)-isoproterenol, or 10⁻¹² M to 10⁻⁴ M of ligand. For studies of antagonist effect, cells were preincubated at 37 °C for 10 min. with 100 μM of ligand tested before addition of 10 nM of (–)-isoproterenol and incubation for another 20 min period. The reaction was stopped by washing once with 1 ml PBS and by immediate addition of 500 μl 1N sodium hydro-

xide. After 20 min. at 37 °C, dissolved cells were collected, buffered with 1N acetic acid and centrifuged at 3000 × g for 10 min at 4 °C. The total amount of cAMP contained in an aliquot of supernatant was determined using the Amersham kit according to the manufacturers instructions. For eight of the full agonists activation constant (K_{act}) was calculated from dose-response curve experiments.

3.3.2. Binding experiments

Competition analysis was carried out using a saturating concentration of [¹²⁵I] ICYP (2 nM), and increasing concentrations of the β₃-selective agonists. CHOK1-β₃ were incubated with drugs for 30 min at 37 °C, before dilution with ice-cold PBS and rapid filtration over Whatmann GF/C glass fiber filters soaked in 0.3% polyethylenimine using a Brandel cell harvester, followed by extensive washing with ice-cold PBS. Radioactivity was assessed by γ-counting. IC₅₀ values (concentration of ligand inducing 50% inhibition of [¹²⁵I] ICYP binding, K_i (inhibition constant) values were calculated from the Cheng and Prusoff equation [13]. K_i and K_{act} parameters were determined using computerized iterative non-linear regression curve fitting, with the INPLOT-4 program (GraphPad software; ©1987 by H. J. Motulsky).

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