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Design of New β_1-Selective Adrenoceptor Ligands as Potential Radioligands for In Vivo Imaging

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Abstract—In general, the failing human heart is characterized by a selective reduction in β_1-adrenoceptors (β_1-ARs) without change in β_2-AR density. Medical imaging techniques, either single photon emission computed tomography (SPECT) or positron emission tomography (PET) with appropriate radioligands, offer the possibility of assessing β-adrenoceptor density non-invasively in humans. To date, neither a SPECT nor a PET radioligand is available for the selective imaging of cardiac β_1-ARs. The aim of this study was to develop potential high affinity β_1-selective AR radioligands for the non-invasive in vivo imaging of the β_1-AR density in the human heart using SPECT or PET. A variety of racemic *N*-aryl-*N*′-[2-[3-aryloxy-2-hydroxy-propylamino]-ethyl]-urea derivatives and chain-elongated analogues, related to the established β_1-AR antagonist, ICI 89,406 **8i**, were synthesized. Competition studies using the non-selective AR ligand, [^{125}I]iodocyanopindolol ([^{125}I]ICYP), and ventricular membrane preparations of wild-type mice revealed nine ligands with higher β_1-AR affinities (up to 76-fold) and β_1-AR selectivities (up to 139-fold) than **8i**. Mostly, these ligands possess a 2-substituted phenoxy group and a 4-substituted phenyl residue in contrast to the lead compound **8i**. The non-radioactive counterparts of the desired SPECT- and PET-radiotracers were synthesized as reference compounds [e.g., **8f**, **8g**, **8h** and **8l** as the non-radioactive analogues of the radioiodinated SPECT radioligands, **8e** and **8h** as the non-radioactive compounds of C-11 labelled PET-tracers (C-11 in the methoxy group)]. The established library of high affinity β_1-selective AR antagonists was screened for chemical precursors for the radiosynthesis of the mentioned radioligands. Furthermore, the library consists of some comparison compounds that are unsubstituted, allyl- and alkyl-substituted or chain-elongated (e.g., **8a**, **8j**, **8o** and **8r–t**). Future steps will include radiolabelling and pharmacokinetic evaluation of the β_1-selective target compounds, which could be applied as sympathetic innervation agents for in vivo investigations and diagnostics in patients suffering from cardiac diseases like heart failure and ventricular arrhythmias.

Introduction

The β-adrenoceptor (AR) family is heterogeneous in nature and is subdivided into at least three distinct subtypes, the β_1- and β_2-AR[7] and the atypical β_3-AR.[8,9]

β_1-AR agonists are responsible for the stimulation of heart rate and cardiac contractility, whereas β_2-selective AR agents mainly stimulate bronchodilation and vasodepression. The atypical β_3-ARs are involved in lipolysis. Additionally, a putative subtype has been identified in cardiac tissue, classified as the β_4-AR.[10]

Within the ventricles of the healthy human heart, the β_1/β_2-AR ratio is approximately 3:1.[1] In heart disease, both the β-AR density and the β_1/β_2-AR ratio may change. Several conditions, including hypertension, heart failure, ischemia, hypertrophic and dilated cardiomyopathy (HCM, DCM) are accompanied by a reduced β-AR density in the heart.[11–16] In addition, the failing human

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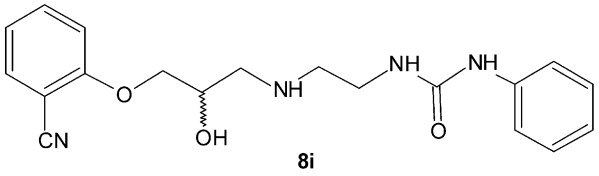


8i

Scheme 1. Lead structure of ICI 89,406 **8i**.

heart is often characterized by a selective reduction in β_1-adrenoceptors (β_1-ARs) without change in β_2-AR density.[1]

Measuring β_1- and β_2-AR densities in vivo independently therefore may prove to be very valuable. Only functional imaging techniques like SPECT or PET allow for the non-invasive investigation of β-AR density and distribution.[2] Selective radiolabelled β-AR antagonists suitable for PET or SPECT, respectively, have yet to be designed to measure β_1- and β_2-AR densities in the human heart.

The only clinically established radioligand for visualization of β-AR density with PET is the non-selective β-AR radioligand (*S*)-[^{11}C]CGP 12177 which was first described as the racemic radioligand in 1991 by Del Forge and coworkers.[17] The recently developed and radiochemically more easily accessible (*S*)-[^{11}C]CGP 12388 has been presented as a promising alternative for non-selective β-AR targeting.[18] Despite the fact that SPECT does not share the unique quantifying capabilities of PET, initial attempts were made in the mid-1990s to develop suitable ligands, for example radioiodinated carazolol and CGP 12177 derivatives for β-AR imaging with SPECT.[19–21]

Up to now, β_1-selective AR antagonists available as drugs for clinical use have been very rare. In cardiac studies of β-ARs, a β_1-selective radioligand is needed precisely because changes of this subtype occur mainly in heart disease. Only few β_1-AR selective radioligands like (±)-[^{11}C]HX-CH 44,[22] (*S*)-[^{11}C]bisoprolol,[23] or [^{11}C]CGP 20712A[24] are in development.[25]

An early example of a designed β_1-selective AR antagonist is the compound ICI 89,406 **8i**,[26] which produces effective β_1-adrenoceptor blockade during exercise in patients with angina pectoris even when applied as the racemate.[27,28] This compound was chosen as lead structure to develop new radiotracers for application in nuclear medical imaging.

The aim of the present study was to develop potential high affinity β_1-selective AR antagonists, based on ICI 89,406 **8i**,[3–6] which could be applied as radioligands with SPECT and PET. The synthesis of ICI 89,406 **8i** was verified and improved by developing a straightforward convergent synthesis via two major fragments, an aromatic epoxide and an aromatic urea, respectively. By inserting different substituents within both aromatic systems a library of compounds was synthesized. The in vitro pharmacology of these β_1-AR antagonists was examined.

Results

Chemistry

ICI 89,406 **8i** (Scheme 1), which is recognized for its β_1-AR selectivity (75- to 195-fold),[29–31] and 20 aromatically functionalized compounds with structural similarities to this (aryloxy)propanolamine lead structure were synthesized. In order to examine the structure–activity relationships (SARs) between the potential ligands and β_1-ARs, the prepared compounds were modified in the pattern of aromatic substituents and in length of the aliphatic chain. These compounds are ligands that may be useful for elucidating the SAR of β_1-ARs, potential precursors of the corresponding radiolabelled derivatives, or the non-radioactive references of these radioactive counterparts. Four of these compounds were previously described in literature as potential β_1-selective AR antagonists (**8a**, **8i**, **8q**, **8t**).[26,32,33] Most of the substances are accessible via a convergent five-step synthesis (Scheme 2), starting with the conversion of the corresponding alcohols **1a–h** and 3-chloro-1,2-epoxypropane

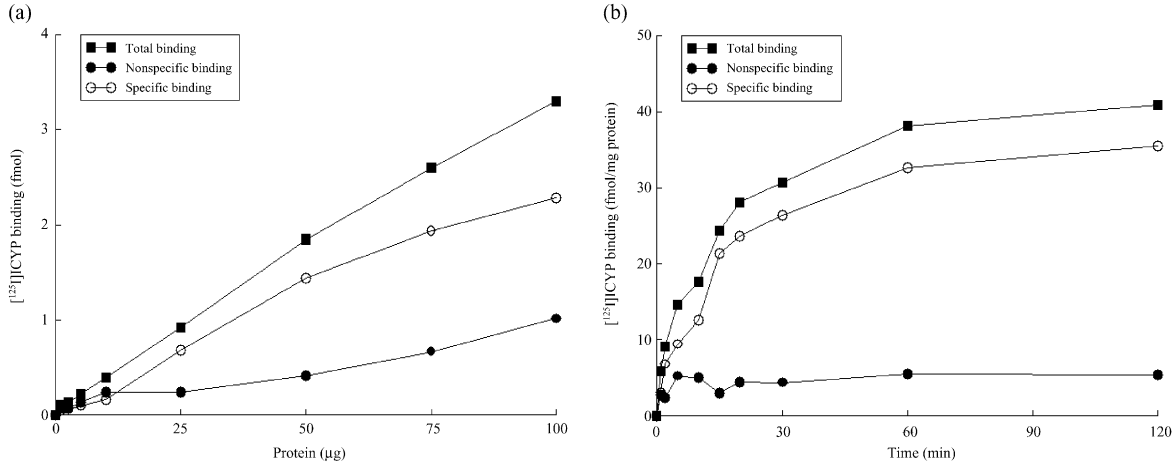
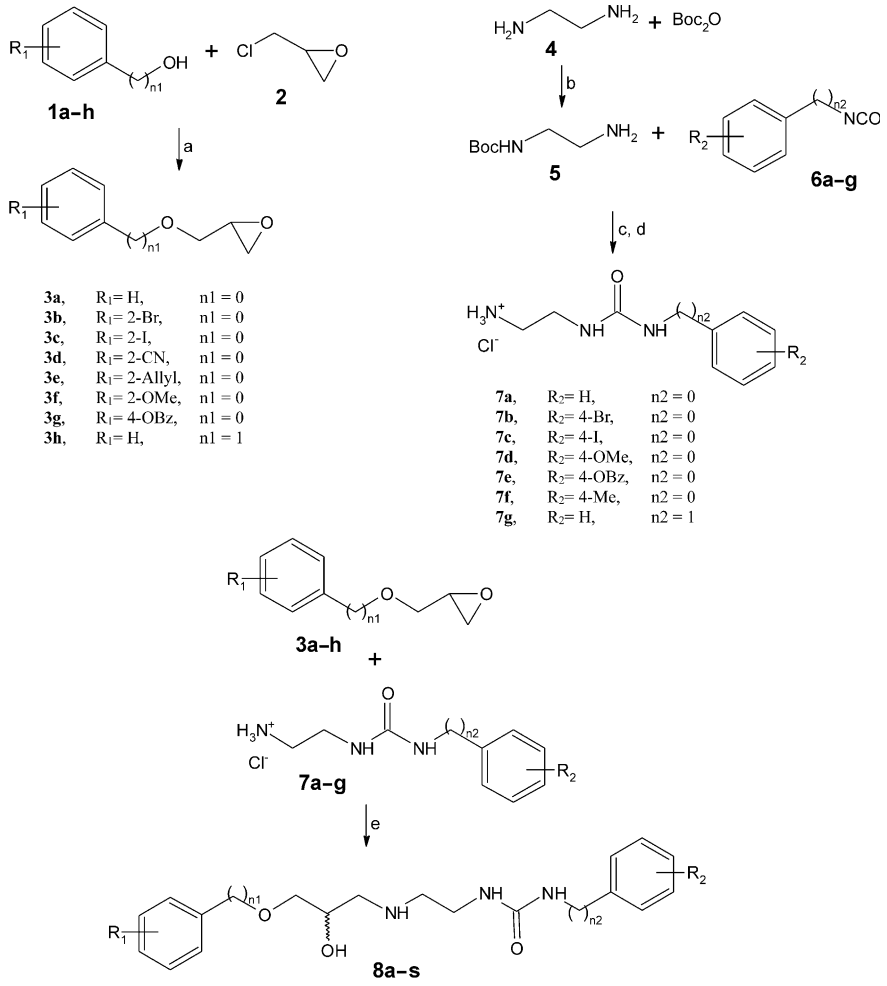


Figure 1. (a) Protein dependence of [^{125}I]ICYP binding to mouse ventricular membrane preparations. The specific binding was linear for up to 50 µg protein/200 µL; (b) time course of [^{125}I]ICYP binding to mouse ventricular membranes. The association rate was linear for 15 min and reached a steady state after 60 min.



Scheme 2. Synthesis of the β_1-AR ligands **8a–s**. Reagents: (a) 2N NaOH or Na, toluene for compound **3h**; (b) CH_2Cl_2; (c) Et_2O or THF; (d) HCl, MeOH; (e) 10N NaOH, *n*-PrOH, H_2O.

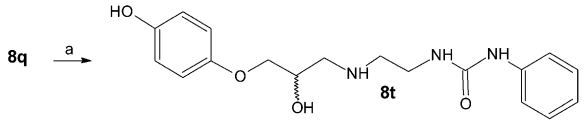
2, which results in the first key compounds, the 1,2-epoxy-3-substituted-propane derivatives **3a–h**. The second key structures, the *N*-(2-amino-ethyl)-urea derivatives **7a–g**, were prepared via a three step sequence (Scheme 2 continued). At first ethylendiamine **4** is protected by a Boc-group in order to couple it with the aromatic isocyanates **6a–g**. After cleavage of the Boc-group, ring opening of the 1,2-epoxy-3-substituted-propanes **3a–h** with the *N*-(2-amino-ethyl)-urea compounds **7a–g** resulted in the desired potential β_1-AR antagonists **8a–s** (Scheme 2 continued and Table 1).

Compound **8t** was prepared via a hydrogenolysis from the benzyloxy substituted precursor **8q** (Scheme 3), while the carbamate **8u** was built by the reaction of **3d** and **5** (Scheme 4).

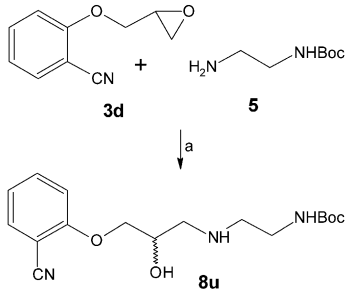
Biology

First, the protein dependence of [^{125}I]ICYP binding to mouse ventricular membrane preparations was investigated. The specific binding increased linearly with the protein concentration for up to 50 μg/200 μL (Fig. 1a). Therefore, 15 μg protein per 200 μL were applied for the in vitro binding studies.

The time course of [^{125}I]ICYP binding to β-ARs is shown in Figure 1b. The binding reaction was linear for approximately 15 min and reached equilibrium after 60 min of incubation. To determine the maximum binding, assays were carried out for 60 min.



Scheme 3. Synthesis of the β_1-AR ligand **8t**. Reagents: (a) H_2, Pd/C, CH_3COOH.



Scheme 4. Synthesis of the β_1-AR ligand **8u**. Reagents: (a) *n*-PrOH.

The binding of the nonselective β-AR antagonist [^{125}I]ICYP to ventricular membranes was specific, saturable and of high affinity (Fig. 2a). Scatchard transformation of the saturation data yielded a linear plot with a correlation coefficient greater than 0.95 (Fig. 2b). The dissociation constant (K_D) and the maximum number of binding sites (B_{max}) determined from three experiments for the binding of [^{125}I]ICYP were 32.3±1.9 pM and 38.6±2.9 fmol mg^{-1} protein, respectively.

Competition studies were carried out to assess the inhibition of the binding of [^{125}I]ICYP by the β_1-selective lead compound ICI 89,406 **8i** and its derivatives (**8a–h** and **8j–u**) (Fig. 3a–e). Non-linear regression analysis using the XMGRACE programme (Linux software) showed that the data of the compounds **8a–o**, **8q** and **8u** fitted a two-site model significantly better than a one-site model ($F=3.52$, $P<0.05$). These compounds show a significantly higher affinity to β_1- than to β_2-ARs (Table 1).

The high- and low-affinity IC_{50} values of the unlabelled derivatives **8a–u** for the β_1- and β_2-ARs, respectively, were calculated by non-linear regression analysis of competition studies using [^{125}I]ICYP and mouse ventricular membrane preparations.

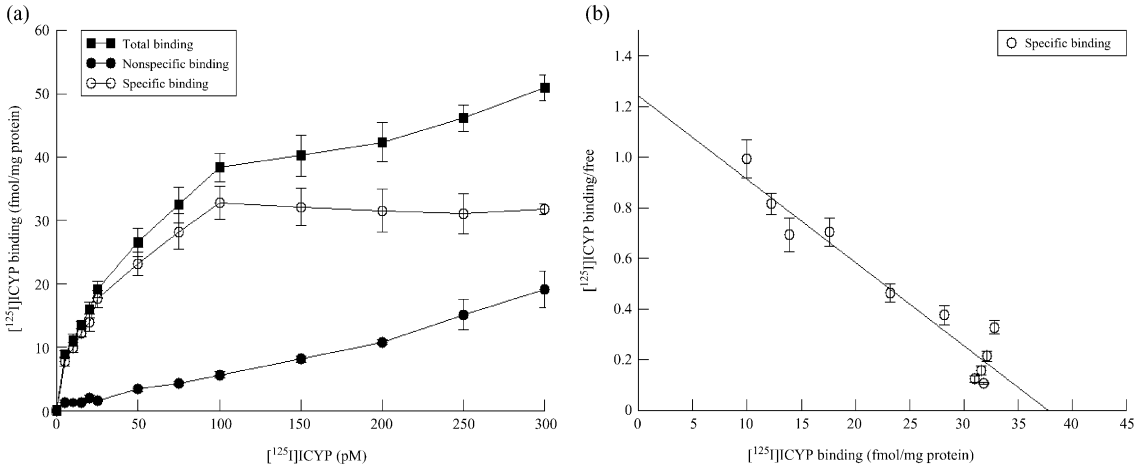


Figure 2. (a) Saturation curve for the specific binding of [^{125}I]ICYP to mouse ventricular membrane preparations. Each point represents the mean±SEM ($n=3$). (b) Scatchard transformation of the saturation isotherm yielded a dissociation constant (K_D) of 32.3±1.9 pM and a maximum density of β-AR (B_{max}) of 38.6±2.9 fmol mg^{-1} protein. Each point represents the mean±SEM ($n=3$).

Table 1. Inhibition constants and calculated β_1-selectivities of compounds **8a–u** obtained via a radioligand binding assay using mouse ventricular membrane preparations, plus calculated logP/logD values

Compd	R_1	n1	n2	R_2	K_{I1} (nM)[a]	K_{I2} (nM)[a]	β_1-selectivity[b]	logP/logD[c]
8a	H	0	0	H	8.1±0.6	940±200	113±16	1.36/0.07
8b	H	0	0	4-Br	1.8±0.4	260±40	143±15	2.25/0.95
8c	2-Br	0	0	H	1.8±0.4	120±20	75±14	2.24/0.94
8d	2-Br	0	0	4-Br	0.19±0.03	74±11	403±79	3.12/1.83
8e	2-Br	0	0	4-OMe	0.04±0.016	140±50	5220±1890	2.19/0.90
8f	2-I	0	0	H	0.045±0.005	12±2	266±28	2.49/1.19
8g	2-I	0	0	4-I	0.043±0.011	34±5	905±194	2.45/1.15
8h	2-I	0	0	4-OMe	0.55±0.11	390±160	783±362	2.45/1.15
8i[d]	2-CN	0	0	H	1.3±0.2	160±40	121±14	1.73/0.44
8j	2-CN	0	0	4-Me	0.017±0.002	89±13	5340±182	1.71/0.41
8k	2-CN	0	0	4-Br	0.017±0.007	260±120	16,800±4200	2.16/0.86
8l	2-CN	0	0	4-I	0.12±0.04	44±16	398±48	2.41/1.11
8m	2-CN	0	0	4-OMe	1.8±0.8	120±60	82±33	1.23/−0.07
8n	2-CN	0	0	4-OBz	0.022±0.006	64±9	4020±1570	2.83/1.54
8o	2-allyl	0	0	H	0.80±0.13	27±5	35±7	1.99/0.69
8p	2-OMe	0	0	H	1205±72	1205±72	1.0[e]	1.33/0.03
8q	4-OBz	0	0	H	30±4	2700±300	96±19	2.93/1.63
8r	2-CN	0	1	H	61±7	61±7	1.0[e]	1.00/−0.29
8s	H	1	1	H	3500±820	3500±820	1.0[e]	0.87/−0.55
8t	4-OH	0	0	H	150±30	150±30	1.0[e]	0.82/−0.94
8u[f]					35±1	16,000±8000	489±260	1.27/−0.03

[a]Displacement of specifically bound non-selective β-AR ligand [^{125}I]ICYP binding at β_1- and β_2-ARs expressed in mouse ventricular membrane preparations, noted as mean±SEM, $n=4$–6.
[b]The ratios of the low- over the high-affinity inhibition constants (K_{I2}/K_{I1}) yield the β_1-selectivities of the unlabelled derivatives **8a–u**, noted as mean±SEM, $n=4$–6.
[c]logP and logD values calculated by Pallas 3.0 (logD = logP at physiological pH 7.4).
[d]**8i** is the lead compound ICI 89,406.
[e]If the two-site model was rejected by the *F*-ratio test the one site model was applied and the β_1-selectivity was set to 1.0.
[f]The structure of **8u** is shown in Scheme 4.

The IC_{50} values were converted into the high- and low-affinity inhibition constants (K_{I1} for the β_1-ARs and K_{I2} for the β_2-ARs) by the method of Cheng–Prusoff[34] using the experimentally determined K_D value of [^{125}I]ICYP (32.3 ± 1.9 pM).

The ratios of the low- to high-affinity inhibition constants (K_{I2}/K_{I1}) yield the β_1-selectivities of the unlabelled derivatives **8a–u** (Table 1). Additionally, the calculated logP and logD values (Pallas 3.0) for compounds **8a–u** are also listed in Table 1 to imply the changes of the lipophilicities caused by the chemical modifications of the lead compound **8i**, ICI 89,406.

Substitution of one phenyl hydrogen in **8a** by a bromine atom in 4-position decreases the K_{I1}-value about 4-fold, so that the β_1-selectivity of **8b** raises by 27%. The exchange of the phenoxy hydrogen by bromine in 2-position results in an opposite effect. In fact the K_{I1}-values of **8b** and **8c** are identical (K_{I1} = 1.8 nM); but in comparison with **8b** the β_1-selectivity of **8c** is nearly halved (143 vs 75). The substitutions of **8b** and **8c** combined in **8d** leads to a very potent β_1-AR ligand, that shows a more than 6-fold higher affinity for β_1-AR (K_{I1} = 0.19 nM) and a 3-fold higher β_1-selectivity (403) in comparison with the lead compound ICI 89,406 **8i**. Formally, changing the bromine in

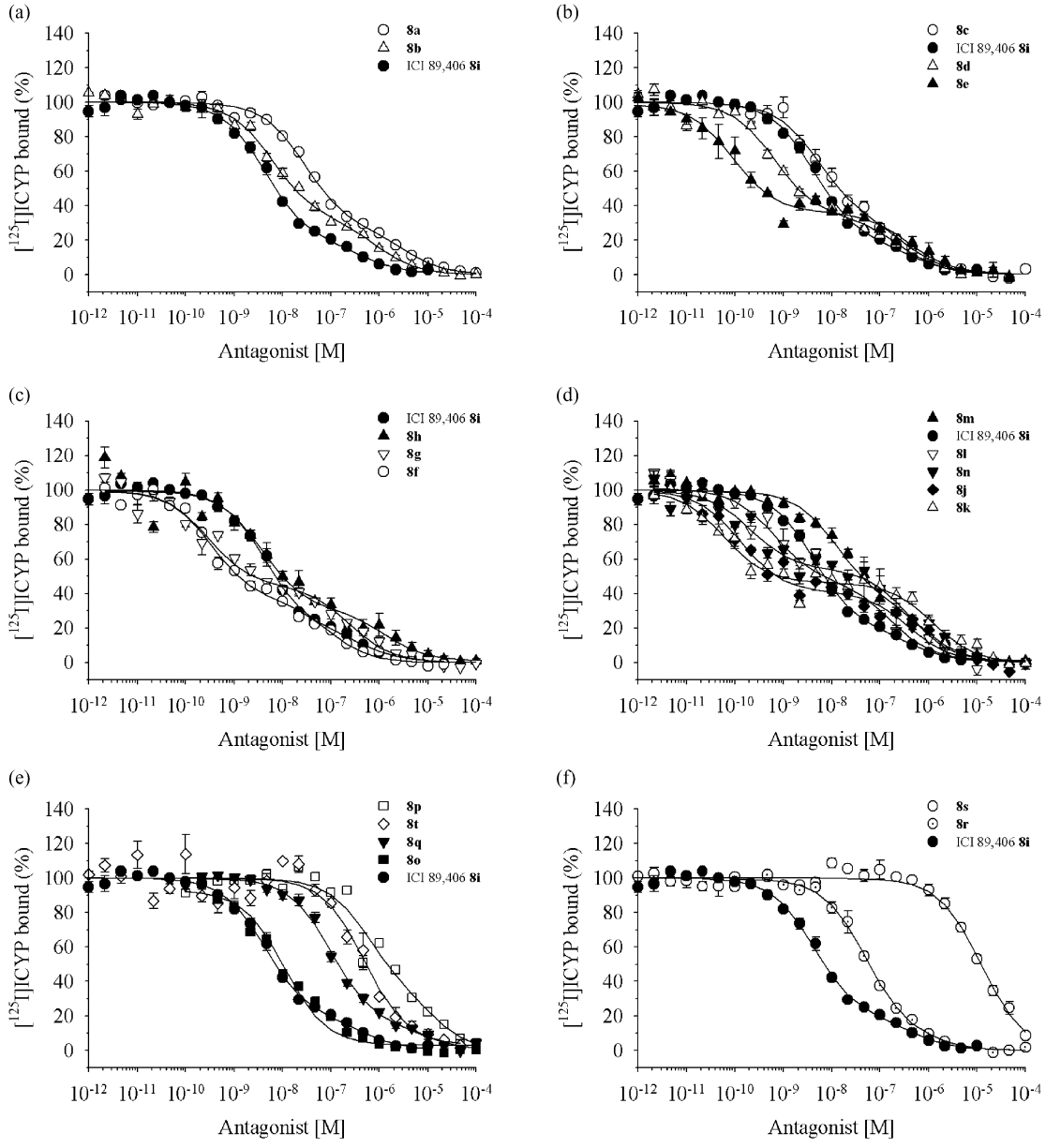


Figure 3. Competition of ICI 89,406 (**8i**) and its derivatives: (a) with a hydrogen substituent replacing the cyano moiety of **8i** (**8a–b**); (b) with a bromine substituent replacing the cyano moiety of **8i** (**8c–e**); (c) with an iodine substituent replacing the cyano moiety of **8i** (**8f–h**); (d) without a substutition of the cyano moiety of **8i** (**8j–n**); (e) with different substituents replacing the cyano moiety of **8i** (**8o–q, 8t**); (f) with a change in the chain length of the carbon skeleton (**8r–s**) with [^{125}I]ICYP binding to mouse ventricular membranes. Ordinate: specific [^{125}I]ICYP binding expressed as percentage of maximum binding in each experiment. Abscissa: concentration of antagonists competing for specific binding of [^{125}I]ICYP. Each point represents the mean ± SEM (n = 4–6). See Table 1 for details of substituents.

the phenyl residue into a methoxy group (**8e**) increases the β_1-selectivity dramatically, because compound **8e** possesses a lower K_{I1}-value (0.04 nM) than **8d**. The bromo derivatives **8b–e** are potential precursor compounds of the corresponding radioiodinated substances (Br/*I-exchange), while the highly β_1-selective compound **8e** can act additionally as the non-radioactive reference for the PET-tracer [methoxy-^{11}C]**8e**, in which the positron emitter C-11 is introduced within the methyl group of the methoxy moiety.

The iodinated compounds **8f** and **8g** exhibit lower affinity-constants K_{I1} and higher β_1-selectivities than the bromo counterparts **8c** and **8d**. In contrast to **8c** and **8d**, their K_{I1}-values are within the same order of magnitude ($\approx$0.04 nM). In comparison with the methoxy compound **8e** the iodinated counterpart **8h** shows a lower affinity to the β_1-AR (0.04 nM vs 0.55 nM). Anyhow, the β_1-selectivity of **8h** ranges between **8f** and **8g**. The mentioned iodinated substances **8f-h** serve as the non-radioactive references for the future radioiodinated and C-11-methylated compounds.

The series of cyano derivatives **8i–n** exhibit very different β_1-affinities and selectivities. Compared with the other cyano substances, the lead structure ICI 89,406 **8i** and compound **8m** possess both moderate affinity inhibition constants K_{I1} and β_1-selectivities (1.3 nM, 121 and 1.8 nM, 82, respectively). These parameters can be improved by introducing a more hydrophobic substituent in 4-position at the phenyl moiety (**8j–l** and **8n**). The exchange of hydrogen in **8i** for a methyl, bromo or benzyloxy function (**8j**, **8k** and **8n**) leads to a potent increase in the β_1-affinities ($\geq$59-fold in comparison with **8i**) and β_1-selectivities ($\geq$33-fold in comparison with **8i**), respectively. A weakening effect has been observed in the case of the iodo analogue **8l**. This potential reference compound for a feasible radioiodinated SPECT-tracer demonstrates a K_{I1}-value of 0.12 nM and a β_1-selectivity of 398.

Replacing the cyano moiety of **8i** by an allyl group, forming compound **8o**, leads to a notable decrease of β_1-selectivity (35). A complete loss of β_1-selectivity can be observed with the 2-methoxy substituted derivative **8p**. In addition, a loss of β_1-selectivity can also be found if the phenyl group of **8i** is replaced by a benzyl residue (**8r**) or if both the phenyl and the phenoxy groups are replaced by the corresponding benzyl and benzyloxy residues (**8s**).

Examples for 4-substituted phenoxy compounds are the benzyloxy derivative **8q** and the hydroxy analogue **8t**. Thereby, **8q** is a moderate β_1-AR ligand (K_{I1} = 30 nM, β_1-selectivity = 96), while **8t** completely loses its β_1-selectivity.

The *tert*-butyl-carbamate **8u**, containing only one aromatic subunit, shows no β_2-affinity (K_{I2} = 16,000 nM) but a moderate β_1-affinity (K_{I1} = 35 nM) resulting in a β_1-selectivity of 489.

Discussion

The human heart contains a heterogenous population of β_1- and β_2-ARs, both mediating positive inotropic, chronotropic, dromotropic and lusitropic effects of catecholamines.[30,35] In the normal human heart, β_1-ARs play the major role in the adrenergic control of myocardial function.[36] They account for approximately 60–80% of cardiac β-Ars.[37,38] In heart failure the cardiac and systemic adrenergic drive is activated in order to maintain cardiac function. This enhanced adrenergic stimulation leads to a downregulation of myocardial β-ARs.[39,40] In most studies a selective downregulation of the β_1-ARs is predominant whereas the β_2-ARs appear to be unaffected.[36,41,42]

With functional imaging procedures like SPECT and PET it is possible to study the behaviour and fate of radiolabelled receptor ligands in vivo, for example their biodistribution, the plasma and tissue kinetics and specific and non-specific binding. Receptors can be studied in their natural environment and their distribution and density can be assessed.

Radioligands suitable for non-invasive β-AR imaging should exhibit high selectivity and affinity (nanomolar K_D) as well as antagonist action for stable receptor-ligand interactions.[25] In addition, stereoselectivity is an important criterion for β-AR ligands. Similar to the endogenous (*S*)-enantiomers of epinephrine and norepinephrine, the (*S*)-enantiomers of β-AR antagonists show approximately a 100-fold higher affinity to β-AR than the corresponding (*R*)-enantiomers.[43] Development of (*S*)-enantiomers of new β-AR radioligands rather than racemates should reduce non-specific binding. Nevertheless, we decided to synthesize the racemic forms of **8a–u** in order to be able to build up a library of compounds in a fast and cost-effective manner. From this library highly selective and affine candidates could be chosen for enantioselective and radiochemical resynthesis for detailed evaluation in vitro and in vivo. A further criterion for cardiac β-AR radioligands is a low lipophilicity. Radioligands with high lipophilicity can traverse the sarcolemmal membrane and bind to internalized, that is inactive, β-ARs. This in turn precludes the exact quantification of cardiac β-AR densities in vivo with PET. In addition, high lipophilicity (e.g., logP $>>$ 2, see also calculated logD values = logP at physiological pH 7.4, Table 1) results in a high degree of nonspecific binding, especially in the lungs. Pulmonary trapping reduces the myocardial contrast of the radioligand after intravenous injection.

Furthermore, low metabolism of the putative radioligand, at least in the myocardium itself, is an important aspect to simplify kinetic modelling and quantification of β-AR densities with PET. In addition, low toxicity and mutagenicity of the radioligand is a prerequisite for applications in the clinical setting. Last but not least, the putative tracer has to be amenable to labelling with gamma emitters for SPECT and positron emitter for PET.

Nonselective radioligands for β-ARs such as [^{11}C]CGP 12177 and [^{11}C]CGP 12388 have been successfully used in vivo with PET.[15,17,44–46] In addition, the β_2-selective radioligand [^{11}C]formoterol may provide an unique approach to quantify β_2-AR density with PET.[47] To date however, no β_1-selective radioligand suitable for the non-invasive assessment of cardiac β_1-ARs has been established clinically either for SPECT or for PET.

For the present study ICI 89,406 **8i** was chosen as the lead compound for β_1-AR radioligand development on the basis of its high affinity and selectivity for β_1-ARs, moderate lipophilicity and amenability to labelling with [^{123}I]iodine and [^{11}C]carbon. Furthermore, this compound produces effective β_1-AR blockade even when applied as racemate.[27,28] In order to find suitable precursor and reference compounds for the development of potential β_1-AR PET and SPECT tracers, a series of new 2-propanol derivatives have been synthesized by modifying ICI 89,406 **8i**. The substituents of the aromatic groups have been slightly altered, partly varying the chain length of the carbon skeleton. A few of the compounds (e.g., non-precursor compounds **8a**, **8j**, **8n**, **8o** and **8q**) function as derivatives that help to clarify the change of β_1-affinity and β_1-selectivity caused by introduction of small substituents into the phenoxy and phenyl residues. The prepared substances were tested in competition studies using [^{125}I]ICYP and mouse ventricular membrane preparations to calculate their high- and low-affinity inhibition constants K_{I1} and K_{I2}. Ten compounds were identified that possess a higher β_1-affinity (K_{I1} <1.3 nM) than the lead compound **8i**, and 11 derivatives had a higher β_1-selectivity (>121) than **8i**. Nine of the derivatives show higher β_1-affinities as well as higher β_1-selectivities.

In eight of these compounds the improvement of both the β_1-affinity and the β_1-selectivity was achieved by substitution of both aromatic groups, especially by altering the phenoxy group at the 2-position and the phenyl group at the 4-position.

The mentioned potent β_1-AR ligands show this characteristic substitution pattern with the exception of **8f**. The combination of the 2-bromo residue and the 4-bromo or 4-methoxy group (**8d** and **8e**), the combination of the 2-iodo and the 4-iodo or 4-methoxy subunit (**8g** and **8h**), and the assembly of the 2-cyano and the 4-methyl, 4-bromo, 4-iodo or 4-benzyloxy functions (**8j**, **8k**, **8l** and **8n**) lead to very β_1-affine and β_1-selective AR compounds that are up to 139-fold more selective than **8i**.

An electrostatic interaction between a conserved aspartate on transmembrane helix 3 in cationic ligand GPCRs (G protein-coupled-receptors), which include the β_1-ARs, and the cationic neurotransmitter is suggested. In compounds **8a–u** the cationic centre is represented by the protonated secondary amine moiety. A second interesting feature of cationic neurotransmitter GPCRs is the presence of a cluster of conserved aromatic residues that encages the ammonium-aspartate ion pair. This hydrophobic aromatic cluster is able to form attractive interactions with the aromatic and/or hydrophobic counterparts of the ligands.[48–51] In fact the disubstituted affine ligands **8d–e**, **8g–h**, **8j–l** and **8n** possess at least one hydrophobic or aromatic substituent in 2- or 4-position of their phenyl cores that obviously increases the β_1-affinities. Thereby the β_2-affinities are not increased in the same manner resulting in high β_1-selectivities.

A monosubstitution of the phenoxy ring in 2-position with a sterical demanding or π-electron-donor moiety (**8c**, **8f**, **8i**, **8o**) increases the β_1-affinities in comparison to the unsubstituted compound **8a**. Obviously, such substituents extends the attractive interactions with the aromatic residue cage of β_1-ARs without improving β_1-selectivities significantly. In contrast, a complete loss of β_1-selectivity can be observed in the case of a methoxy group inserted at the 2-position of the phenoxy unit (**8p**). Additionally, a benzyloxy or especially a hydroxy moiety in 4-position (**8q**, **8t**) decreases the β_1-affinities and β_1-selectivities in comparison to the unsubstituted counterpart **8a**.

The carbon skeleton of **8i** tolerates neither one phenyl-to-benzyl substitution (**8r**) nor a replacement of both phenyl subunits of **8a** by benzyl groups (**8s**) because both substitutions lead to a loss of β_1-selectivity and decreased β_1-affinity. A similar effect was observed by Nudelman et. al. comparing classical 3-aryloxy-2-propanolamine β_1-AR ligands with their more rigid 4-aryl-3-butenyl-2-ol-amine analogues. The variation of increased rigidity also results in decreased β_1-affinity.[52]

Conclusion

A library of derivatives of the known β_1-selective antagonist ICI 89,406 **8i** was synthesized and their binding to murine ventricular membranes examined in vitro in order to identify potent β_1-affine and β_1-selective AR ligands as possible radiotracers for assessing β_1-ARs in vivo via SPECT and PET. The next step is to chose a β_1-selective AR compound and prepare its (*S*)-enantiomer and optical pure radiolabelled analogue as a possible β_1-AR radioligand for in vivo diagnostics of the human heart.

The bromo species **8c**, **8d**, **8e** and **8k** could function as precursor compounds for the radioiodination of the hot counterpart of **8f**, **8g**, **8h** and **8l** via the commonly used [*I]iodo-debromination-reaction.

The highly β_1-affine and β_1-selective methoxy derivatives **8e** und **8h** may represent the non-radioactive analogues of the C-11 labelled PET-tracers, that may be prepared from the corresponding phenolic compounds by methylation using [^{11}C]methyliodide.

Further investigations concerning the radiosynthesis of the above mentioned compounds as feasible radioligands in β_1-AR imaging and the in vivo behaviour of these 2-propanol derivatives are in progress. SPECT and PET using these β_1-selective radioligands may have

a great impact on the diagnostic and therapeutic management of patients with cardiac diseases in the future.

Experimental

Synthetic methods

All chemicals, reagents, and solvents for the synthesis of the compounds were analytical grade and purchased from commercial sources.

The melting points (uncorrected) were determined on a Stuart Scientific SMP3 capillary melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 200, ARX 300, and AMX 400 spectrometer, respectively. Mass spectrometry was performed via a Varian MAT 212 (EI = 70 eV) spectrometer and a Bruker MALDI-TOF-MS Reflex IV (matrix: DHB). GC–MS analysis was undertaken with a Dani 8521 gas chromatograph containing a DB 5, 30 m column (temperature program: start 60 °C, gradient 20 °C/min, end 280 °C) combined with an ITD 800 Finnigan MAT (EI = 70 eV) mass spectrometer. Elemental analysis was realised by a Vario EL III analyser (see Table 2).

Synthesis of oxirane derivatives 3a–g: general procedure (Scheme 2)

3 Equivalents **2**, 1 equivalent phenol compound **1a–g** and 1.4 equivalent 2 N NaOH were heated to 50 °C for 1.5–2.5 h.

At rt, the mixture was extracted three times with $CHCl_3$ or CH_2Cl_2, the combined organic layers were dried ($MgSO_4$) and the solvent and volatile compounds were evaporated. In most cases, the residue was destilled in vacuo fractionally or in a Kugelrohr to provide the epoxide derivatives as colorless liquids.

3d and **3g** were purified via crystallization from diethylether to provide colorless solids.

2-Phenoxymethyl-oxirane 3a. Yield: 74%. Bp (0.94 mbar): 115 °C [lit.: 105 °C (0.08 Torr)[53]]. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 7.55–7.50 (m, 2H, H_{Aryl}), 7.24–7.15 (m, 3H, H_{Aryl}), 4.42 (dd, ^2J = 11.1 Hz, ^3J = 3.0 Hz, 1H, 1 CH_2), 4.15 (dd, ^2J = 11.1 Hz, ^3J = 5.7 Hz, 1H, 1 CH_2), 3.58–3.52 (m, 1H, CH), 3.08 (dd, ^2J = 4.8 Hz, ^3J = 4.2 Hz, 1H, 1 CH_2), 2.90 (dd, ^2J = 5.0 Hz, ^3J = 2.6 Hz, 1H, 1 CH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ (ppm): 158.98, 129.77, 121.64, 115.27, 69.16, 50.53, 45.04.

2-(2-Bromo-phenoxymethyl)-oxirane 3b. Yield: 71%. Bp (1.4 mbar): 135 °C [lit.: 131 °C (3 Torr)[54]]. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 7.48 (dd, ^3J = 7.8 Hz, ^4J = 1.8 Hz, 1H, H_{Aryl}), 7.22–7.16 (m, 1H, H_{Aryl}), 6.87 (dd, ^3J = 8.4 Hz, ^4J = 1.8 Hz, 1H, H_{Aryl}), 6.80 (dt, ^3J = 7.6 Hz, ^4J = 1.3 Hz, 1H, H_{Aryl}), 4.23 (dd, ^2J = 11.3 Hz, ^3J = 2.9 Hz, 1H, 1 CH_2), 3.99 (dd, ^2J = 11.1 Hz, ^3J = 5.1 Hz, 1H, 1 CH_2), 3.35–3.30 (m, 1H, CH), 2.84 (dd, ^2J = 5.1 Hz, ^3J = 4.2 Hz, 1H, 1 CH_2), 2.78 (dd, ^2J = 5.1 Hz, ^3J = 2.7 Hz, 1H, 1 CH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ (ppm): 154.94, 133.41, 128.42, 122.50, 113.97, 112.47, 69.61, 49.98, 44.54.

2-(2-Iodo-phenoxymethyl)-oxirane 3c. Yield: 75%. Bp (3.0 mbar): 140 °C [lit.: 118–123 °C (0.2 Torr)[55]] ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 7.84 (dd, ^3J = 7.3 Hz, ^4J = 1.6 Hz, 1H, H_{Aryl}), 7.38–7.34 (m, 1H, H_{Aryl}), 6.91 (dd, ^3J = 8.4 Hz, ^4J = 1.2 Hz, 1H, H_{Aryl}), 6.80 (dt, ^3J = 7.6 Hz, ^4J = 1.5 Hz, 1H, H_{Aryl}), 4.35 (dd, ^2J = 11.2 Hz, ^3J = 2.8 Hz, 1H, 1 CH_2), 4.13 (dd, ^2J = 11.2 Hz, ^3J = 5.2 Hz, 1H, 1 CH_2), 3.48–3.44 (m, 1H, CH), 3.00–2.89 (m, 2H, 2 CH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ (ppm): 157.55, 139.98, 129.91, 123.58, 113.22, 87.14, 70.12, 50.47, 45.13.

2-(2-Cyano-phenoxymethyl)-oxirane 3d. Yield: 71%. Mp 65 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 7.51–7.42 (m, 2H, H_{Aryl}), 6.99–6.93 (m, 2H, H_{Aryl}), 4.30 (dd, ^2J = 11.5 Hz, ^3J = 3.0 Hz, 1H, 1 CH_2), 4.05 (dd, ^2J = 11.3 Hz, ^3J = 5.2 Hz, 1H, 1 CH_2), 3.34–3.29 (m, 1H, CH), 2.84 (dd, ^2J = 5.0 Hz, ^3J = 4.0 Hz, 1H, 1 CH_2), 2.76 (dd, ^2J = 5.1 Hz, ^3J = 2.6 Hz, 1H, 1 CH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ (ppm): 160.51, 134.75, 133.97, 121.78, 116.57, 113.20, 102.74, 69.71, 50,18, 44.81.

2-(2-Allyl-phenoxymethyl)-oxirane 3e. Yield: 66%. Bp. (2.2 mbar): 130 °C [lit.: 109–114 °C (0.8 Torr)[56]]. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm): 7.24–7.13 (m, 2H, H_{Aryl}), 6.94–6.81 (m, 2H, H_{Aryl}), 6.06–5.89 (m, 1H, =CH), 5.11–5.00 (m, 2H, $=CH_2$), 4.22 (dd, ^2J = 11.1 Hz, ^3J = 3.1 Hz, 1H, 1 CH_2), 3.98 (dd, ^2J = 11.0 Hz, ^3J = 5.3 Hz, 1H, 1 CH_2), 3.37–3.31 (m, 3H, CH_2 and CH), 2.91 ('t', ^3J = 4.5 Hz, 1H, 1 CH_2), 2.78 (dd, ^2J = 5.0 Hz, ^3J = 2.7 Hz, 1H, 1 CH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ (ppm): 156.11, 136.83, 129.90, 129.00, 127.24, 121.11, 115.35, 111.66, 68.74, 50.20, 44.49, 34.26.

2-(2-Methoxy-phenoxymethyl)-oxirane 3f. Yield: 71%. Bp (1.2 mbar): 120 °C [lit.: 131 °C (3 Torr)[57]]. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 6.95–6.82 (m, 4H, H_{Aryl}), 4.19 (dd, ^2J = 11.4 Hz, ^3J = 3.6 Hz, 1H, 1 CH_2), 4.00 (dd, ^2J = 11.4 Hz, ^3J = 5.4 Hz, 1H, 1 CH_2), 3.83 (s, 2H, CH_2), 3.36–3.31 (m, 1H, CH), 2.84 (dd, ^2J = 5.0 Hz, ^3J = 4.1 Hz, 1H, 1 CH_2), 2.69 (dd, ^2J = 5.0 Hz, ^3J = 2.6 Hz, 1H, 1 CH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ (ppm): 149.88, 148.18, 122.06, 120.95, 114.66, 112.25, 70.40, 55.97, 50.25, 44.90.

2-(4-Benzyloxy-phenoxymethyl)-oxirane 3g. Yield: 71%. Mp 62 °C (lit.: 63–66 °C[58]). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 7.43–7.30 (m, 5H, H_{Aryl}), 6.92–6.82 (m, 4H, H_{Aryl}), 5.01 (s, 2H, 2 CH_2), 4.15 (dd, ^2J = 11.1 Hz, ^3J = 3.3 Hz, 1H, 1 CH_2), 3.91 (dd, ^2J = 11.3 Hz, ^3J = 5.6 Hz, 1H, 1 CH_2), 3.34–3.29 (m, 1H, CH), 2.87 (dd, ^2J = 5.0 Hz, ^3J = 4.1 Hz, 1H, 1 CH_2), 2.72 (dd, ^2J = 5.1 Hz, ^3J = 2.7 Hz, 1H, 1 CH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ (ppm): 137.38, 152.88, 137.19, 128.46, 127.80, 127.36, 115.94, 115.71, 70.66, 69.48, 50.16, 44.62.

2-Benzyloxymethyl-oxirane 3h. This was prepared according to ref 59, but was purified via Kugelrohr-destillation. Yield: 3%. Bp (1.2 mbar): 90-100 °C [lit.:

85–86 °C (2.0 Torr)[60]]. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 7.32–7.28 (m, 5H, H_{Aryl}), 4.62 (d, ^2J = 12.0 Hz, 1H, 1 CH_2), 4.55 (d, ^2J = 12.0 Hz, 1H, 1 CH_2), 3.76 (dd, ^2J = 11.6 Hz, ^3J = 3.2 Hz, 1H, 1 CH_2), 3.45 (dd, ^2J = 11.4 Hz, ^3J = 5.7 Hz, 1H, 1 CH_2), 3.21–3.16 (m, 1H, CH), 2.79 ('t', ^3J = 4.5 Hz, 1H, 1 CH_2), 2.61 (dd, ^2J = 5.0 Hz, ^3J = 2.6 Hz, 1H, 1 CH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ (ppm): 137.98, 128.44, 127.76, 73.37, 70.85, 50.86, 44.30.

Synthesis of *N*-mono(*tert*-butyloxycarbonyl)ethylenediamine 5 (Scheme 2 continued). 16.35 g (74.9 mmol) Boc_2O, dissolved in 150 mL anhydrous CH_2Cl_2, were added slowly (3 h) to a solution of 14.08 g (234 mmol) **4** in 150 mL anhydrous CH_2Cl_2. The mixture was stirred 21 h at rt and filtered by suction. The solid filter cake was washed with 50 mL CH_2Cl_2 three times and the combined organic layers were evaporated to dryness. The residue was fractionated in vacuo, using a short vigreux-column, to give **5** as a pale yellow oil. Yield: 9.01 g (56.2 mmol), 75%. Bp (30 mbar): 102–105 °C [lit.: 105 °C (20 Torr)[61]]. ^{1}H NMR (300 MHz, $CDCl_3$, TMS intern): δ (ppm): 4.90 (broad, s, 1H, NH), 3.16 (q, ^3J = 5.9 Hz, 2H, CH_2), 2.81 (t, ^3J = 5.9 Hz, 2H, CH_2), 1.45 (s, 9H, 3 CH_3), 1.21 (s, 2H, NH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$, TMS intern): δ (ppm): 156.27, 79.20, 43.56, 41.96, 28.31.

Synthesis of urea hydrochloride derivatives 7a–g: general procedure (Scheme 2 continued)

5 was dissolved in anhydrous diethylether or THF (approximately 1.7 mL per mmol **5**). At 0 °C an equimolar amount of **6a–g** was added within 30 min and the mixture was stirred for further 30 min at 0 °C.

It was filtered, the filter cake was washed with diethylether and dried in vacuo to provide the raw *N*-aryl/benzyl-*N*′-[2-[(*tert*-butoxycarbonyl)amino]ethyl]-urea derivatives as colorless solids.

These compounds were dissolved in a concd HCl/MeOH mixture (1:1 v/v, approximately 720 μL per mmol). The solvent was evaporated at 45–50 °C in vacuo (99–53 mbar) within 2.5 h. The oily residues were treated with anhydrous acetone (approximately 2.2 mL per mmol) and the solvent was removed in vacuo. This procedure was repeated four times to provide **7a–g** as colorless solids.

***N*-(2-Amino-ethyl)-*N*′-phenyl-urea hydrochloride 7a.** Yield: 84%. Mp 189 °C (lit.: 190–191 °C[62]). ^{1}H NMR (200 MHz, DMSO-d_6, TMS intern): δ (ppm): 9.09 (s, 1H, NH), 8.06 (s, broad, 3H, NH_3^+), 7.41 (dd, ^3J = 8.6 Hz, ^4J = 1.0 Hz, 2H, H_{Aryl}), 7.21 (t, ^3J = 7.9 Hz, 2H, H_{Aryl}) 6.92–6.84 (m, 1H, H_{Aryl}), 6.73 (t, ^3J = 5.7 Hz, 1H, NH), 3.33 (t, ^3J = 6.0 Hz, 2H, CH_2), 2.92–2.86 (m, 2H, CH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 156.20, 140.75, 128.92, 121.50, 118.14, 37.49, 33.32.

***N*-(2-Amino-ethyl)-*N*′-(4-bromo-phenyl)-urea hydrochloride 7b.** Yield: 51%. Mp 234 °C (decomposition). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm): 9.29 (s, 1H, NH), 8.02 (s, broad, 3H, NH_3^+), 7.42–7.34 (m, 4H, H_{Aryl}), 6.74 (t, ^3J = 5.9 Hz, 1H, NH), 3.32 (q, ^3J = 6.1 Hz, 2H, CH_2), 2.87 (q, ^3J = 6.2 Hz, 2H, CH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 156.11, 140.40, 131.78, 120.13, 112.92, 39.72, 37.61.

***N*-(2-Amino-ethyl)-*N*′-(4-iodo-phenyl)-urea hydrochloride 7c.** Yield: 78%. Mp 215 °C (decomposition). ^{1}H NMR (200 MHz, DMSO-d_6, TMS intern): δ (ppm): 9.12 (s, 1H, NH), 7.90 (s, broad, 3H, NH_3^+), 7.54 (d, ^3J = 8.8 Hz, 2H, H_{Aryl}), 7.27 (d, ^3J = 8.9 Hz, 2H, H_{Aryl}), 5.91 (s, broad, 1H, NH), 3.32–3.27 (m, 2H, CH_2), 2.87–2.80 (m, 2H, CH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 155.67, 140.41, 137.27, 120.19, 83.86, 37.26.

***N*-(2-Amino-ethyl)-*N*′-(4-methoxy-phenyl)-urea hydrochloride 7d.** Yield: 83%. Mp 185 °C (decomposition). ^{1}H NMR (200 MHz, DMSO-d_6, TMS intern): δ (ppm): 8.84 (s, 1H, NH), 8.04 (s, broad, 3H, NH_3^+), 7.33–7.26 (m, 2H, H_{Aryl}), 6.84–6.78 (m, 2H, H_{Aryl}), 6.15 (s, broad, 1H, NH), 3.68 (s, 3H, CH_3), 3.31 (t, ^3J = 5.7 Hz, 2H, CH_2), 2.86 (q, ^3J = 5.8 Hz, 2H, CH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm):. 156.22, 154.20, 133.68, 119.74, 114.05, 55.29, 39.52, 27.35.

***N*-(2-Amino-ethyl)-*N*′-(4-benzyloxy-phenyl)-urea hydrochloride 7e.** Yield: 98%. Mp 205 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm): 8.83 (s, 1H, NH), 8.05 (s, broad, 3H, NH_3^+), 7.44–7.28 (m, 7H, H_{Aryl}), 6.91–6.87 (m, 2H, H_{Aryl}), 6.59 (t, ^3J = 5.9 Hz, 1H, NH), 5.03 (s, 2H, CH_2), 3.32 (t, ^3J = 6.1 Hz, 2H, CH_2), 2.87 (t, ^3J = 6.2 Hz, 2H, CH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 156.38, 153.47, 137.73, 134.10, 128.71, 128.05, 127.95, 119.90, 115.30, 69.84, 39.78, 37.58.

***N*-(2-Amino-ethyl)-*N*′-(4-methyl-phenyl)-urea hydrochloride 7f.** Yield: 88%. Mp 213 °C (decomposition). ^{1}H NMR (200 MHz, DMSO-d_6, TMS intern): δ (ppm): 8.98 (s, 1H, NH), 8.08 (s, broad, 3H, NH_3^+), 7.29 (d, ^3J = 8.1 Hz, 2H, H_{Aryl}), 7.03–6.99 (m, 2H, H_{Aryl}), 6.69 (s, broad, 1H, NH), 3.37–3.28 (m, 2H, CH_2), 2.95–2.83 (m, 2H, CH_2), 2.20 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 156.26, 138.16, 130.26, 129.33, 118.31, 37.54, 20.64.

***N*-(2-Amino-ethyl)-*N*′-benzyl-urea hydrochloride 7g.** Yield: 83%. Mp 160 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm): 8.17 (s, broad, 3H, NH_3^+), 7.75 (s, 1H, NH), 7.30–7.18 (m, 5H, H_{Aryl}), 4.19 (s, 2H, CH_2), 3.25 (t, ^3J = 6.3 Hz, 2H, CH_2), 2.80 (q, ^3J = 6.0 Hz, 2H, CH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 158.79, 140.81, 128.41, 127.25, 126.73, 43.16, 39.75, 37.69.

Synthesis of the *N*-aryl-*N*′-[2-[3-aryloxy-2-hydroxy-propylamino]-ethyl]-urea and chain-elongated derivatives 8a–s: general procedure (Scheme 2 continued)

1.00 mmol **3a–h**, an equimolar amount of **7a–g** and 1.05 equivalents of 10 N NaOH were heated in approximately 3.0 mL *n*-propanol and 0.5 mL water up to 90 °C for 30 min.

As much water was added at rt until the mixture gets a slight turbidity. The suspension was cooled down to

+4 °C for about 20 h, the product was filtered off, washed with water, thereafter with diethylether and dried in vacuo. Compounds **8a–s** were isolated as colorless solids, in some cases as hemihydrates (**8e**, **8h**, **8l** and **8n**).

***N*-[2-(3-Phenoxy - 2 - hydroxy - propylamino) - ethyl]-*N*′-phenyl-urea 8a.** Yield: 30%. Mp 136 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.51 (s, 1H, NH), 7.37 (dd, ^3J = 8.6 Hz, ^4J = 1.0 Hz, 2H, H_{Aryl}), 7.27–7.17 (m, 4H, H_{Aryl}), 6.93–6.84 (m, 4H, H_{Aryl}), 6.14 (t, ^3J = 5.4 Hz, 1H, NH), 4.93 (s, 1H, OH), 3.96–3.14 (m, 5H, CH_2CH and CH_2), 2.71–2.57 (m, 4H, 2 CH_2), 1.85 (s, broad, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm): 157.05, 153.65, 138.96, 127.77, 126.94, 118.77, 119.23, 115.92, 112.85, 69.02, 66.55, 53.97, 50.64. Anal. ($C_{18}H_{23}N_3O_3$) C, H, N.

***N*-(4-Bromo-phenyl)-*N*′-[2-(3-phenoxy-2-hydroxy-propylamino)-ethyl]-urea 8b.** Yield: 42%. Mp 157 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.68 (s, 1H, NH), 7.36–7.23 (m, 6H, H_{Aryl}), 6.93–6.88 (m, 3H, H_{Aryl}), 6.19 (t, ^3J = 5.4 Hz, 1H, NH), 4.93 (s, 1H, OH), 3.97–3.13 (m, 5H, CH_2CH and CH_2), 2.71–2.56 (m, 4H, 2 CH_2), 1.81 (s, broad, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm): 160.48, 156.88, 141.84, 133.10, 131.14, 122.22, 121.26, 116.28, 113.93, 72.45, 69.99, 54.06, 51.02, 40.73. Anal. ($C_{18}H_{22}BrN_3O_3$) C, H, N.

***N*-[2-[3 - (2 - Bromo - phenoxy) - 2 - hydroxy - propylamino]-ethyl]-*N*′-phenyl-urea 8c.** Yield: 41%. Mp 158 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.51 (s, 1H, NH), 7.54 (dd, ^3J = 7.8 Hz, ^4J = 1.8 Hz, 1H, H_{Aryl}), 7.38–7.28 (m, 3H, H_{Aryl}), 7.19 (t, ^3J = 8.0 Hz, 2H, H_{Aryl}), 7.10 (dd, ^3J = 8.2 Hz, ^4J = 1.4 Hz, 1H, H_{Aryl}), 6.88–6.84 (m, 2H, H_{Aryl}), 6.15 (t, ^3J = 5.4 Hz, 1H, NH), 4.95 (s, 1H, OH), 4.01–3.14 (m, 5H, CH_2CH and CH_2), 2.79–2.62 (m, 4H, 2 CH_2), 1.73 (s, broad, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm): 156.03, 155.62, 141.35, 133.64, 129.67, 129.32, 122.69, 122.53, 121.61, 118.43, 111.85, 72.29, 68.74, 52.98, 50.03, 40.00. Anal. ($C_{18}H_{22}BrN_3O_3$) C, H, N.

***N*-[2-[3-(2-Bromo - phenoxy) - 2 - hydroxy - propylamino] - ethyl] - *N*′ - (4 - bromo - phenyl) - urea 8d.** Yield: 30%. Mp 159 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.68 (s, 1H, NH), 7.54 (dd, ^3J = 7.8 Hz, ^4J = 1.8 Hz, 1H, H_{Aryl}), 7.35–7.28 (m, 5H, H_{Aryl}), 7.10 (dd, ^3J = 8.2 Hz, ^4J = 1.4 Hz, 1H, H_{Aryl}), 6.88–6.86 (m, 1H, H_{Aryl}), 6.19 (t, ^3J = 5.4 Hz, 1H, NH), 4.94 (s, 1H, OH), 4.00–3.15 (m, 5H, CH_2CH and CH_2), 2.75–2.62 (m, 4H, 2 CH_2), 1.86 (s, broad, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm): 155.45, 155.25, 140.41, 133.27, 131.67, 129.30, 122.32, 119.83, 114.27, 112.50, 111.48, 71.91, 68.37, 52.59, 49.56, 39.64. Anal. ($C_{18}H_{21}Br_2N_3O_3$) C, H, N.

***N*-[2-[3-(2-Bromo-phenoxy) - 2 - hydroxy - propylamino] - ethyl] - *N*′ - (4 - methoxy - phenyl) - urea 8e.** Yield: 43%. Mp 140 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm): 8.31 (s, 1H, NH), 7.69 (dd, ^3J = 7.7 Hz, ^4J = 1.7 Hz, 1H, H_{Aryl}), 7.34–7.25 (m, 3H, H_{Aryl}), 7.11 (dd, ^3J = 8.3 Hz, ^4J = 1.4 Hz, 1H, H_{Aryl}), 6.90–6.78 (m, 3H, H_{Aryl}), 6.05 (t, ^3J = 5.4 Hz, 1H, NH), 4.83 (s, broad, 1H, OH), 4.05–3.88 (m, 3H, CH_2CH), 3.68 (s, 3H, CH_3), 3.14 (q, ^3J = 5.9 Hz, 2H, CH_2), 2.80–2.55 (m, 4H, 2 CH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 155.92, 155.28, 154.24, 134.15, 133.27, 129.30, 122.32, 119.71, 114.30, 111.51, 71.96, 68.39, 55.52, 52.63, 49.76. MS (EI): *m*/*z* (intensity%): 439 ($M^{\bullet+}$, 2), 437 ($M^{\bullet+}$, 2), 260 (88), 258 (91), 149 (41), 123 (100), 108 (69). Anal. ($C_{19}H_{24}BrN_3O_4 \cdot 0.5H_2O$) C, H, N.

***N*-[2-[3-(2-Iodo-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-phenyl-urea 8f.** Yield: 62%. Mp 158–159 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.51 (s, 1H, NH), 7.74 (dd, ^3J = 8.2 Hz, ^4J = 1.8 Hz, 1H, H_{Aryl}), 7.38–7.36 (m, 2H, H_{Aryl}), 7.34–7.29 (m, 1H, H_{Aryl}), 7.19 (t, ^3J = 8.0 Hz, 2H, H_{Aryl}), 7.00 (dd, ^3J = 8.4 Hz, ^4J = 1.0 Hz, 1H, H_{Aryl}), 6.86 (t, ^3J = 7.4 Hz, 1H, H_{Aryl}), 6.72 (dt, ^3J = 7.5 Hz, ^4J = 1.0 Hz, 1H, H_{Aryl}), 6.14 (t, ^3J = 5.6 Hz, 1H, NH), 4.92 (s, 1H, OH), 4.00–3.15 (m, 5H, CH_2CHCH_2), 2.83–2.63 (m, 4H, CH_2CH_2), 2.10 (s, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm): 156.45, 154.61, 139.93, 138.20, 129.01, 127.91, 120.20, 116.96, 111.99, 85.91, 70.82, 67.29, 51.65, 48.62, 38.60. Anal. ($C_{18}H_{22}IN_3O_3$) C, H, N.

***N*-[2-[3-(2-Iodo-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-(4-iodo-phenyl)-urea 8g.** Yield: 61%. Mp 163 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.66 (s, 1H, NH), 7.74 (dd, ^3J = 7.6 Hz, ^4J = 1.6 Hz, 1H, H_{Aryl}), 7.52–7.21 (m, 5H, H_{Aryl}), 6.99 (dd, ^3J = 8.4 Hz, ^4J = 1.2 Hz, 1H, H_{Aryl}), 6.74–6.69 (m, 1H, H_{Aryl}), 6.19 (t, ^3J = 5.4 Hz, 1H, NH), 4.93 (s, 1H, OH), 4.00–3.14 (m, 5H, CH_2CH and CH_2), 2.82–2.64 (m, 4H, 2 CH_2), 2.00 (s, broad, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm): 158.56, 156.48, 141.97, 140.34, 138.56, 131.15, 124.05, 121.32, 114.01, 88.07, 84.77, 72.92, 69.40, 53.74, 50.63, 40.72. Anal. ($C_{18}H_{21}I_2N_3O_3$) C, H, N.

***N*-[2-[3-(2-Iodo-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-(4-methoxy-phenyl)-urea 8h.** Yield: 53%. Mp 148 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm): 8.29 (s, 1H, NH), 7.69 (dd, ^3J = 7.7 Hz, ^4J = 1.7 Hz, 1H, H_{Aryl}), 7.65–7.59 (m, 1H, H_{Aryl}), 7.30–7.24 (m, 3H, H_{Aryl}), 7.07 (dt, ^3J = 7.4 Hz, ^4J = 0.7 Hz, 1H, H_{Aryl}), 6.82–6.77 (m, 2H, H_{Aryl}), 6.03 (t, ^3J = 5.4 Hz, 1H, NH), 5.01 (s, 1H, OH), 4.19–3.92 (m, 3H, CH_2CH), 3.68 (s, 3H, CH_3), 3.18–3.11 (m, 2H, CH_2), 2.76–2.55 (m, 4H, 2 CH_2), 1.92 (s, broad, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 157.51, 155.94, 154.24, 139.27, 134.15, 130.08, 122.87, 119.72, 114.25, 113.16, 87.00, 71.90, 68.35, 55.53, 52.70, 49.77. MS (EI): *m*/*z* (intensity%): 306 (84), 149 (98), 123 (100), 108 (86). Anal. ($C_{19}H_{24}IN_3O_4 \cdot 0.5H_2O$) C, H, N.

***N*-[2-[3-(2-Cyano - phenoxy) - 2 - hydroxy - propylamino] - ethyl] - *N*′ - phenyl-urea 8i.** Yield: 41%. Mp 154 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm): 8.52 (s, 1H, NH), 7.67 (dd, ^3J = 7.7 Hz, ^4J = 1.7 Hz, 1H, H_{Aryl}), 7.62–7.57 (m, 1H, H_{Aryl}), 7.39–7.15 (m, 5H, H_{Aryl}), 7.06 (t, ^3J = 7.2 Hz, 1H, H_{Aryl}), 6.86 (t, ^3J = 7.2 Hz, 1H, H_{Aryl}), 6.16 (t, ^3J = 5.5 Hz, 1H, NH), 5.05 (s, 1H, OH), 4.15–3.11 (m, 5H, CH_2CH and CH_2), 2.78–2.59 (m, 4H, 2 CH_2). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm):

160.27, 155.21, 140.52, 134.91, 133.55, 128.49, 120.89, 117.47, 116.35, 113.10, 100.56, 71.50, 67.81, 51.86, 49.08. Anal. ($C_{19}H_{22}N_4O_3$) C, H, N.

***N*-[2-[3-(2-Cyano-phenoxy) - 2 - hydroxy - propylamino] - ethyl] - *N′* - (4 - methyl - phenyl)-urea 8j.** Yield: 63%. Mp 149 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm): 8.35 (s, 1H, NH), 7.69 (dd, ^3J = 7.7 Hz, ^4J = 1.4 Hz, 1H, H_{Aryl}), 7.64–7.58 (m, 1H, H_{Aryl}), 7.24 (d, ^3J = 8.4 Hz, 3H, H_{Aryl}), 7.06 (dt, ^3J = 7.6 Hz, ^4J = 0.8 Hz, 1H, H_{Aryl}), 6.99 (d, ^3J = 8.1 Hz, 2H, H_{Aryl}), 6.06 (t, ^3J = 5.4 Hz, 1H, NH), 4.99 (d, ^3J = 4.8 Hz, 1H, OH), 4.16–3.89 (m, 3H, CH_2CH), 3.14 (q, ^3J = 5.8 Hz, 2H, CH_2), 2.75–2.60 (m, 4H, 2 CH_2), 2.19 (s, 3H, CH_3), 1.80 (s, broad, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 160.74, 155.75, 138.41, 135.33, 133.99, 129.96, 129.33, 121.33, 118.21, 116.78, 113.58, 112.50, 101.07, 71.98, 68.32, 52.32, 49.56. Anal. ($C_{20}H_{24}N_4O_3$) C, H, N.

***N*-(4-Bromo - phenyl) - *N′* - [2 - [3 - (2 - cyano - phenoxy) - 2 - hydroxy - propylamino] - ethyl] - urea 8k.** Yield: 58%. Mp 148 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm): 8.65 (s, 1H, NH), 7.69 (dd, ^3J = 7.7 Hz, ^4J = 2.0 Hz, 1H, H_{Aryl}), 7.64–7.58 (m, 1H, H_{Aryl}), 7.38–7.35 (m, 4H, H_{Aryl}), 7.24 (d, ^3J = 8.4 Hz, 1H, H_{Aryl}), 7.06 (dt, ^3J = 7.6 Hz, ^4J = 1.0 Hz, 1H, H_{Aryl}), 6.17 (t, ^3J = 5.4 Hz, 1H, NH), 4.99 (d, ^3J = 4.8 Hz, 1H, OH), 4.16–3.89 (m, 3H, CH_2CH), 3.15 (q, ^3J = 5.9 Hz, 2H, CH_2), 2.75–2.60 (m, 4H, 2 CH_2), 1.84 (s, broad, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 160.73, 155.47, 140.42, 135.32, 131.65, 121.33, 119.85, 116.78, 113.56, 112.50, 101.06, 138.41, 135.33, 133.99, 129.96, 121.33, 118.21, 116.78, 113.58, 101.07, 72.00, 68.33, 52.36, 49.71. Anal. ($C_{19}H_{21}BrN_4O_3$) C, H, N.

***N*-[2-[3-(2-Cyano - phenoxy) - 2 - hydroxy - propylamino] - ethyl] - *N′* - (4 - iodo - phenyl)-urea 8l.** Yield: 90%. Mp 150 °C (decomposition). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm): 8.64 (s, 1H, NH), 7.69 (dd, ^3J = 7.7 Hz, ^4J = 1.4 Hz, 1H, H_{Aryl}), 7.65–7.59 (m, 1H, H_{Aryl}), 7.52–7.48 (m, 2H, H_{Aryl}), 7.25–7.21 (m, 2H, H_{Aryl}), 7.06 (dt, ^3J = 7.6 Hz, ^4J = 0.8 Hz, 1H, H_{Aryl}), 6.17 (t, ^3J = 5.4 Hz, 1H, NH), 5.00 (s, broad, 1H, OH), 4.16–3.91 (m, 3H, CH_2CH), 3.22–3.08 (m, 2H, CH_2), 2.78–2.60 (m, 4H, 2 CH_2), 2.15 (s, broad, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 160.54, 155.24, 140.71, 137.30, 135.14, 133.80, 121.15, 120.08, 116.60, 113.38, 100.87, 83.50, 71.79, 68.13, 52.13, 49.37. MS (EI): *m*/*z* (intensity%): 245 (100), 219 (73), 205 (16) 99 (30). Anal. ($C_{19}H_{21}IN_4O_3 \cdot 0.5H_2O$) C, H, N.

***N*-[2-[3-(2-Cyano-phenoxy) - 2 - hydroxy - propylamino] - ethyl]-*N′*-(4-methoxy-phenyl)-urea 8m.** Yield: 65%. Mp 137 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm): 8.31 (s, 1H, NH), 7.75 (dd, ^3J = 7.7 Hz, ^4J = 1.7 Hz, 1H, H_{Aryl}), 7.35–7.24 (m, 3H, H_{Aryl}), 7.00 (dd, ^3J = 8.3 Hz, ^4J = 1.4 Hz, 1H, H_{Aryl}), 6.79 (dd, ^3J = 6.8 Hz, ^4J = 2.3 Hz, 2H, H_{Aryl}), 6.73 (dd, ^3J = 7.6 Hz, ^4J = 1.1 Hz, 1H, H_{Aryl}), 6.04 (t, ^3J = 5.4 Hz, 1H, NH), 4.91 (s, broad, 1H, OH), 4.01–3.90 (m, 3H, CH_2CH), 3.68 (s, 3H, CH_3), 3.16 (q, ^3J = 5.9 Hz, 2H, CH_2), 2.84–2.62 (m, 4H, 2 CH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 160.74, 155.94, 154.26, 135.35, 134.10, 133.99, 121.35, 119.87, 116.79, 114.24, 113.57, 101.05, 71.99, 68.32, 55.52, 52.34, 49.74. MS (EI): *m*/*z* (intensity%): 205 (45), 149 (45), 123 (100) 108 (100). Anal. ($C_{20}H_{24}N_4O_4$) C, H, N.

***N*-(4-Benzyloxy-phenyl)-*N′*-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea 8n.** Yield: 73%. Mp 105 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm): 8.32 (s, 1H, NH), 7.70 (dd, ^3J = 7.7 Hz, ^4J = 1.6 Hz, 1H, H_{Aryl}), 7.64–7.59 (m, 1H, H_{Aryl}), 7.43–7.24 (m, 8H, H_{Aryl}), 7.06 (t, ^3J = 7.4 Hz, 1H, H_{Aryl}), 6.87 (d, ^3J = 9.4 Hz, 2H, H_{Aryl}), 6.04 (t, ^3J = 5.4 Hz, 1H, NH), 5.05–5.02 (m, 3H, OH and CH_2), 4.16–3.12 (m, 5H, CH_2CH and CH_2), 2.75–2.61 (m, 4H, 2 CH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 160.74, 155.90, 153.28, 137.77, 135.35, 134.37, 133.99, 128.71, 128.04, 127.94, 121.35, 119.67, 116.80, 115.33, 113.58, 101.05, 71.99, 69.84, 68.32, 52.34, 49.73. Anal. ($C_{26}H_{28}N_4O_4 \cdot 0.5H_2O$) C, H, N.

***N*-[2-[3-(2-Allyl-phenoxy)-2-hydroxy-propylamino]-ethyl] -*N′*-phenyl-urea 8o.** Yield: 42%. Mp 143 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.51 (s, 1H, NH), 7.37 (dd, ^3J = 8.6 Hz, ^4J = 1.0 Hz, 2H, H_{Aryl}), 7.21–7.08 (m, 4H, H_{Aryl}), 6.93 (d, ^3J = 8.4 Hz, 1H, H_{Aryl}), 6.88–6.83 (m, 2H, H_{Aryl}), 6.14 (t, ^3J = 5.5 Hz, 1H, NH), 5.98–5.92 (m, 1H, =CH), 5.05–4.97 (m, 2H, $=CH_2$), 4.90 (s, 1H, OH), 3.94–3.14 (m, 7H, CH_2CH and 2 CH_2), 2.72–2.48 (m, 4H, 2 CH_2), 2.00 (s, broad, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm): 156.15, 155.22, 140.54, 137.01, 129.38, 128.51, 127.97, 127.30, 120.80, 120.24, 117.49, 115.31, 111.56, 70.59, 68.16, 52.25, 49.25, 33.86. Anal. ($C_{21}H_{27}N_3O_3$) C, H, N.

***N*-[2-[3-(2-Methoxy - phenoxy) - 2 - hydroxy - propylamino] - ethyl] - *N′* - phenyl - urea 8p.** Yield: 48%. Mp 143 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.51 (s, 1H, NH), 7.37 (d, ^3J = 7.6 Hz, 2H, H_{Aryl}), 7.20 (t, ^3J = 8.0 Hz, 2H, H_{Aryl}), 6.88–6.73 (m, 5H, H_{Aryl}), 6.14 (t, ^3J = 5.6 Hz, 1H, NH), 4.90 (s, 1H, OH), 3.92–3.78 (m, 3H, CH_2CH), 3.67 (s, 3H, CH_3), 3.16 (q, ^3J = 6.0 Hz, 2H, CH_2), 2.72–2.48 (m, 4H, 2 CH_2), 2.02 (s, broad, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm): 154.54, 152.54, 151.99, 139.80, 127.83, 120.12, 116.81, 114.65, 113.81, 70.60, 67.49, 54.58, 51.55, 48.56. Anal. ($C_{19}H_{25}N_3O_4$) C, H, N.

***N*-[2-[3-(4-Benzyloxy-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N′*-phenyl-urea 8q.** Yield: 48%. Mp 140 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.52 (s, 1H, NH), 7.43–7.30 (m, 7H, H_{Aryl}), 7.20 (t, ^3J = 8.0 Hz, 2H, H_{Aryl}), 6.91–6.81 (m, 5H, H_{Aryl}), 6.15 (t, ^3J = 5.4 Hz, 1H, NH), 5.01 (s, 2H, CH_2), 4.91 (s, 1H, OH), 3.89–3.14 (m, 5H, CH_2CH and CH_2), 2.69–2.55 (m, 4H, 2 CH_2), 1.67 (s, broad, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm): 154.57, 152.20, 151.61, 139.89, 136.63, 127.86, 127.62, 126.95, 126.83, 120.14, 116.83, 114.96, 114.65, 70.59, 68.95, 67.50 51.57, 48.58, 38.53. Anal. ($C_{25}H_{29}N_3O_4$) N, H, C: calcd, 68.95; found, 68.43.

***N*-Benzyl-*N′*-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propyl-amino]-ethyl]-urea 8r.** Yield: 32%. Mp 144 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm): 7.70–7.59 (m, 2H,

H_{Aryl}), 7.31–7.19 (m, 6H, H_{Aryl}), 7.06 (dd, $^3J = 7.5$ Hz, $^4J = 0.9$ Hz, 1H, H_{Aryl}), 6.36 (t, $^3J = 6.0$ Hz, 1H, NH), 5.91 (t, $^3J = 5.6$ Hz, 1H, NH), 4.97 (s, 1H, OH), 4.20–3.90 (m, 5H, CH_2CH and CH_2), 3.10 (q, $^3J = 6.0$ Hz, 2H, CH_2), 2.74–2.56 (m, 4H, 2 CH_2) 1.86 (s, broad, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 160.77, 158.55, 141.31, 135.33, 133.99, 128.52, 127.37, 125.84, 121.34, 116.77, 113.61, 101.10, 72.03, 68.35, 52.40, 50.04, 43.33. Anal. ($C_{20}H_{24}N_4O_3$) C, H, N: calcd, 15.21; found, 14.66.

***N*-Benzyl-*N*′-[2-(3-benzyloxy-2-hydroxy-propylamino)-ethyl]-urea 8s.** Yield: 19%. Mp 116 °C. ^1H NMR (400 MHz, DMSO-d_6): δ (ppm): 7.36–7.20 (m, 10H, H_{Aryl}), 6.39 (t, $^3J = 6.0$ Hz, 1H, NH), 5.91 (t, $^3J = 5.6$ Hz, 1H, NH), 4.67 (s, 1H, OH), 4.47 (s, 2H, CH_2) 4.19 (d, $^3J = 6.0$ Hz, 2H, CH_2), 3.71–3.35 (m, 5H, CH_2CH and CH_2), 2.61–2.45 (m, 4H, 2 CH_2) 1.80 (s, broad, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm): 157.88, 140.68, 138.35, 127.90, 127.88, 127.15, 127.02, 126.71, 126.20, 72.90, 71.98, 68.39, 52.36, 49.37, 42.64, 39.37. Anal. ($C_{20}H_{27}N_3O_3$) C, H, N.

Synthesis of *N*-[2-[3-(4-hydroxy-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-phenyl-urea 8t (Scheme 3). 850 mg (1.95 mmol) **8q** were dissolved in 20 mL acetic acid and 92 mg Pd/C (10%) were added. The mixture was stirred under a H_2-atmosphere for 69 h. The catalyst was filtered off and the filtrate was evaporated to dryness. 20 mL acetonitrile were added. After stirring for 20 h at rt 10 mL acetonitrile were added. Then the mixture was refluxed and decanted from the residual oily brown solid. This procedure was repeated two times. The solution was cooled down to −15 °C for 3 h and the precipitate was filtered off to provide the acetate of **8t** as a colorless solid. Yield: 172 mg (0.42 mmol), 22%. Mp 134 °C. ^1H NMR (300 MHz, DMSO-d_6): δ (ppm): 8.57 (s, 1H, NH), 7.35 (d, $^3J = 7.8$ Hz, 2H, H_{Aryl}), 7.17 (t, $^3J = 8.0$ Hz, 2H, H_{Aryl}), 6.84 (t, $^3J = 7.5$ Hz, 1H, H_{Aryl}), 6.71 (d, $^3J = 9.0$ Hz, 2H, H_{Aryl}), 6.62 (d, $^3J = 9.0$ Hz, 2H, H_{Aryl}), 6.24 (t, $^3J = 5.1$ Hz, 1H, NH), 5.00 (s, broad, 3H, OH and NH_2^+), 3.82–3.12 (m, 5H, CH_2CH and CH_2), 2.68–2.54 (m, 4H, 2 CH_2), 1.86 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm): 172.63, 155.73, 151.89, 151.55, 141.01, 128.94, 121.23, 117.97, 116.06, 115.85, 71.72, 68.37, 52.54, 49.56, 21.73. MALDI-TOF: 345 $(M+H)^+$, 368 $(M+Na)^+$, 384 $(M+K)^+$. Anal. ($C_{20}H_{27}N_3O_6$) C, H, N.

Synthesis of *N*-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-carbamic acid *tert*-butyl ester 8u (Scheme 4). 187 mg (1.07 mmol) **3d** and 171 mg (1.07 mmol) **5** were heated in 1.5 mL *n*-propanol over 10 min at 100 °C. The solvent was removed in vacuo and the residue became completely solid after 1 day at rt. It was extracted with 10 mL hot diethylether, the organic layer was cooled down to +4 °C (1 h), the resulting solid was filtered off, washed with *n*-pentane and dried. The isolated **8u** was a colorless solid. Yield: 147 mg (0.44 mmol), 41%. Mp 55 °C. ^1H NMR (400 MHz, DMSO-d_6): δ (ppm): 7.69 (dd, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz, 1H, H_{Aryl}), 7.63 (ddd, $^3J = 8.6$ Hz, $^3J = 7.3$ Hz, $^4J = 1.5$ Hz, 1H, H_{Aryl}), 7.24 (d, $^3J = 8.4$ Hz, 1H, H_{Aryl}), 7.09–7.05 (m, 1H, H_{Aryl}), 6.67 (s, broad, 1H, NH), 4.98 (s, 1H, OH), 4.11 (dd, $^2J = 10.0$ Hz, $^3J = 6.0$ Hz, 1H, 1 CH_2), 4.05 (dd, $^2J = 10.0$ Hz, $^3J = 4.6$ Hz, 1H, 1 CH_2), 3.89–3.86 (m, 1H, CH), 2.99 (q, $^2J = 5.9$ Hz, 2H, CH_2), 2.69–2.54 (m, 4H, 2 CH_2), 1.75 (s, broad, 1H, NH), 1.36 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm): 159.24, 154.48, 133.83, 132.49, 119.81, 115.26, 112.04, 99.52, 76.29, 70.44, 66.84, 50.77, 47.98, 38.97, 27.09. Anal. ($C_{17}H_{25}N_3O_4$) C, H, N.

Table 2. Elemental analytical data of the target compounds **8a–u**

No.	Formula	Calcd			Found		
		C	H	N	C	H	N
8a	$C_{18}H_{23}N_3O_3$	65.63	7.04	12.76	65.34	6.95	12.66
8b	$C_{18}H_{22}BrN_3O_3$	52.95	5.43	10.29	52.79	5.46	10.12
8c	$C_{18}H_{22}BrN_3O_3$	52.95	5.43	10.29	52.88	5.48	10.25
8d	$C_{18}H_{21}Br_2N_3O_3$	44.38	4.34	8.63	44.22	4.39	8.48
8e	$C_{19}H_{24}BrN_3O_4 \cdot 0.5H_2O$	51.02	5.63	9.39	51.08	5.41	9.28
8f	$C_{18}H_{22}IN_3O_3$	47.48	4.87	9.23	47.41	4.93	8.88
8g	$C_{18}H_{21}I_2N_3O_3$	37.20	3.64	7.23	37.43	3.77	7.05
8h	$C_{19}H_{24}IN_3O_4 \cdot 0.5H_2O$	46.17	5.10	8.50	46.31	4.88	8.40
8i	$C_{19}H_{22}N_4O_3$	64.39	6.26	15.81	64.02	6.30	15.52
8j	$C_{20}H_{24}N_4O_3$	65.20	6.57	15.21	64.80	6.55	15.17
8k	$C_{19}H_{21}BrN_4O_3$	52.67	4.88	12.93	52.49	4.84	12.96
8l	$C_{19}H_{21}IN_4O_3 \cdot 0.5H_2O$	46.64	4.53	11.45	46.92	4.48	11.62
8m	$C_{20}H_{24}N_4O_4$	62.49	6.29	14.57	62.19	6.34	14.58
8n	$C_{26}H_{28}N_4O_4 \cdot 0.5H_2O$	66.50	6.23	11.93	66.24	6.06	11.92
8o	$C_{21}H_{27}N_3O_3$	68.27	7.37	11.37	67.99	7.37	11.05
8p	$C_{19}H_{25}N_3O_4$	63.49	7.01	11.69	63.09	6.96	11.58
8q	$C_{25}H_{29}N_3O_4$	68.95	6.71	9.65	68.43	6.64	9.43
8r	$C_{20}H_{24}N_4O_3$	65.20	6.57	15.21	64.87	6.48	14.66
8s	$C_{20}H_{27}N_3O_3$	67.20	7.61	11.76	67.03	7.54	12.04
8t	$C_{20}H_{27}N_3O_6$	59.25	6.71	10.36	59.07	6.81	10.35
8u	$C_{17}H_{25}N_3O_4$	60.88	7.51	12.53	60.90	7.66	12.92

Pharmacological methods

The chemicals were reagent grade.

Tissue preparation

The biological profile of the compounds at the β_1- and β_2-ARs listed in Table 1 was assessed on ventricular membrane preparations of wild-type DAB mice. Microsomes were prepared by homogenizing ventricles at 4 °C for 90 s in 1 mL of a buffer A containing 10 mM EDTA, 10 mM HEPES, 0.1 mM benzamidine (pH 7.4), using a Polytron PT 3000 (Kinematica, Lucerne, Switzerland). Homogenates were centrifuged at 45,000×g_{max} for 15 min at 4 °C. The pellets were again resuspended in 1 mL of a buffer B containing 1 mM EDTA, 10 mM HEPES, 0.1 mM benzamidine (pH 7.4) and recentrifuged at 45,000×g_{max} for 15 min at 4 °C. The pellets were resuspended in 1 mL of buffer B and centrifuged at 10,000×g_{max} for 10 min at 4 °C. The supernatants were recentrifuged at 45,000×g_{max} for 15 min at 4 °C. The pellets, partially enriched membranes, were resuspended in 50 mM Tris–HCl, 5 mM $MgCl_2$ (pH 7.4), and stored frozen at −80 °C. Membranes were used for the measurement of β-AR density.

[^{125}I]ICYP binding experiments

The β-AR antagonist [^{125}I]ICYP represents the most useful ligand for labelling β-ARs on cell membrane

preparations because it is characterized by very high affinity, which results in low non-specific binding at the dissociation constant (K_D) concentration range, and by radioiodination, requiring only small tissue amounts to be added to the assay.[63] Furthermore, [^{125}I]ICYP is considered as nonselective on the basis of linear Scatchard plots derived from saturation binding experiments on tissues containing a mixed population of β-ARs.[64,65]

First, the protein dependence of [^{125}I]ICYP binding to mouse ventricular membrane preparations was investigated. [^{125}I]ICYP binding was linear up to a protein amount of 50 μg in a total volume of 200 μL. Non-specific binding was simultaneously determined in a parallel experiment using the same membrane and ligand conditions in the presence of 20 μM alprenolol. A protein amount of 15 μg in 200 μL was subsequently chosen for kinetic studies.

Subsequently, association kinetics of [^{125}I]ICYP binding to β-ARs were investigated. The association kinetics were linear for approximately 15 min and an equilibrium was reached after 60 min. Therefore, assays were carried out for 60 min to calculate kinetic parameters.

Total β-AR density (B_{max}) and the dissociation constant (K_D) of β-ARs were determined by incubating 15 μg of membrane protein with increasing concentrations of [^{125}I]ICYP (1–300 pM) in a buffer of 10 mM Tris·HCl, 154 mM NaCl, 0.1 mM ascorbic acid (pH 7.4). The nonspecific binding was measured in the presence of 20 μM alprenolol (Fig. 2a). Binding reactions (200 μL) were incubated at 37 °C for 1 h prior to vacuum filtration onto Whatman GF/B filters. Subsequently, membrane bound [^{125}I]ICYP was determined in a gamma-counter. The maximum number of binding sites (B_{max}) and the dissociation constants (K_D) were calculated from plots according to the method of Scatchard (Fig. 2b).[66]

For competition binding studies, 15 μg of membranes were incubated with [^{125}I]ICYP ($c = 80$ pM) and with 24 concentrations of ICI 89,406 **8i** and its derivatives (1 pM–100 μM), respectively. Reactions were conducted at 37 °C for 60 min. Samples were filtered onto Whatman GF/B filters, washed three times, and the membrane bound activity was determined in a gamma-scintillation counter. This screening method was applied for the indirect determination of the half maximum inhibitory concentrations (IC_{50}) of the selective β_1-AR antagonists as compared to a nonselective β-AR radioligand. Subsequently, the obtained IC_{50} values were converted into the high- and low-affinity inhibition constants K_{I1} and K_{I2}, respectively.

Calculation of K_i values

Each competition binding curve was analyzed according to the following equation describing the interaction of a selective competitor with two classes of binding sites:

$$B = B_{max1}/(1 + I/IC_1) + B_{max2}/(1 + I/IC_2)$$

where B is the amount of radioligand bound, B_{max1} and B_{max2} are the total numbers of binding sites labelled by this concentration of radioligand, I is the free concentration of the competing ligand, and IC_1 and IC_2 are the concentrations of the competing ligand that inhibit 50% of the binding of each subtype. Analysis of the competition curves by nonlinear regression analysis with the XMGRACE program (Linux Software) yielded the high- and low-affinity IC_{50} values of ICI 89,406 **8i** and its derivatives.[67] In addition, all inhibition data were fitted to the equation describing the binding of a non-selective β-AR antagonist with a single binding site: $B = B_{max}/(1 + I/IC)$. The F-test was performed in order to test for a significant improvement of the two-site fit as compared to the one-site fit. If the two-site model was rejected the one-site model was applied and the β_1-selectivity was set to 1.0.

The IC_{50} values were converted into the high- and low-affinity inhibition constants (K_{I1} and K_{I2}) by the method of Cheng–Prusoff[34] using the equation $K_i = IC_{50}/(1 + S/K_d)$, where S is the concentration of [^{125}I]ICYP, and K_D is the dissociation constant of β-ARs for [^{125}I]ICYP determined independently by Scatchard analysis.[66] The ratios between K_{I2} and K_{I1} represent the β_1-selectivities of the compounds tested.

Statistics

The experimental data given in the text, Figures 2 and 3, and Table 1 are means ± SEM. Statistical analysis was performed using the Student's t-test. $P < 0.05$ was considered as denoting statistical significance. The dissociation constants (K_D) and the maximum number of binding sites (B_{max}) were calculated from plots according to Scatchard.[66] Analysis of the inhibition of [^{125}I]ICYP binding by ICI 89,406 **8i** and its derivatives was performed by nonlinear regression analysis, as described by Engel et al.[64] Statistical analysis of the competition experiments was performed using the F-ratio test to measure the goodness of fit of the competition curves for either one or two binding sites. The IC_{50} values were converted to K_I values according to the method of Cheng and Prusoff.[34]

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