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Tetrahydro-β-carboline derivatives targeting fatty acid amide hydrolase (FAAH) and transient receptor potential (TRP) channels

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ABSTRACT

A series of twenty-five derivatives of tetrahydro-β-carbolines **1**–**3** was synthesized and assayed on FAAH and TRPV1 and TRPA1 channels. Four carbamates, that is, **5a**,**c**,**e**, and **9b** inhibited FAAH with significant potency and interacted also effectively with TRPV1 and TRPA1 nociceptive receptors, while ureas **7b**,**d**,**f**, and **8a**,**b** were endowed with specific submicromolar TRPV1 modulating activities.

The transient receptor potential vanilloid type 1 (TRPV1) is the founding member of a large family of transient receptor potential (TRP) channels, which typically acts as a molecular detector of noxious signals in primary sensory neurons.[1] The therapeutic potential of targeting TRPV1 by agonists and antagonists, in particular for chronic pain relief, has attracted an enormous amount of attention over the past ten years.[2] A wide range of stimuli are responsible for TRPV1 activation including noxious heat, low extracellular pH and a variety of chemical mediators. The prototypical endocannabinoid anandamide (AEA) has been the first endogenous modulator of TRPV1 to be identified, followed by other fatty acid conjugates of biogenic amines such as *N*-arachidonoyldopamine, *N*-oleoylethanolamine, and *N*-arachidonoylserotonin (AA-5-HT).[3] In particular, AA-5-HT exhibits interesting analgesic properties in models of both inflammatory and neuropathic pain,[4] owing to its peculiar profile of 'hybrid' TRPV1 channel blocker and inhibitor of the endocannabinoid degradative enzyme, fatty acid amide hydrolase (FAAH), another attractive target involved in nociception.[5] FAAH inhibitors have in fact definitely demonstrated therapeutic benefit in a variety of pain models.[5]

Two natural indoloquinazolone alkaloids endowed with vanilloid activity, evodiamine[6] and rutaecarpine[7] (Fig. 1), can be viewed as conformationally restricted tryptamine derivatives, thus bearing a certain resemblance with AA-5-HT.

More recently, TRPV1 affinity has been also demonstrated for certain acyl amides of salsolinol (e.g., *N*-arachidonoylsalsolinol, Fig. 1), an endogenous tetrahydroisoquinoline formed by a Pictet–Spengler condensation of dopamine with acetaldehyde.[8]

Tetrahydro-β-carbolines such as 6-hydroxy-1,2,3,4-tetrahydro-β-carboline (**1**),[9] 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (**2**),[10] and 1,2,3,4-tetrahydro-β-carboline (**3**),[11] occurring in mammals as a result of a reaction of serotonin or tryptamine with formaldehyde or acetaldehyde,[10,12] represent three constrained serotonin or tryptamine analogues. Therefore, we decided, as a continuation of our previous studies on AA-5-HT,[4] its analogues,[13] and other FAAH/TRP dual ligands based on the piperazinylcarbamate and urea chemotypes,[14] to prepare a number of derivatives of carbolines **1**–**3** (compounds **4**–**9**), and thus evaluate the influence of the rigidification of the aminoethyl side chain of the serotonin moiety of AA-5-HT on TRPV1 and FAAH activities (Table 1). All compounds were tested on transient receptor potential ankyrin type-1 (TRPA1) as well.[15] TRPA1 channel is expressed in the polymodal C- and A-δ fiber sensory neurons of the dorsal root and trigeminal ganglia, is coexpressed with TRPV1 in a subset of TRPV1-containing neurons, and is activated by noxious cold and a variety of natural nocifensive or producing burning sensations compounds, thus playing an important role in pain sensing.[16] Since all TRP channels are also rapidly desensitized by their agonists

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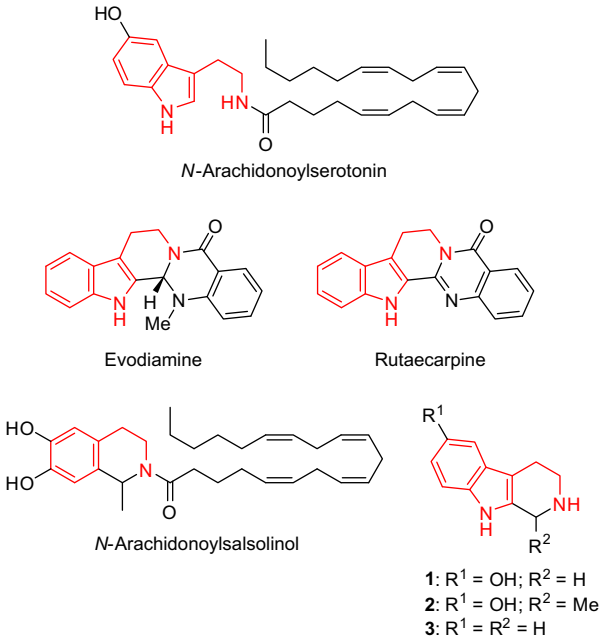
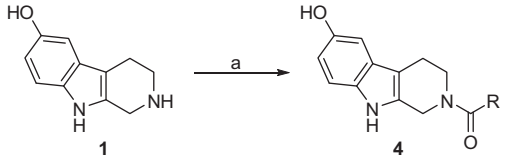


Figure 1. Structures of *N*-arachidonoylserotonin, alkaloids evodiamine and rutaecarpine, *N*-arachidonoylsalsolinol, and tetrahydro-β-carbolines **1**–**3** (tryptamine and phenethylamine moieties are highlighted in red).



Scheme 1. Synthesis of compounds **4**. Reagents and conditions: (a) RCO_2H, HOBt/EDC, DMF, rt, 1 h, then **1**, Et_3N, DMF, rt, 16 h.

after activation,[17] we also tested the capability of the compounds to inhibit the activation of TRPV1 or TRPA1 by their canonical agonists, capsaicin and allyl isothiocyanate. Therefore, for each compound, both EC_{50} (for activation) and IC_{50} (for desensitization) values were calculated. However, when exhibiting an EC_{50} >10 μM and an IC_{50} in the low μM range, a compound is likely to behave as a 'true' antagonist rather than as a desensitizer.

The synthesis of amides **4** was carried out by condensation of **1** (as the hydrochloride) with the appropriate carboxylic acids using 1-hydroxybenzotriazole (HOBt)/*N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) as the carboxylate activator (Scheme 1). Carbamates **5**, **6**, **9a**,**b** and ureas **7**, **8**, **9c**,**d** were synthesized by condensation of β-carbolines **1** (as the hydrochloride), **2**, and **3** (as the hydrochloride) with the appropriate aryl chloroformates or isocyanates (Scheme 2).[18]

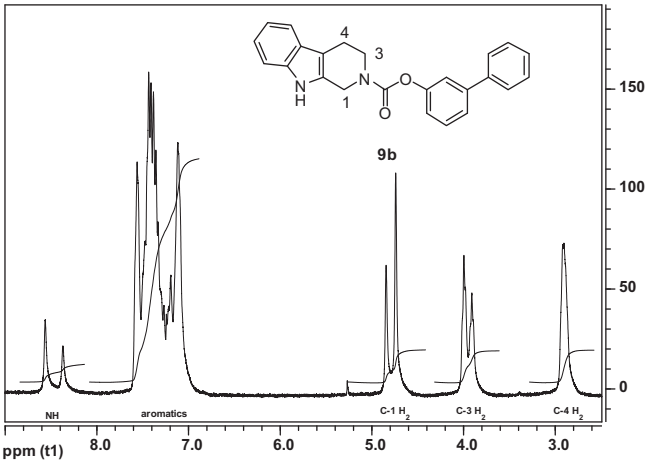
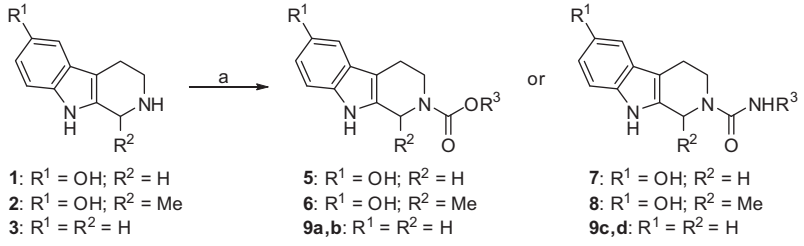


Figure 2. ^1H NMR spectrum of compound **9b** in DMSO-d_6 at room temperature.

Most of compounds **4**–**9** displayed complex ^1H and ^{13}C NMR spectra at room temperature, indicating their existence as interconverting rotamers. As an example, the ^1H NMR spectrum of compound **9b** is shown in Figure 2.

In FAAH assays, amides **4** and ureas **7**, **8**, **9c**,**d** were all essentially inactive, whereas some of the carbamates (compounds **5a**–**c**,**e** and **9b**) exhibited significant FAAH inhibitory activities. Amides **4**, with the partial exception of **4d**,**e**, had also modest effects on TRPV1 and TRPA1 channels. Carbamates with *meta*-substituted aromatic rings were more potent on FAAH than the *para*-substituted ones (compare compounds **5a**,**e** and **5d**,**f**), in line with previous results on AA-5-HT analogues,[13] piperazinyl carbamates and ureas,[14] and arylcarbamic acid aryl esters.[19] The presence of a methyl group on the tetrahydro-β-carboline moiety was detrimental to the inhibitory potency (compare compounds **5e** and **6**). In contrast, removal of the 6-OH group from carbamate **5e** resulted in a ~six fold increase in potency (compound **9b**), a result opposite to that previously described by Fowler et al. for *N*-arachidonoyltryptamine, a 2.3-fold weaker inhibitor of AEA hydrolysis than AA-5-HT.[20] Most of the carbamates were able to modulate both TRPV1 and TRPA1 channels with EC_{50} and/or IC_{50} values <10 μM (compounds **5a**,**c**–**f**, and **9a**,**b**). Four carbamates, that is **5a**,**c**,**e**, and **9b**, acted as efficient triple FAAH/TRPV1/TRPA1 ligands and could conceivably represent useful leads for novel analgesic compounds targeting FAAH and additional players in pain. Carbamate **5b** was the only dual FAAH/TRPV1 blocker identified, but with potencies lower than those of AA-5-HT.

With the exception of **7c**, all ureas showed good and selective TRPV1 modulating activities. Particularly interesting in this respect appeared to be compound **7b**, endowed with selective activity in the low nanomolar range. Recent investigations have been devoted to the identification of TRPV1 regions involved in the recognition of some ligands and in the prediction of their binding modes.[21] The



Scheme 2. Synthesis of compounds **5**–**9**. Reagents and conditions: (a) R^3OCOCl or R^3NCO, Et_3N, DMF, rt, 16 h.

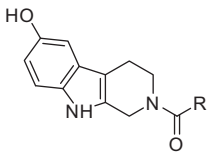
presence of a 4-*t*-Bu phenyl moiety in the lipophilic arm of TRPV1 modulators that can be traced back to capsaicinoids and resiniferatoxin (RTX) was found frequently to be critical for potency, presumably related to optimal π–π stacking and hydrophobic interactions.[22] 1-Methyl group on the carboline moiety reduced the activity of ureas for TRPV1 (compare compounds **7b**,**d** and **8a**,**b**).

The influence of the removal of the 6-OH substituent on the TRP activities was not uniform and both a reduction (compare compounds **5d**, **7b** and **9a**,**c**) and an improvement (compare compounds **5e** and **9b**) of potencies, but not necessarily of efficacy, was observed. Therefore, no definite conclusion can be drawn as to the role of the 6-OH group in TRPV1 and TRPA1 modulation. Anyway, among carbamates and ureas devoid of the 6-OH substituent, **9b** and **9c** exhibited submicromolar FAAH inhibitory/low efficacy TRPA1 agonist activity and 'true'[23] TRPV1 antagonist properties, respectively, and deserve a special mention.

The EC_{50} and IC_{50} values at the same TRP channel were generally of the same order of magnitude and thus, in most cases, activation and desensitization potencies were comparable; some discordances were, however, also evident, particularly for the TRPA1 channel (see compounds **4a**–**c**, **7b**,**f**), suggesting in these cases either the occurrence of antagonism (when the $IC_{50} << EC_{50}$) or a poor capability of producing desensitization (when the $EC_{50} << IC_{50}$).

Comparison of AA-5-HT and its analogues[13] and tetrahydro-β-carboline derivatives with identical R (or R^3) groups revealed that rigidification of the serotonin side chain generally reduced the activity for FAAH and TRPV1, with only few exceptions (i.e., compounds **5a**,**d** for TRPV1).

Table 1
Results of FAAH, TRPV1, and TRPA1 assays of derivatives of tetrahydro-β-carbolines **1**,**2**, and **3**[a]



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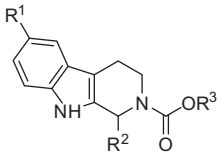
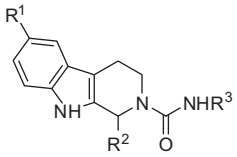
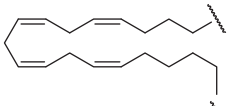
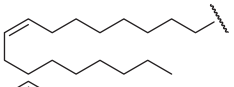
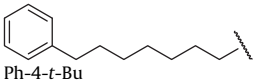


5: R^1 = OH; R^2 = H
6: R^1 = OH; R^2 = Me
9a,**b**: $R^1 = R^2$ = H



7: R^1 = OH; R^2 =H
8: R^1 = OH; R^2 = Me
9c,**d**: $R^1 = R^2$ = H

Compound	R or R^3	FAAH (IC_{50}, μM)	TRPV1[b] (efficacy)	TRPV1 (EC_{50}, μM)	TRPV1[c] (IC_{50}, μM)	TRPA1[d] (efficacy)	TRPA1 (EC_{50}, μM)	TRPA1[e] (IC_{50}, μM)
4a		>10	<10	ND	9.30 ± 0.90	60.2 ± 0.1	5.24 ± 0.01	35.49 ± 0.77
4b		>10	<10	ND	10.60 ± 0.30	< 10	ND	11.67 ± 0.81
4c		>10	<10	ND	9.60 ± 0.03	16.7 ± 0.1	ND	20.17 ± 0.35
4d	Ph-4-*t*-Bu	>10	71.8 ± 0.9	0.54 ± 0.04	0.44 ± 0.01	64.9 ± 0.8	16.51 ± 0.59	30.60 ± 0.83
4e	Ph-4-Ph	>50	49.0 ± 1.6	6.42 ± 0.54	3.13 ± 0.35	83.5 ± 2.0	4.09 ± 0.56	12.90 ± 0.65
4f	CH_2Ph-3-Ph	>50	48.6 ± 0.5	11.80 ± 0.39	19.45 ± 0.30	109.6 ± 2.9	7.78 ± 0.86	15.30 ± 0.60
5a	Ph-3-*t*-Bu	3.69 ± 0.84	52.3 ± 0.5	5.1 ± 2.3	6.20 ± 0.10	65.4 ± 0.9	4.95 ± 0.10	10.47 ± 0.84
5b	Ph-3-CF_3	6.77 ± 0.85	<10	ND	6.40 ± 0.10	113.0 ± 0.8	14.30 ± 0.25	26.32 ± 0.46
5c	Ph-3-$(CH_2)4CH_3$	6.25 ± 0.82	<10	ND	5.10 ± 0.10	81.4 ± 2.6	1.68 ± 0.32	3.25 ± 0.36
5d	Ph-4-*t*-Bu	>10	54.4 ± 0.4	0.4 ± 0.01	0.55 ± 0.5	73.9 ± 4.6	4.29 ± 1.03	3.40 ± 0.51
5e	Ph-3-Ph	1.74 ± 0.03	12.5 ± 0.1	5.2 ± 0.1	9.60 ± 0.20	77.5 ± 0.4	1.70 ± 0.04	3.48 ± 0.17
5f	Ph-4-Ph	>10	37.6 ± 0.8	7.75 ± 0.57	11.38 ± 0.37	73.4 ± 0.1	5.04 ± 0.03	9.76 ± 0.30
6	Ph-3-Ph	>10	< 10	ND	ND	59.1 ± 0.1	1.71 ± 0.02	5.36 ± 0.20
7a	Ph-3-*t*-Bu	>10	30.7 ± 0.2	1.89 ± 0.07	5.35 ± 0.42	38.1 ± 0.8	17.04 ± 1.09	34.87 ± 0.12
7b	Ph-4-*t*-Bu	>10	69.2 ± 0.7	0.019 ± 0.0012	0.001 ± 0.00001	17.9 ± 0.1	ND	21.11 ± 0.57
7c	Ph-3-CF_3	>10	15.5 ± 1.3	7.43 ± 0.50	13.82 ± 0.77	88.8 ± 1.1	19.57 ± 0.75	55.36 ± 2.72
7d	Ph-4-CF_3	>10	57.3 ± 0.2	0.26 ± 0.07	0.33 ± 0.02	91.6 ± 2.1	21.03 ± 0.10	24.56 ± 0.15
7e	Ph-3-Ph	>50	78.3 ± 1.6	2.27 ± 0.20	1.86 ± 0.11	136.5 ± 1.5	14.76 ± 0.86	17.03 ± 0.36
7f	Ph-4-Ph	>50	73.9 ± 2.6	0.17 ± 0.03	0.14 ± 0.01	93.3 ± 5.8	1.82 ± 0.75	18.84 ± 0.67
8a	Ph-4-*t*-Bu	>50	68.2 ± 0.1	1.2 ± 0.02	0.098 ± 0.05	117.5 ± 5.7	10.77 ± 0.10	22.50 ± 0.68
8b	Ph-4-CF_3	>50	67.4 ± 0.3	0.47 ± 0.01	0.31 ± 0.01	70.5 ± 1.0	13.77 ± 0.62	35.12 ± 2.85
9a	Ph-4-*t*-Bu	>50	17.30 ± 0.01	5.29 ± 0.01	13.26 ± 1.23	117.8 ± 3.5	9.81 ± 0.83	3.36 ± 0.38
9b	Ph-3-Ph	0.275 ± 0.04	20.6 ± 1.8	2.40 ± 0.94	7.01 ± 0.29	33.4 ± 0.9	0.20 ± 0.04	2.84 ± 0.43
9c	Ph-4-*t*-Bu	>50	<10	ND	0.75 ± 0.01	52.6 ± 1.2	1.92 ± 0.29	3.90 ± 0.18
9d	Ph-4-CF_3	>50	<10	ND	4.36 ± 0.67	102.2 ± 1.8	3.71 ± 0.25	7.14 ± 0.27

ND, not determined when efficacy is lower than 10%.
[a] Data are means ± SEM of $N = 3$ determinations.
[b] As percent of ionomycin (4 μM).
[c] Determined against the effect of capsaicin (0.1 μM).
[d] As percent of allyl isothiocyanate (100 μM).
[e] Determined against the effect of allyl isothiocyanate (100 μM).

In conclusion, in this Letter we have presented a series of tetrahydro-β-carboline derivatives designed as rigid analogues of AA-5-HT and its congeners and have identified: (1) some carbamates that inhibit FAAH with significant potency and interact also effectively with TRPV1 and TRPA1 nociceptive receptors, and (2) a number of ureas endowed with submicromolar TRPV1 modulating activities.

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15. *FAAH assays*. The effect of increasing concentrations of the new synthetic compounds on the enzymatic hydrolysis of [^{14}C]anandamide was studied by using membranes prepared from rat brain. In brief, the whole rat brain was homogenized at 4 °C in 50 mM Tris-HCl buffer, pH 7.0, by using an ultraturrax and a dounce homogenizer. Homogenates were first centrifuged at 800 g to get rid the debris and the supernatant was centrifuged at 10,000 g. The pellet from this latter centrifugation was used for the assay. Membranes (70–100 μg) were incubated with increasing concentrations (up to 50 μM) of the test compounds and [^{14}C]AEA (10,000 cpm, 1.8 μM) in 50 mM Tris–HCl, pH 9, for 30 min at 37 °C. [^{14}C]Ethanolamine produced from [^{14}C]AEA hydrolysis was used to calculate FAAH activity and was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with 2 volumes of $CHCl_3$/CH_3OH (1:1 by volume). Data are expressed as the concentration exerting a half-maximal inhibition (IC_{50}). *TRPV1 and TRPA1 channel assays*. HEK293 (human embryonic kidney) cells stably over-expressing recombinant rat TRPA1, or human TRPV1 were grown on 100 mm diameter Petri dishes as mono-layers in minimum essential medium (EMEM) supplemented with non-essential amino acids, 10% foetal bovine serum, and 2 mM glutamine, and maintained at 5% CO_2 at 37 °C. Stable expression of each channel was checked by quantitative PCR (data not shown). The effect of the substances on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined by using Fluo-4, a selective intracellular fluorescent probe for Ca^{2+}. On the day of the experiment, cells were loaded for 1 h at room temperature with the methyl ester Fluo-4-AM (4 μM in dimethyl sulfoxide containing 0.02% Pluronic F-127, Invitrogen) in EMEM without foetal bovine serum, then were washed twice in Tyrode's buffer (145 mM NaCl, 2.5 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM D-glucose, and 10 mM HEPES, pH 7.4), resuspended in the same buffer, and transferred (about 100,000 cells) to the quartz cuvette of the spectrofluorimeter (Perkin-Elmer LS50B equipped with PTP-1 Fluorescence Peltier System; PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) under continuous stirring. The changes in $[Ca^{2+}]_i$ were determined before and after the addition of various concentrations of test compounds by measuring cell fluorescence (λ_{EX} = 488 nm, λ_{EM} = 516 nm) at 25 °C. Curve fitting (sigmoidal dose-response variable slope) and parameter estimation were performed with GraphPad Prism® (GraphPad Software Inc., San Diego, CA). Potency was expressed as the concentration of test substances exerting a half-maximal agonist effect (i.e., half-maximal increases in $[Ca^{2+}]_i$) (EC_{50}). In the case of TRPV1 assays, the efficacy of the agonists was first determined by normalizing their effect to the maximum Ca^{2+} influx effect on $[Ca^{2+}]_i$ observed with application of 4 μM ionomycin (Alexis). When significant, the values of the effect on $[Ca^{2+}]_i$ in wild-type (i.e., not transfected with any construct) HEK293 cells were taken as baseline and subtracted from the values obtained from transfected cells. The effects of TRPA1 agonists are expressed as a percentage of the effect obtained with 100 μM allyl isothiocyanate (AITC). Antagonist/desensitizing behaviour was evaluated against AITC (100 μM) for TRPA1 and capsaicin (0.1 μM) for TRPV1, by adding the test compounds in the quartz cuvette 5 min before stimulation of cells with agonists. Data are expressed as the concentration exerting a half-maximal inhibition of agonist-induced $[Ca^{2+}]$i elevation (IC_{50}), which was calculated again using GraphPad Prism® software. The effect on $[Ca^{2+}]_i$ exerted by agonist alone was taken as 100%. Dose response curves were fitted by a sigmoidal regression with variable slope. All determinations were performed at least in triplicate. Statistical analysis of the data was performed by analysis of variance at each point using ANOVA followed by the Bonferroni's test.
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18. *General procedure for the synthesis of compounds **4***. To a stirred solution of the appropriate carboxylic acid (0.20 mmol) in DMF (1 mL) HOBt (35 mg, 0.24 mmol) and EDC (40 mg, 0.24 mmol) were added at 0 °C. The mixture was stirred for 15 min at 0 °C and for 1 h at room temperature. Then **1** (as hydrochloride, 54 mg, 0.24 mmol) and Et_3N (34 μL, 0.24 mmol) were added, and the mixture was stirred overnight at room temperature. The mixture was diluted with brine and extracted with AcOEt. The organic phase was washed with 2 N HCl solution, saturated $NaHCO_3$, and brine, dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by column chromatography. *General procedure for the synthesis of compounds **5**, **6**, **9a**,**b***. To a stirred 20% phosgene solution in toluene (0.60 mL, 1.15 mmol) a solution of the appropriate phenol (0.29 mmol) and Et_3N (47 μL, 0.34 mmol) in dry toluene (2.9 mL) was added dropwise at 0 °C. The reaction mixture was stirred for 3 h at room temperature and evaporated under vacuum. The residue of the crude chloroformate was dissolved in dry CH_2Cl_2 (2 mL) and a solution of **1** (as hydrochloride) or **2** or **3** (as hydrochloride) (0.29 mmol) and Et_3N (in the case of **1** and **3**, 81 μL, 0.58 mmol; in the case of **2**, 41 μL, 0.29 mmol) in dry DMF (1 mL) was added dropwise at room temperature with stirring. The reaction mixture was stirred overnight at room temperature, diluted with water, and extracted with AcOEt. The organic phase was washed twice with brine, dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by column chromatography. *General procedure for the synthesis of compounds **7**, **8**, **9c**,**d***. A solution of the appropriate isocyanate (0.24 mmol), **1** (as hydrochloride) or **2** or **3** (as hydrochloride) (0.24 mmol), and Et_3N (in the case of **1** and **3**, 67 μL, 0.48 mmol; in the case of **2**, 34 μL, 0.24 mmol) in dry DMF (1 mL) was stirred overnight at room temperature. The mixture was diluted with brine and extracted with AcOEt. The organic phase was washed twice with brine, dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by column chromatography. *Data for selected compounds*: Compound **4d**: yield 31%; mp 256 °C; IR (KBr) 3312, 3120, 2960, 1585, 1448, 1367, 1203 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ1.32 (9H, s), 2.51 and 2.69 (2H, 2 br s), 3.63 and 3.96 (2H, 2 br s), 4.59 and 4.77 (2H, 2 br s), 6.56–7.13 (3H, m), 7.40 (2H, d, J = 7.5 Hz), 7.49 (2H, d, J = 7.5 Hz), 8.66 (1H, s), 10.32 and 10.62 (1H, 2 br s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.14, 21.68, 30.33, 30.92, 34.46, 45.49, 101.81, 110.66, 111.21, 124.80, 125.01, 125.11, 126.64, 127.17, 130.31, 131.07, 133.27, 150.35, 152.17. Compound **5e**: yield 43%; oil; IR ($CHCl_3$) 3468, 3359, 2926, 1708, 1600, 1417, 1259, 1184, 1074 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 2.74 and 2.79 (2H, 2 m), 3.84 and 3.94 (2H, 2 m), 4.68 and 4.83 (2H, 2 br s), 6.64–7.57 (12H, m); ^{13}C NMR (75 MHz, CD_3OD) δ 21.93, 22.51, 43.59, 43.71, 44.28, 49.84, 103.25, 111.92, 111.94, 112.32, 121.40, 121.64, 123.18, 125.12, 127.86, 127.96, 128.29, 128.66, 128.84, 129.85, 130.71, 131.88, 141.30, 143.94, 151.32, 153.10, 153.13, 155.92, 155.95. Compound **7b**: yield 70%; mp 158–161 °C; IR (KBr) 3328, 2960, 1636, 1593, 1518, 1417, 1239 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.25 (9H, s), 2.68 (2H, br s), 3.80 (2H, br s), 4.64 (2H, s), 6.57 (1H, d, J = 8.6 Hz), 6.74 (1H, br s), 7.10 (1H, J = 8.6 Hz), 7.25 (2H, d, J = 8.7 Hz), 7.39 (2H, d, J = 8.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.14, 31.21, 33.76, 42.18, 42.39, 101.84, 106.17, 110.49, 111.16, 119.60, 119.71, 124.77, 127.33, 130.40, 132.12, 137.84, 144.01, 150.27, 155.54. Compound **9b**: yield 49%; mp 156–159 °C; IR (KBr) 3384, 3046, 2845, 1698, 1624, 1433, 1416, 1226, 1180, 1077 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.91 (2H, br s), 3.90 and 3.99 (2H, 2 br s), 4.74 and 4.84 (2H, 2 br s), 7.11–7.56 (13H, m), 8.37 and 8.56 (1H, 2 br s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.02, 21.60, 42.61, 43.11, 110.99, 111.06, 117.87, 118.08, 119.45, 119.59,

120.55, 120.58, 121.72, 121.89, 124.27, 127.17, 127.64, 128.79, 129.66, 136.29, 140.20, 142.79, 151.74, 154.56.
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23. Inhibition without agonism per se, and hence not due to desensitization.