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Thienopyrimidines as β3-adrenoceptor agonists: Hit-to-lead optimization

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ABSTRACT

Resulting from a vHTS based on a pharmacophore alignment on known β3-adrenoceptor ligands, an aryloxypropanolamine scaffold comprising a thienopyrimidine moiety was further optimized as a human β3-AR agonist, yielding a lead compound with an excellent cellular activity of EC_{50} = 20 pM, selectivity over hβ1- and hβ2-adrenoceptors and a promising safety profile.

The human β3-adrenergic receptor (hβ3-AR) is distributed in different tissues like white and brown adipocytes, urinary bladder detrusor, gastrointestinal tract, near-term myometrium, brain and the heart. Consequently, several indications emerged for a potential treatment with hβ3-AR ligands, in particular agonists: obesity, type II diabetes, overactive bladder, irritable bowel syndrome (IBS), preterm labor, anxiety and major depression disorder.[1] Antagonists have recently been under evaluation for the treatment of chronic heart failure.[2] However, first clinical trials conducted with hβ3-AR agonists for the treatment of obesity and diabetes failed. This was mainly due to differing expression levels of this receptor especially on white adipocytes and a differently weighted role in lipolysis as compared to rodents, in which respective animal models were performed. Given these results, the major focus shifted towards the treatment of overactive bladder.[1,3]

Over the last decade, several phenylethanolamine hβ3-AR agonists (Fig. 1) advanced to clinical trials with variable success: development of Solabegron was not continued upon completion of phase II for the treatment of overactive bladder and IBS. Amibegron was not further pursued upon two phase III trials, one on anxiety, the other on major depressive disorder. Mirabegron and KUC-7483 are still under evaluation in active phase III trials for the treatment of overactive bladder, with a new drug application filing being expected for the former to be submitted in 2011.[4,5] Even though hβ3-AR agonists based on a second major general scaffold, aryloxypropanolamines, emerged already in the mid 1980s,[6] such

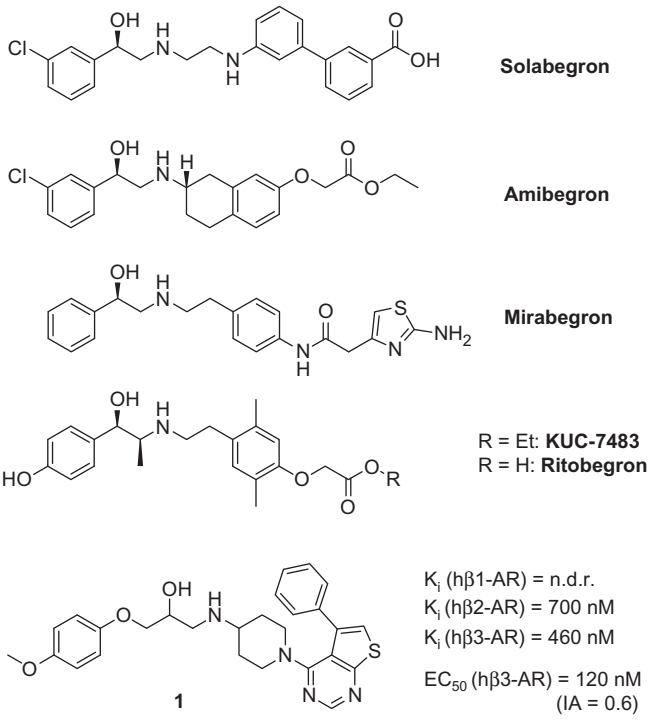

Figure 1. hβ3-AR agonists under clinical evaluation and hit compound **1** deduced from a vHTS approach.[7,9] IA = intrinsic activity; n.d.r. = no dose–response.

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substrates have failed so far to reach any phase III clinical trials. To our knowledge, LY-377604 is the only aryloxypropanolamine currently in phase II. It is under investigation for the treatment of obesity in combination with Sibutramine, a serotonin and norepinephrine reuptake inhibitor.[4]

As starting point for a program on hβ3-adrenoceptors, a pharmacophore alignment of a virtual library of 4.6 Mio commercially available compounds on known hβ3-AR agonists—mainly of the phenylethanolamine array—was chosen. This led to the identification of intriguing hit classes of the aryloxypropanolamine-type displaying K_i values already in the submicromolar range.[7] The further structural evaluation of one of these hits, thienopyrimidine **1** (Fig. 1), is described within this contribution. It already displayed a certain selectivity over hβ1-AR and proved to be a partial agonist at hβ3-AR. As a commercially available compound, it has already been described as an antagonist on neurokinin (=tachykinin) receptors [K_i(NK1) = 53 nM; K_i(NK2) = 1700 nM].[8] Notwithstanding, it served as an excellent starting point for structural variations leaving the realm of NK ligands towards selective hβ3-AR agonists.

For an evaluation of the pharmacologically relevant molecular texture of the compounds, a set of different assays has been established. Binding constants (K_i) were obtained from radioligand binding assays for subtypes hβ1-, hβ2- and hβ3-AR.[10] A functional cellular assay was used to assess hβ3-AR agonism.[11] Structure–activity relationships (SAR) were based not only on affinity and activity,[12] but also on a combined metric which utilizes K_i and EC_{50} values as well as the intrinsic activity (IA) of the respective ligand (relative to the maximum cellular response triggered by treatment with 100 μM isoproterenol). This *relative efficacy*[13] is reflected by the term ρ_{Resp}:

$$\rho_{Resp} = \frac{RL * EC_{50}\ [\mu M]}{K_i\ [\mu M] * (IA - RL) + (RL * EC_{50}\ [\mu M])} * 100$$

RL = pre-defined response level, can be adjusted in between 0.01 and 1.0 to include partial agonists in analysis

IA = intrinsic activity, with 1.0 representing the 100% level achieved with 100 μM isoproterenol

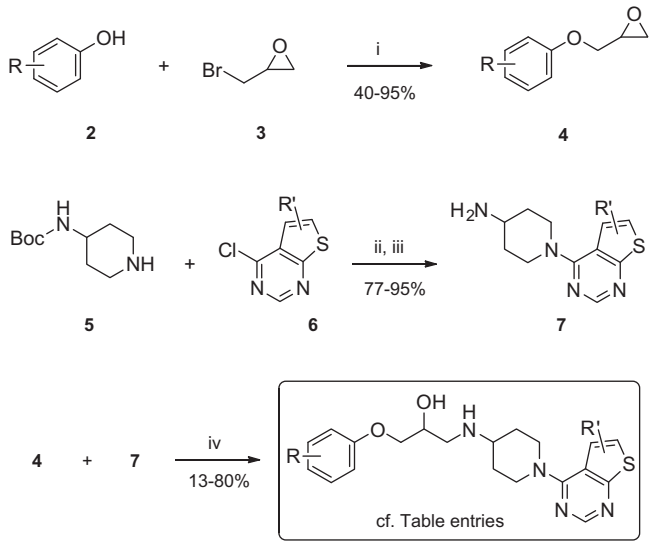
ρ_{Resp} represents the percentage of receptors required to be occupied by a given ligand to result in a cellular response equal to a given percentage of the isoproterenol signal (=response level RL). In our case RL was chosen to be 0.25 (that is 25%) in order to allow for an evaluation of partial agonists down to an IA of 0.25. Throughout the text body, discussion of relative efficacy is based on the ρ_{Resp} values calculated according to this equation.

With this concept established one is able to discriminate efficacy-driven from affinity-driven agonists. Accordingly, tissue selectivity can be addressed and target related side effects might be avoided: for the activation of a certain cellular response, less efficacious ligands (those with higher ρ_{Resp}) would require a higher receptor density on the respective cells, thus allowing for a selective stimulation of tissue with higher receptor expression levels.[14] The primary focus, however, was defined as to identify highly potent and efficacious ligands (that is, with low ρ_{Resp}) for triggering a maximum cellular response, thus enabling an evaluation of one of these ligands in a first tissue model.

A general synthetic route towards thienopyrimidine-substituted aryloxypropanolamines can be deduced from Scheme 1. Additional details are elaborated within the Supplementary data. Standard synthesis for aryloxymethyloxiranes **4** involved treatment of phenols **2** with epibromohydrine (**3**) and K_2CO_3 in butanone at 80 °C—usually succeeding in good to excellent yields of 40–95%.[15] N-Substituted 4-aminothienopyrimidines **7** were easily attained by conversion of the respective 4-chlorothienopyrimidine **6**[16] with a *N*-Boc-protected diamine like **5** in ethylene glycol[17] and subsequent in situ Boc-removal with HCl in dioxane. Yields of 77–95% for this transformation represent the outcome for all combinations of chloro-thienopyrimidines and secondary amines utilized within the following SAR investigations. Only when utilizing primary amines like 1-Boc-4-aminopiperidine and 1-Boc-4-(aminomethyl)piperidine (cf. derivatives in Fig. 2), yields deteriorated to about 40%. For 4-chloro-2-methylthieno[2,3-*d*]pyrimidine (**6**) and 4-chlorothieno[3,2-*d*]pyrimidine (en route to compounds **49**–**51** and **52**–**54**, respectively; Table 3), this sequence resulted in precipitation of the product as the respective hydrochloride upon Boc-removal, which was simply filtered off. For all other amine building blocks **7**, alkaline extraction yielded the free amines. Finally, amines **7** and oxiranes **4** were heated together up to 80 °C in *i*PrOH or *i*PrOH/DMSO mixtures depending on solubility of the respective reaction partners.[18] Thus the desired aryloxypropanolamine substrates for biological evaluation on adrenergic receptors were attained. When amine **7** was used as the hydrochloride salt, DIEA had to be added for achieving a smooth conversion. For multiple step syntheses of more complexly substituted phenols **2** and additional functional group transformations upon construction of the aryloxypropanol array, refer to the Supplementary data.

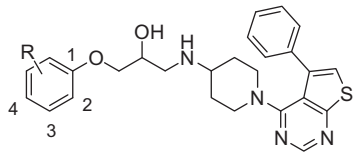
Due to the commercial availability of numerous differently substituted phenols **2**, an extensive SAR analysis was initiated around the aryloxy portion of the hit molecule **1**, an excerpt of which can be seen in Table 1.

Based on binding data, it became obvious for basically all variations that selectivity for hβ3-AR over hβ1-AR might be achieved more easily as over hβ2-AR: affinities for hβ2-AR were found to be similar or even better as compared to the K_i values for hβ3-AR (except for only compound **24**). When pure hydrogen bond acceptor groups were located in 3- or 4-position (OMe, OCF_3, F; entries **12**, **1**, **13**, **15** and **16**), partial agonism on hβ3-AR was detected in a functional assay. Intrinsic activities ranged from 0.4 to 0.8, resulting in a poor relative efficacy reflected by ρ_{Resp} = 17–51% (i.e., 17–51% of the β3-receptors in one cell have to be occupied by these compounds in order to trigger a functional response equal to 25% of the maximum response achieved with 100 μM isoproterenol). 3-CN substitution (compound **23**) was in line with these findings, even though now resulting in a full agonist and thus a somewhat better relative efficacy. Interestingly, with hydrogen bond acceptor groups installed in 2-position (OMe, F; entries **11** and **14**), highest affinities for hβ3-AR (K_i values around 12 nM) and good one-digit



Scheme 1. Reagents and conditions: (i) K_2CO_3, butanone, 80 °C, 24–72 h; (ii) ethylene glycol, 110 °C, 4 h; (iii) HCl in dioxane (4.0 M), rt, 3 h; (iv) depending on solubility: *i*PrOH or *i*PrOH/DMSO (up to 1:1), DIEA (when amine present as hydrochloride), 60–80 °C, 2–24 h; yields are not optimized.

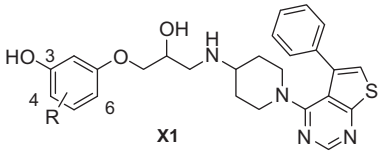
Table 1
Mono-substitution at the aryloxy moiety[9]



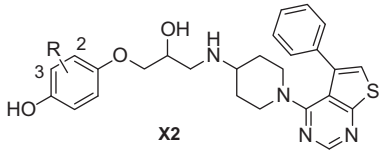
Compd	R	Binding assay, K_i [nM]			Agonist assay hβ3-AR		Antagonist assay hβ3-AR, IC_{50} [nM]
		hβ1-AR	hβ2-AR	hβ3-AR	EC_{50} [nM] (IA)	ρ_{Resp}[a] [%]	
8	H	159	9.0	17	18 (1.1)	23	–
9	3-OH[b]	60	6.0	8.5	0.62 (1.1)	2.1	–
10	4-OH[b]	975	81	148	1.6 (1.3)	0.26	–
11	2-OMe	215	11	11	2.5 (1.2)	5.5	–
12	3-OMe	938	30	42	21 (0.4)	44	–
1[c]	4-OMe	–	700	460	120 (0.6)	17	–
13	3-OCF_3	996	68	59	113 (0.8)	49	–
14	2-F	433	25	14	8.3 (1.0)	16	–
15	3-F	293	30	17	41 (0.8)	51	–
16	4-F	268	55	88	92 (0.8)	32	–
17	3-CH_2OH	34,400	18	49	26 (1.0)	15	–
18	4-CH_2OH	–	93	290	–	–	162
19	3-Et	289	10	63	264 (1.0)	58	–
20	4-Et	462	88	107	–	–	8064
21	2-Allyl	150	6.0	86	79 (1.1)	22	–
22	2-*i*Pr	100	10	64	100 (1.0)	35	–
23	3-CN	700	42	47	35 (1.0)	19	–
24	3-$NHCONH_2$	2560	139	37	150 (0.8)	64	–
25	3-NHAc	2018	117	85	–	–	107
26	3-CONHMe[b]	1427	370	331	–	–	402
27	3-CO-morpholin[b]	2902	135	149	55 (1.1)	9.4	–
28	3-COOH[b]	–	>10,000	2646	2410 (0.7)	36	–

'–' refers to no dose–response in the respective assay.
[a] ρ_{Resp} equation, RL set to 0.25.
[b] For synthetic details, cf. Supplementary data.
[c] Compound from a commercial source.

Table 2
Di-substitution at the aryloxy moiety[a,9]



X1



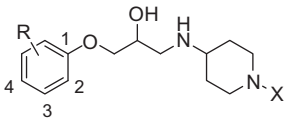
X2

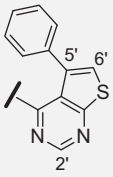
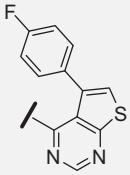
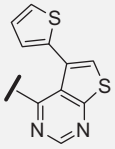
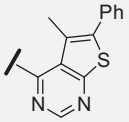
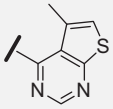
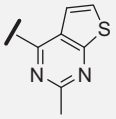
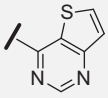
Compd	Scaffold	R	Binding assay, K_i [nM]			Agonist assay hβ3-AR	
			hβ1-AR	hβ2-AR	hβ3-AR	EC_{50} [nM] (IA)	ρ_{Resp}[b] [%]
9	X1	H	60	6.0	8.5	0.62 (1.1)	2.1
29	X1	4-CH_2OH	–	259	620	97 (1.1)	4.7
30	X1	6-COOH	–	517	919	n.d.	n.d.
10	X2	H	975	81	148	1.6 (1.3)	0.26
31	X2	3-OH	8560	873	932	1.2 (1.3)	0.03
32	X2	2-CH_2OH	1480	25	72	1.7 (1.1)	0.70
33	X2	3-CH_2OH	629	14	7.3	0.02 (1.1)	0.08
34	X2	3-CH_2OMe	484	4.9	1.0	0.04 (1.1)	1.2
35	X2	2-Et	236	7.0	30	2.2 (1.1)	2.0
36	X2	3-Et	287	11	9.0	0.14 (1.1)	0.46
37	X2	3-COOH	6710	–	3500	310 (1.0)	2.8

'–' refers to no dose–response in the respective assay; n.d. = not determined.
[a] For synthetic details, cf. Supplementary data.
[b] ρ_{Resp} equation, RL set to 0.25.

nanomolar agonistic effects were achieved. These represented slight improvements over the unsubstituted parent compound **8**. As to incorporation of hydroxy groups within the *para*-substituted series, 4-hydroxymethyl substitution (compound **18**) was not well tolerated with regard to binding affinity for hβ3-AR (K_i = 290 nM). Furthermore, this derivative displayed antagonistic activity. In contrast, direct 4-attachment of a hydroxy group (compound **10**) resulted in the most efficacious hβ3-AR agonist of the whole SAR series based on mono-substituted aryloxy derivatives reflected by an ρ_{Resp} value of 0.26%. In comparison, 3-OH incorporation (com-

Table 3
Variations of the thienopyrimidine unit[a,9]



Compd	Scaffold X	R	Binding assay, K_i [nM]			Agonist assay hβ3-AR	
			hβ1-AR	hβ2-AR	hβ3-AR	EC_{50} [nM] (IA)	ρ_{Resp}[b] [%]
8		H	159	9	17	18 (1.1)	23
10		4-OH	975	81	148	1.6 (1.3)	0.26
33		3-CH_2OH–4-OH	629	14	7.3	0.02 (1.1)	0.08
38		H	114	19	23	10 (1.0)	13
39		4-OH	600	175	75	3.1 (1.1)	1.2
40		H	33	4	11	4.7 (1.0)	13
41		4-OH	129	28	76	1.0 (1.3)	0.31
42		3-CH_2OH–4-OH	1049	20	62	0.50 (1.2)	0.21
43		H	230	100	90	96 (1.3)	20
44		4-OH	560	270	499	7.8 (1.4)	0.35
45		3-CH_2OH–4-OH	2810	94	300	3.1 (1.2)	0.27
46		H	450	80	151	7.1 (1.1)	1.4
47		4-OH	3507	2600	1390	20 (1.1)	0.41
48		3-CH_2OH–4-OH	9330	223	989	2.9 (1.2)	0.08
49		H	72	189	113	15 (1.2)	3.4
50		4-OH	4880	589	1300	24 (1.1)	0.54
51		3-CH_2OH–4-OH	4980	440	670	1.7 (1.1)	0.07
52		H	30	14	41	2.8 (1.0)	2.2
53		4-OH	5600	961	1385	33 (1.1)	0.74
54		3-CH_2OH–4-OH	3970	165	600	2.0 (1.1)	0.10

[a] For synthetic details, cf. Supplementary data.
[b] ρ_{Resp} equation, RL set to 0.25.

pound **9**) gave rise to a slightly superior EC_{50} value (0.62 vs 1.6 nM for derivative **10**), with a decreased relative efficacy, though, due to a significantly enhanced hβ3-AR affinity. As already found for the 4-hydroxymethyl derivative **18**, hβ1-AR affinity was virtually lost as well for the respective 3-derivative **17**. In the latter case, however, a decent agonistic effect on hβ3-AR was maintained, comparable to the unsubstituted variant **8**. Other polar groups than hydroxy functionalities in 3-position were not well tolerated (entries **24**–**27**): a 3-ureido group resulted in low relative efficacy (ρ_{Resp} only 64%; compound **24**), 3-acetylamino and 3-(*N*-methylcarboxamide) substitution again switched functional activity to antagonism with IC_{50} values in the range of 100–400 nM (derivatives **25** and **26**). A 3-(morpholinocarbonyl) substitution proved yet to be the best among those polar groups with an EC_{50} value of 55 nM paralleled by a relative efficacy ρ_{Resp} around 10%. Placing different alkyl groups in 2- or 3-position (entries **19**, **21** and **22**) resulted in a certain selectivity for hβ2-AR based on K_i values. As to 2-substitution (entries **21** and **22**), affinities for hβ1-AR improved simultaneously, thus resulting in a rather general unselective hβ-AR binding. Agonistic activities on hβ3-AR were diminished for 2- and 3-alkyl derivatives as compared to the parent derivative **8** with EC_{50} values now found between 79 and 264 nM. In contrast, respective 4-ethyl substitution resulted in comparable affinity levels for hβ1- and hβ3-ARs with K_i values around 100 nM, but led now to an antagonistic effect on hβ3-AR in the functional assay. Incorporation of carboxy groups (in 3- or 4-position) as well as of endocyclic nitrogens (e.g., a 2-pyridyloxy unit) drastically decreased affinities for hβ3-AR. Within this subset of compounds, best results were attained for derivative **28**, still displaying a poor K_i value of 2.6 μM.

With regard to selectivity for the hβ3-AR over the other two β-receptors, mono-substitution at the aryloxy-unit seemed appropriate to serve a certain tuning of selectivity over hβ1-AR based on K_i values. A 3-hydroxymethyl group was identified as the most effec-

tive substituent in the latter context (compound **17**) among hβ3-AR agonists. Best factor towards hβ2-AR affinity was observed for the urea derivative **24**, displaying a factor of only 3.8—accompanied by a weak hβ3-AR partial agonism with an EC_{50} value of merely 150 nM, though. Based on the SAR attained so far, a combination of two substituents within the aryloxy portion was envisaged next, with a 4-hydroxy-3-hydroxymethyl pattern appearing to be most promising: the phenolic hydroxy group for attaining highest relative efficacy and the hydroxymethyl unit for tuning selectivity over hβ1-AR.

When comparing corresponding hydroxymethyl derivatives within the 4-hydroxyaryloxy series **X2** and the 3-hydroxyaryloxy variations **X1** (compound **29** vs **33**; Table 2), the **X2**-derivative displayed significantly higher relative efficacy and activity on hβ3-AR as compared to the **X1**-compound, now even with an EC_{50} value of 20 pM (compound **33**). The rather poor results attained for both carboxylic acid derivatives **30** and **37** and the 2-ethyl compound **35** were perfectly in line with the trends already observed with respective mono-substituted derivatives (Table 1): a poor affinity and/or activity on hβ3-AR for carboxylic acid derivatives and a loss of selectivity for 2-alkyl variants were observed. For all other examples, selectivity over hβ1-AR (based on affinity data) was generally improved upon addition of another substituent as compared to the respective parent compounds **9** and **10**. Especially 4-hydroxy variants **X2** bearing a hydroxymethyl, methoxymethyl or ethyl group in 3-position (entries **33**, **34** and **36**) should be highlighted in this context—particularly compound **33**, as originally anticipated based on the data of Table 1: selectivity factors over hβ1-AR between 86 and 480 (ratio of K_i values) and sub-nanomolar agonistic activity at hβ3-AR were determined. Comparing the relative efficacies of these three compounds, different additional substitution of the 4-hydroxyphenyloxy unit might allow for an efficacy fine-tuning in the end if required (1.2% and 0.46% vs 0.08% for compound **33**)—on a highly efficacious level, though. With regard to selectivity for hβ3-Ar over hβ2-AR based on K_i values, however, no significant improvement was observed as compared to mono-substituted derivatives of Table 1. Affinities were still found to be in the same range for both receptors. Best factor of almost 5 was displayed by compound **34**, which is still rather similar to that identified for the 3-ureido substituted derivative **24** (factor 4).

Effects of variations within the thienopyrimidine portion were investigated next, the corresponding data is depicted in Table 3. Two subsets of compounds might be formed for comparison: 5′-aryl derivatives showed very similar trends, as did all 5′-non-aryl variants, with the 5′-methyl-6′-phenyl set being somewhat in-between. Within the 5′-Ar subset, 4-OH substitution always resulted in superior EC_{50} values by a factor of 3–11 as compared to the unsubstituted parent compounds, paralleled by a significant increase in relative efficacy (compounds **8**, **38** and **40** vs **10**, **39** and **41**, respectively). Both values were further optimized upon additional substitution with a 3-hydroxymethyl group, slightly for derivative **42**, significantly for compound **33**. For the latter, the EC_{50} value decreased by a factor of 80 (**33** vs **10**). Affinities for all β-ARs deteriorated from the unsubstituted parent compounds **8**, **38** and **40** to the 4-hydroxy derivatives **10**, **39** and **41**. By adding the hydroxymethyl group, an increase of selectivity (based on K_i values) for hβ3-AR over hβ1-AR was experienced for compounds **33** and **42** by a factor of at least 10, either by gaining hβ3-AR affinity or losing hβ1-AR affinity. Still, no tuning of selectivity over hβ2-AR was detectable.

Within the three sets of smaller thienopyrimidines, hβ3-AR affinity was generally diminished as compared to the 5′-aryl variants, resulting in poor affinities of around 1.35 μM for all 4-hydroxy derivatives **47**, **50** and **53**. Significantly better affinities could not even be detected with the as yet most promising 4-hydroxy-3-hydroxymethyl substitution pattern in place (compounds **48**, **51** and **54**; K_i values between 600 and 990 nM). The diminution in hβ3-AR affinity observed upon incorporation of the 4-hydroxy group into the parent molecules **46**, **49** and **52** was accompanied by a slight to significant decrease of functional activity (factor 1.5–12), which is in clear contrast to the results out of the 5′-aryl subsets. Agonistic effects, however, were still decent with EC_{50} values in the one-digit nanomolar range for the 4-hydroxy-3-hydroxymethyl derivatives. This resulted in excellent relative efficacies comparable to the respective compounds out of the 5′-aryl series. Due to the generally elevated K_i level on hβ3-AR within the 5′-non-aryl subsets, selectivity over hβ1-AR based on K_i values became less favorable (all below a factor of 10 as compared to 86 for **33**).

The 5′-methyl-6′-phenyl derivatives displayed a similar development of relative efficacy as observed for the 5′-thienyl derivatives **40**–**42**, from the parent compound **43** to the 4-OH derivative **44** and the 4-hydroxy-3-hydroxymethyl compound **45**. However, both the affinity and the activity on hβ3-AR were diminished significantly for these derivatives, thus rendering this array less favorable.

Upon altering both 'ends' of the molecule, the final set of variations was performed around the 2-propanolamine bridge and the piperidine spacer unit. Several derivatizations are depicted in Figure 2, all of which were quite detrimental to affinity and activity on hβ3-AR. Alkylations—with both small and large groups, lipophilic and polar—and acylation of the secondary amine of the propanolamine unit, as well as cyclization including both the amine and the hydroxy group resulted in derivatives with poor agonistic activity on hβ3-AR. This effect was paralleled by low relative efficacy data (high ρ_{Resp}). An evaluation of different spacer units was performed on the level of affinity for hβ3-AR: inverting the 4-aminopiperidine unit resulted in a decrease of affinity from K_i = 23 nM

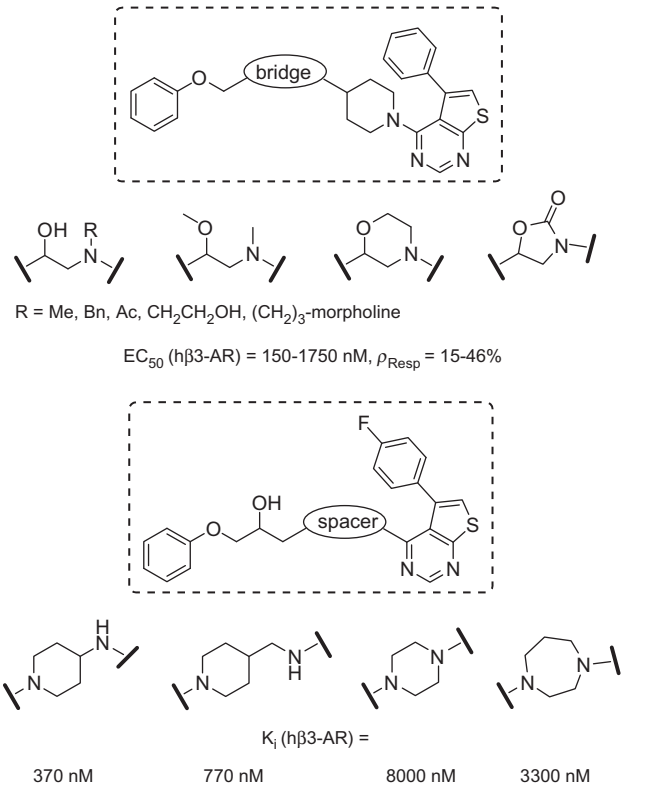


Figure 2. Bridge and spacer variations.[9] For synthetic details, cf. Supplementary data. ρ_{Resp} values were calculated using RL = 0.25.

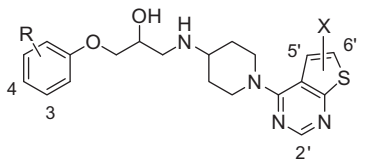
for compound **38** to 370 nM for the new derivative. Implementation of other diamines like (4-aminomethyl)piperidine, piperazine or homopiperazine were tolerated even less. Keeping in mind the central role of this spacer with regard to an overall orientation of the thienopyrimidine moiety versus the aryloxy portion, such a finding was not unexpected: these new spacer variants significantly manipulate distance and angle between the two distal aromatic regions.

The next questions to be addressed were whether (1) the selectivity over hβ1-AR would prevail on a functional level (reflecting relative efficacies on hβ-AR subtypes), and (2) a certain selectivity over hβ2-AR might be achievable based on different relative efficacies on these two hβ-AR subtypes.

And indeed, a certain selectivity for hβ3-AR over hβ1-AR could be maintained on a cellular level, even though not predictably with a loss or gain as compared to the binding events. The overall trend was identified to be rather a (slight) deterioration of this selectivity factor as compared to a selectivity ratio determined by K_i values: for three out of twelve compounds, selectivity ratios based on K_i values and on EC_{50} values were similar (compounds **12**, **41** and **47**; Table 4 vs Tables 1 and 3), for two data pairs, the EC_{50} ratio was favorable (compounds **14** and **33**; Table 4 vs Tables 1 and 2) and for nine data pairs, the EC_{50} ratio was less favorable as compared to the K_i ratios (compounds **9**, **11**, **17**, **24**, **38**, **39** and **49**; Table 4 vs Tables 1 and 3), for compounds **17** and **24** even drastically. Such deviations between K_i ratios and EC_{50} ratios reflect differences in relative efficacies of respective compounds on different hβ-AR subtypes: relative efficacies ρ_{Resp} on hβ1- and hβ3-AR varied by factors of 1.4–3.0 in favor of hβ3-AR (=higher relative efficacy on hβ3-AR = lower ρ_{Resp} for hβ3-AR in Table 4; valid for five out of twelve compounds) and by factors of 1.5–100 in favor of hβ1-AR (for seven out of twelve compounds). The higher factors in favor of hβ1-AR reflect the more general loss of selectivity towards this adrenoceptor on a functional level, now resulting in no selectivity for hβ3-AR over hβ1-AR for compound **9** or even in a slight selectivity for hβ1-AR for derivatives **24**, **38** and **49**. As to selectivity for hβ3-AR over hβ2-AR, antagonism at hβ2-AR was identified for all derivatives tested in a cellular assay. This result emphasizes the importance of founding a hit-to-lead optimization process not only on binding data alone. Based on functional data, compound **33** represented the most promising compound displaying good to excellent selectivities towards hβ1- and hβ2-AR. Other thienopyrimidine variants than 5′-phenyl usually displayed no significant selectivity for hβ3-AR over hβ1-AR based on EC_{50} values (compounds **38**, **39**, **41**, **47** and **49**; best selectivity factor of 3.3 for **47**). Any slight selectivity based on K_i values was retained at best, in most cases it was diminished. Selectivity for hβ3-AR over hβ2-AR on a cellular level, on the other hand, seemed to be quite decent for the latter compounds.

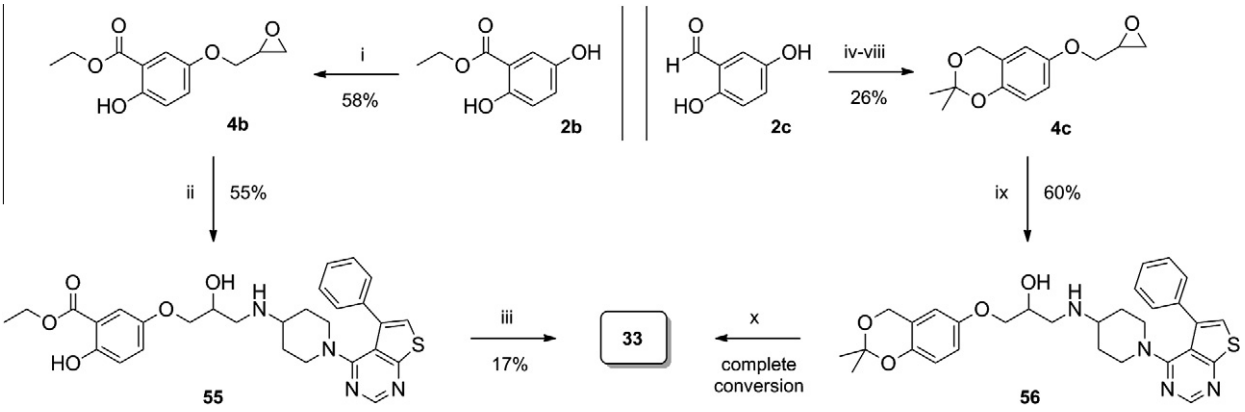
Concluding on these SAR investigations, compound **33** was selected for further evaluations: a HitProfilingScreen® on 30 primary molecular targets (GPCRs, ion channels and enzymes),[19] a toxicity profiling, and a determination of physicochemical properties were initiated. With regard to toxicity uncritical ED_{50} values of 29 and 60 μM were measured in a PBMC viability and HepG2 assay, respectively. Functional inhibition of the hERG channel was not representing an issue either (determined electrophysiologically, no inhibition at 2 μM). As to be expected from a log *P* of 2.90 (determined by capillary electrophoresis) and a TPSA of 110 (calcd),[20] solubility in aq. media was found to be quite excellent (>100 μM in a pH range from 4 to 7.4). Membrane permeability as evaluated in a PAMPA was determined to be moderate to good (about 10 nm/s at pH 5 and 7.4). The compound proved to be completely stable in artificial gastric juice, simulated intestinal fluids and human plasma within a test period of 24 h. The HitProfilingScreen®[19] was further complemented by an evaluation on a set of GPCRs comprising dopamine D4.2, 5-HT1 and the neurokinin receptors NK1, NK2 and NK3. As the original hit molecule **1** resulting from the vHTS campaign was described in the literature to possess antagonistic effects on the latter three receptors,[8] at least the binding potential to these receptors had to be examined for compound **33** as well. Compared to an on-target activity of 20 pM, the whole set of binding data at a compound concentration of 10 μM proved to be quite promising: only at α1b-AR (rat), the human norepinephrine transporter and the dopamine receptor D4.2 radioligand displacement was observed with 88–97% at this concentration. For these off-targets, dose–response curves and functional data have to be determined next. Affinities for neurokinin

Table 4
Functional data on hβ1-, hβ2- and hβ3-ARs for selected compounds[a,9]



Compd	X	R	Agonist assay hβ1-AR		Antagonist assay hβ2-AR, IC_{50} [nM]	Agonist assay hβ3-AR	
			EC_{50} [nM] (IA)	ρ_{Resp}[b] [%]		EC_{50} [nM] (IA)	ρ_{Resp}[b] [%]
9	5′-Ph	3-OH	0.57 (1.0)	0.31	100	0.62 (1.1)	2.1
11	5′-Ph	2-OMe	9.6 (1.0)	1.5	205	2.5 (1.2)	5.5
12	5′-Ph	3-OMe	450 (0.3)	71	250	21 (0.4)	44
14	5′-Ph	2-F	600 (0.6)	50	220	8.3 (1.0)	16
17	5′-Ph	3-CH_2OH	70 (0.6)	0.15	245	26 (1.0)	15
24	5′-Ph	3-NHCONH$_2$	110 (0.7)	2.3	1280	150 (0.8)	64
33	5′-Ph	3-CH_2OH–4-OH	3.5 (1.1)	0.15	2250	0.02 (1.1)	0.08
38	5′-(4-F-Ph)	H	6.6 (1.0)	1.9	123	10 (1.0)	13
39	5′-(4-F-Ph)	4-OH	6.3 (1.0)	0.34	3000	3.1 (1.1)	1.2
41	2′-(2-Thienyl)	4-OH	1.8 (1.1)	0.43	327	1.0 (1.3)	0.31
47	5′-Me	4-OH	65 (1.0)	0.61	5500	20 (1.1)	0.41
49	2′-Me	H	5.0 (1.0)	2.2	862	15 (1.2)	3.4

[a] EC_{50} values given for agonistic effects, IC_{50} values represent antagonistic activity.
[b] ρ_{Resp} equation, RL set to 0.25.



Scheme 2. Reagents and conditions: (i) epibromohydrine, K_2CO_3, acetone, 55 °C, 48 h; (ii) amine **7**, *i*PrOH, 80 °C, 6 h; (iii) $LiAlH_4$ (1 M in THF), CH_2Cl_2, 0 °C to rt, 6 h, 4–36%; (iv) *t*BDMS-Cl, imidazole, CH_2Cl_2, rt, 18 h, 84%; (v) $NaBH_4$, EtOH, 0 °C to rt, 1.5 h, 99% (crude); (vi) 2,2-dimethoxypropane, *p*TsOH (cat), CH_2Cl_2, rt, 48 h, 51%; (vii) KF, DMF, rt, 2 h, 75%; (viii) epibromohydrine, K_2CO_3, 2-butanone, 80 °C, 48 h, 81%; (ix) amine **7**, $LiClO_4$, DIEA, CH_3CN, rt, 20 h; (x) $HOAc/H_2O$ (5:2), 70 °C, 0.5 h.

receptors were not significant, best binding event was identified for NK2 with 52% displacement at 10 μM.

Advancing towards a first tissue assay and in vivo investigations, bulk material of compound **33** was required. Following the general synthetic route depicted in Scheme 1, which corresponds to the sequence on the left in Scheme 2, a $LiAlH_4$ reduction of the ester group in compound **55** to give the hydroxymethyl unit of lead compound **33** was scheduled as the final step (cf. also Supplementary data). This, however, was only achieved with low reproducibility and yield (4–36%; average yield 17%). The outcome was compromised by an unfavorable workup procedure due to the presence of aluminum salts remaining from the reducing agent and the occasional necessity of removing byproducts. Consequently, the total synthesis was re-evaluated, adapting the functional group transformation strategy for the aryloxy portion from the synthesis of Salmeterol:[21] starting from aldehyde **2c**, the carbaldehyde functionality was reduced to a hydroxymethyl group,[22] which was incorporated into a cyclic acetal together with the adjacent phenolic hydroxy group (Scheme 2). However, this route called for an extension of this sequence by two additional synthetic steps as a direct generation and transformation of 2-(hydroxymethyl)benzene-1,4-diol was unsuccessful: a *t*BDMS protection of the unhindered hydroxy group[23] prior to the reduction of the carbaldehyde functionality and its deprotection after acetal formation. Thus the preparation of the aryloxymethyloxirane **4c** now became a 5-step-synthesis with an overall yield of 26% as compared to a one-step procedure (58%) for the respective 3-ethoxycarbonyl derivative **4b** incorporated within the original sequence. Despite this drawback the total synthesis of **33** became advantageous following the alternative route due to its last step: final deprotection of **56** to give **33** now succeeded with complete conversion without any formation of byproducts. Nucleophilic oxirane opening of **4c** with amine **7** could be slightly optimized using $LiClO_4$ as Lewis acid catalyst.[24]

Using a pharmacophore alignment on known hβ3-AR agonists, not including a single aryloxypropanolamine, a vHTS approach followed by medicinal chemistry explorations around the resulting hit molecule led to the identification of a highly potent and efficacious hβ3-AR agonist (EC_{50} = 20 pM) of the aryloxypropanolamine scaffold. This lead compound possesses promising selectivity over hβ1- and hβ2-AR on a functional level and a favorable safety profile. Based on these results, an evaluation of the relaxing potential on pre-contracted human bladder detrusor strips was envisaged and compound **33** advanced to the lead optimization stage, the results of which will be disclosed in due course.

Acknowledgments

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Supplementary data

Supplementary data (additional synthetic Schemes, spectral data for compound **33**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.039.

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9. All aryloxypropanolamine derivatives were tested as racemates. For a further lead optimization process, the (*S*)-enantiomers proved to be more active than the respective (*R*)-enantiomers.
10. Membrane preparations (CHO-K1 cell line) expressing human β1-, β2- or β3-ARs (B_{max} = 3.78, 1.68 and 47.2 pmol/mg protein, respectively), were purchased from Euroscreen (now Euroscreen FAST or Perkin Elmer). Binding assays were performed according to the manufacturer's instructions. The radioligand for all three receptor subtypes was [^{125}I]-cyanopindolol (Amersham) (final concentration of 0.05, 0.05 and 1.5 nM, respectively). K_i values were calculated using the Cheng–Prusoff equation on IC_{50} determinations, which were based on concentration curves using eight concentrations (half-logarithmic) in duplicate.
11. Functional response of cells (agonistic or antagonistic) to the test compounds was tested by measurement of cyclic AMP formation by HTRF® (Homogeneous Time-Resolved Fluorescence) technology (Cisbio International) using a stable cell line CHO-K1 expressing the human recombinant β3-AR (Euroscreen, now Euroscreen FAST or Perkin Elmer; B_{max} = 23 pmol/mg protein) according to the manufacturer's instructions. EC_{50} (agonists) and IC_{50} values (antagonists) were determined by dose–response curves based on eight concentrations (logarithmic) determined in quadruplicate in a 96 half-well plate in a final volume of 100 μl. The antagonistic effect was determined by preincubation with a test compound for 10 min followed by agonist stimulation (0.05 nM

isoproterenol) for 30 min. All compounds were evaluated in both assay formats.
Functional data on hβ1-AR and hβ2-AR were determined at Euroscreen (both, in an agonist and an antagonist assay; B_{max} = 11.1 and 2.3 pmol/mg protein for hβ1-AR and hβ2-AR, respectively). EC_{50} and IC_{50} values, respectively, were determined by dose–response curves based on seven concentrations (logarithmic) determined in duplicate.
12. The term 'affinity' is strictly used for the description of binding data, whereas 'activity' refers to functional data from cellular assays throughout this Letter.
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