Bioorganic & Medicinal Chemistry 18 (2010) 3387–3402

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Serotonin derivatives as a new class of non-ATP-competitive receptor tyrosine kinase inhibitors

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ARTICLE INFO

Article history:
Received 24 February 2010
Revised 29 March 2010
Accepted 1 April 2010
Available online 04 April 2010

Keywords:
Receptor tyrosine kinases
Serotonin
Carbazole
Inhibitor

ABSTRACT

The discovery of new templates and their subsequent elaboration to clinically useful receptor tyrosine kinase (RTK) inhibitors continues to be an important issue. RTKs are a class of enzymes responsible for the activation of different cellular signal transduction cascades. The majority of the known small molecules RTK inhibitors are ATP-competitive and they are multiple targeted inhibitors. We describe here serotonin derivatives as a new class of multiple targeted RTK inhibitors. In contrast to most other RTK inhibitors they act via a non-ATP-competitive (allosteric) mechanism. Furthermore, they are able to inhibit the proliferation of HUVE cells, fibroblasts and two cancer cell lines.

1. Introduction

Receptor tyrosine kinase (RTK) are enzymes able to catalyze the transfer of a phosphate group from ATP to tyrosine residues of proteins and polypeptides. The human genome contains about 90 tyrosine kinase (TK) genes. Of them 58 encode transmembrane RTK's distributed into 20 subfamilies.[1] RTK's when mutated or altered structurally can become oncogenes that lead to cell transformation and cancer. After validation of RTK's as suitable pharmacological targets for anticancer therapy several small molecules were designed and developed to inhibit the tyrosine kinase domain of the receptor, thereby inhibiting intracellular signalling resulting in regulation of mitosis, gene transcription, and cell differentiation. Important examples are Imatinib, Gefitinib, Lapatinib, Sorafenib, and Erlotinib.[2] Most of the known small molecules RTK inhibitors including the examples mentioned above are multiple targeted inhibitors, that is, they inhibit more than one tyrosine kinase.[3]

Considering the observed development of tumour resistance to tyrosine kinase inhibitors, the discovery of new templates and their subsequent elaboration to clinically useful RTK inhibitors continues to be an important issue.[4] In the past structural biology of receptor tyrosine kinases has given important insights into the flexibility of the catalytic domain and has provided a rational basis for obtaining inhibitors including selective ones. Future direction in RTK drug discovery include finding new ways to inhibit the enzymatic activity of these proteins. One such approach is the discovery of non-ATP-competitive (allosteric) inhibitors.[5]

The majority of the known RTK inhibitors belong to the Type I binding mode, that is, they bind in the ATP-binding site of a kinase and are therefore ATP-competitive. The protein kinase is in an activated state and the binding mode can be described by Traxler's pharmacophore model.[6] Type II inhibitors are also ATP-competitive but they also interact with the extended ATP-binding site of an inactive protein kinase. Conformational changes of the protein kinase induced by the inhibitor open a new hydrophobic pocket that is called Deep Pocket or Phe Pocket. Type II inhibitors specifically interact with this pocket. Examples are Imatinib, Sorafenib, Lapatinib and Nilotinib. Type III inhibitors interact with allosteric binding sites and are non-ATP-competitive. Only a few such kinase inhibitors are known[7] and of them only three target Receptor Tyrosine Kinases: ON012380[8] as well as GNF-1 and GNF-2 which are small molecules inhibitors of Bcr-Abl kinase.[9] Type II inhibitors like Imatinib are often referred to as allosteric inhibitors. However 'this is not correct according to enzymological definition of an allosteric inhibitor' and 'the rigid experimental criterion for the distinction between allosteric and non-allosteric binders is the competitiveness of the inhibitor with respect to ATP'.[7a]

Herein we report the discovery of serotonin derivatives as a novel type of non-ATP-competitive RTK inhibitors. In the frame of our project of development of Aurora A kinase inhibitors we synthesized a small library of serotonin derivatives. Whereas our compounds proved to be inactive against Aurora A kinase we found derivatives **1** and **2** to be inhibitors at the one digit micromolar range against four different receptor tyrosine kinases (Table 1).

In order to get more insights into their mode of action and to investigate structure–activity relationships, we decided to synthesize a library of several analogues of derivatives **1** and **2**.

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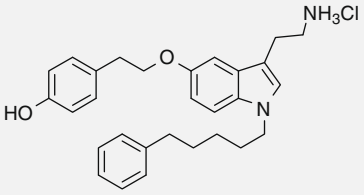
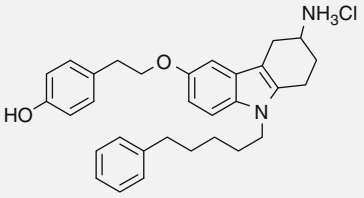
doi:10.1016/j.bmc.2010.04.001

Table 1
IC_{50} (μM) values of receptor tyrosine kinase (RTK) inhibition by derivatives **1** and **2**

No.	Structure	EGFR	IGF-1R	VEGFR-2	VEGFR-3
1		1.63 ± 0.13	2.96 ± 0.41	2.83 ± 0.52	2.68 ± 0.73
2		1.01 ± 0.06	0.93 ± 0.04	1.06 ± 0.36	2.17 ± 0.15

2. Results and discussion

2.1. Chemistry

An overview of the synthetic route is given in Scheme 1. Treatment of serotonin **3** with Boc_2O afforded the corresponding Boc-protected derivative which was transformed to compound **4** after reaction with 4-(methoxymethoxy)phenethyl 4-methylbenzene sulfonate. Subsequently, several substituents were introduced at the indole N using different arylalkyl halogenides to produce after deprotection derivatives **1**, **5** and **6**. On the other hand treatment of compound **4** with *p*-iodobenzyl bromide afforded the corresponding *N*-iodobenzyl-derivative, which was transformed to derivatives **7**–**9** containing a biphenyl moiety after reaction with different boronic acids in a Suzuki reaction. Deprotection of **4** using acidic conditions yielded derivative **10**. In order to prove the importance of a non-basic side chain as well as the importance of the free phenol moiety **6** was converted to carbamate **11** and into derivative **12**.

For the synthesis of the tetrahydrocarbazoles **20**–**25** derivative **13** was transformed in two steps to ketone **14**. Furthermore, 4-benzyloxyaniline hydrochloride **15** was converted to the hydrazine hydrochloride **16** by diazotation followed by Sn(II) reduction. Reaction between ketone **14** and derivative **16** under the conditions of Fischer indole synthesis afforded tetrahydrocarbazole **17** which after hydrogenolysis in the presence of Boc_2O yielded tetrahydrocarbazole **18**. Treatment of the later with 4-(methoxymethoxy)phenethyl-4-methylbenzene sulfonate gave compound **19**. Subsequent N-alkylation and deprotection as described above furnished the derivatives **2** and **20**–**25**.

2.2. Biological evaluation

2.2.1. Inhibition of receptor tyrosine kinases

All these derivatives as well as the commercially available serotonin hydrochloride **3** and 4-(2-methoxyethyl) phenol **26** were tested for their activity to inhibit EGFR, IGF-1R, VEGFR-2 and VEGFR-3. They were proven to be multi-RTK-inhibitors like derivative **1** and **2**. The results are summarized in Table 2.

All inhibitory active compounds posses a free amino group and a phenylethyl substituent in 5-position (**5**–**10**, **12**, **20**–**25**), respectively, and showed IC_{50} values in the lower micromolar range against the tested receptors. Serotonin hydrochloride **3** as well as derivatives **11** and **26** were inactive.

From these results it is obvious that in the series of the serotonin analogues the alkyl chain at the indole N has not a significant effect on the potency of the inhibitors. By contrast in the tetrahydrocarbazole series of compounds the alkyl chain at the nitrogen at the indole moiety is important: the inhibitory activity of tetrahydrocarbazole **25** against the tested RTK's is in part more than one order of magnitude weaker in comparison to derivatives **20**–**24**. The lack of activity of derivative **11** indicates the necessity of a basic amino group at the side chain. Comparison of the inhibitory properties of derivatives **6** and **12** showed that a free phenolic OH group is not essential for activity. Moreover, the inactivity of serotonin **3** as well as of derivative **11** and of the simple phenol ether **26** allow the presumption that the inhibitors described here are not substrate analogues of the tyrosine side chain. In summary it can be noticed that the free amino group of the serotonin derivatives and the phenylethyl substituent in 5-position are essential for inhibitory activity. The substituent of the indole nitrogen only slightly influences the inhibitory activity.

2.2.2. RTK inhibitors act via an allosteric mechanism

In order to obtain insights into the mechanism of RTK inhibition we determined the IC_{50} values of derivatives **2** and **7** against selected RTK's at different ATP concentrations. As shown in Table 3 the measured IC_{50} values are independent of the ATP concentration indicating a non-ATP-competitive (allosteric) mechanism of action. Similar results were obtained also for the other three RTK's.

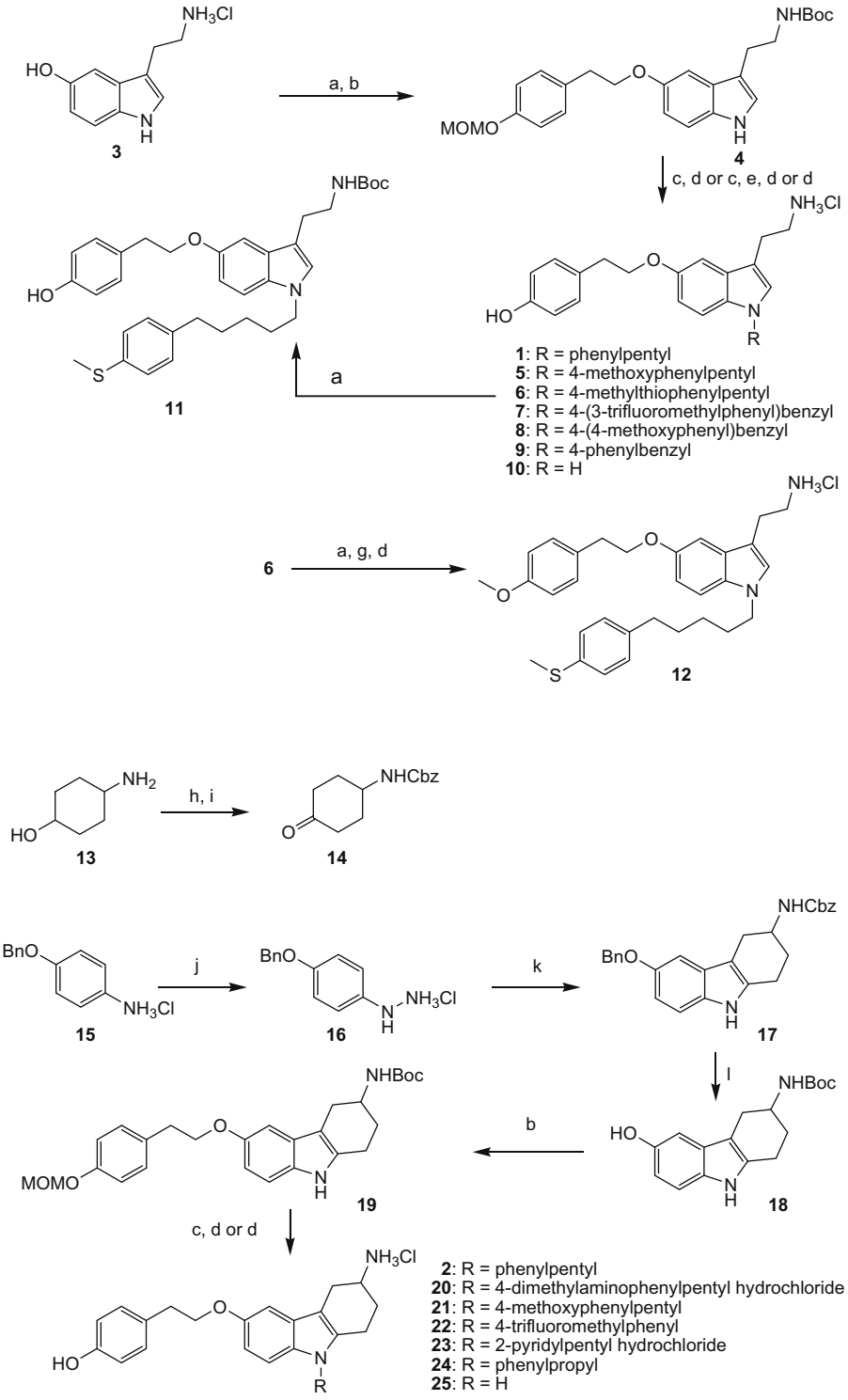
2.2.3. Cell toxicity and inhibition of proliferation

Four different derivatives were tested for their antiproliferative activity and toxicity against HUVE cells, the epithelial breast cancer cell line MCF-7, the epithelial colorectal adenocarcinoma cell line HT29 and a fibroblast cell line (HEPM) (Table 4).

The IC_{50} values of the cell proliferation were comparable to the IC_{50} values obtained by the ELISA-RTK-assay with isolated enzymes indicating a good cell permeability of the inhibitors. Furthermore, the IC_{50} values for antiproliferative activity were always lower than these for toxicity although they were in the same order of magnitude. This may be explained by the fact that our derivatives are multiple targeted inhibitors.

3. Conclusion

In search of novel kinase inhibitors we succeeded to synthesize and identify serotonin derivatives as multi-RTK-inhibitors. These compounds inhibit EGFR, IGF-1R, VEGFR-2 and VEGFR-3 via a non-ATP-competitive (allosteric) mechanism. Our results indicate the existence of an allosteric site common at least to the tested RTK's that is addressed by the serotonin derivatives. This motif can be further exploited for the design of more potent and more selective RTK inhibitors using the serotonin skeleton as lead structure.



Scheme 1. Synthesis of indole derivatives. Reagents and conditions: (a) Boc_2O, Et_3N, THF, rt, 87%-quant.; (b) 4-(methoxymethoxy)phenethyl-4-methylbenzene sulfonate **29**, Cs_2CO_3, acetone, reflux, 24–48 h, 65–84%; (c) NaH, DMF, 0 °C, 15 min, then alkylbromide, rt, 2–5 h; (d) 0.8 M HCl in MeOH, 50 °C, 2–4 h, 70–93% (2–3 steps); (e) boronic acid, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, Cs_2CO_3, dioxane/water 3:1, reflux, 4–12 h; (f) benzoylchloride, Et_3N, DMAP, CH_2Cl_2, rt, 4 h, 40%; (g) MeI, K_2CO_3, DMF/acetone 1:1, 50 °C, 12 h, 86%; (h) benzyloxycarbonylchloride, Na_2CO_3, CH_2Cl_2, 0 °C, 30 min, quant.; (i) IBX, EtOAc, reflux, 4 h, 88%; (j) $NaNO_2$, HCl, water, −5 °C, 1 h, then $SnCl_2$, 0 °C, 2 h, 75%; (k) **14**, AcOH, 80 °C, 3 h, 83%; (l) Boc_2O, $Pd(OH)_2/C$, H_2, MeOH, 85%.

4. Experimental part

4.1. Chemistry

All reagents were commercially obtained from Acros, Aldrich, Alfa Aesar and Fluka and used without further purification. Melting points were measured with a Boetius-micro hot stage and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded by using a Varian Gemini 200 (200 MHz for ^1H NMR; 50 MHz for ^{13}C), a Varian Gemini 300 (300 MHz for ^1H NMR; 75 MHz for ^{13}C NMR) and Bruker Avance-DRX 400 (400 MHz for ^1H NMR; 100 MHz for ^{13}C NMR); the residual solvent peak was used as an internal reference.

Table 2
Inhibition of receptor tyrosine kinases by indole derivatives

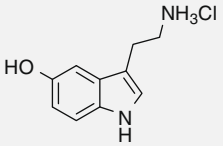
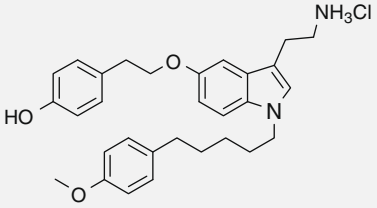
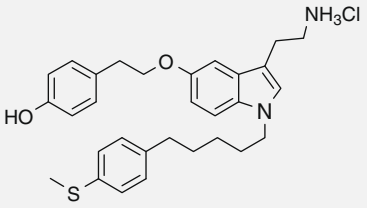
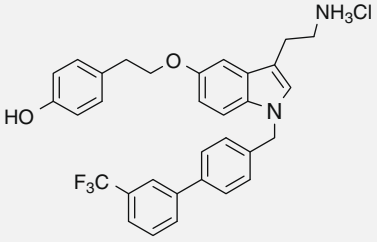
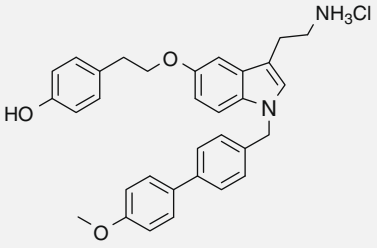
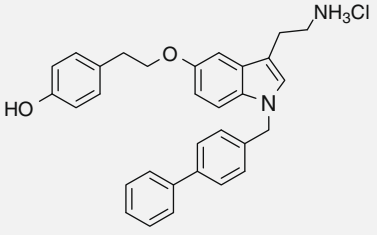
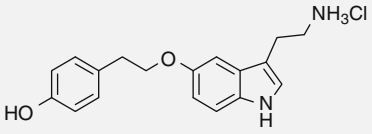
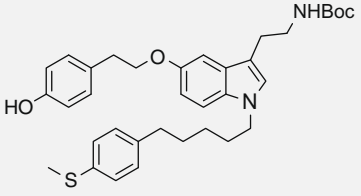
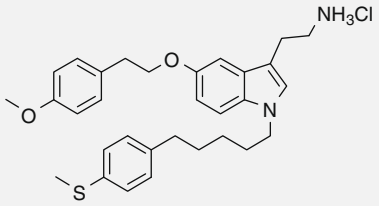
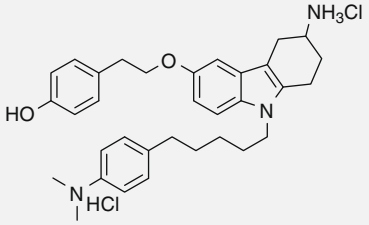
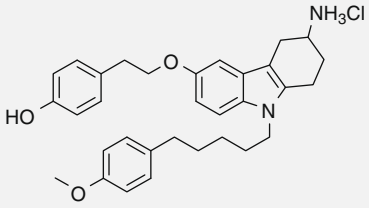
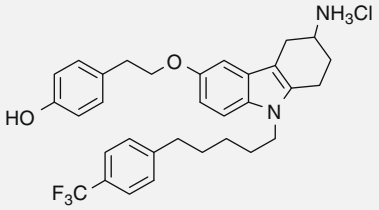
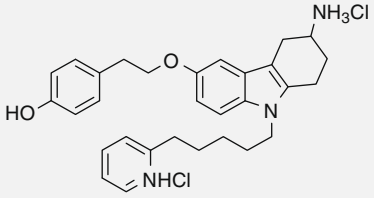
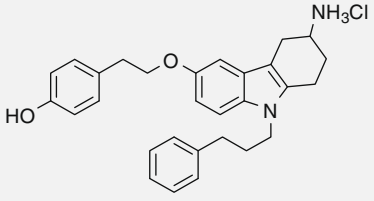
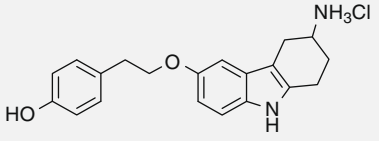
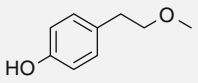
No.	Structure	EGFR	IGF-1R	VEGFR-2	VEGFR-3
3		n.a.	n.a.	n.a.	n.a.
5		4.40 ± 0.16	3.21 ± 0.30	2.17 ± 0.95	2.05 ± 0.48
6		1.92 ± 0.36	2.25 ± 0.34	1.22 ± 0.11	2.53 ± 0.47
7		1.11 ± 0.10	1.07 ± 0.55	1.56 ± 0.15	3.01 ± 0.25
8		1.25 ± 0.13	1.79 ± 0.24	1.01 ± 0.06	0.96 ± 0.40
9		1.30 ± 0.11	2.36 ± 0.41	1.17 ± 0.15	4.07 ± 0.47
10		2.07 ± 0.19	1.78 ± 0.20	2.03 ± 0.09	2.64 ± 0.85
11		n.a.	n.a.	n.a.	n.a.

Table 2 (*continued*)

No.	Structure	EGFR	IGF-1R	VEGFR-2	VEGFR-3
12		2.45 ± 0.85	0.98 ± 0.09	1.45 ± 0.15	1.31 ± 0.07
20		1.91 ± 0.21	2.76 ± 0.12	1.26 ± 0.11	1.66 ± 0.20
21		1.37 ± 0.37	2.86 ± 0.17	0.94 ± 0.02	1.81 ± 0.20
22		2.75 ± 0.21	5.15 ± 0.32	2.18 ± 0.32	2.27 ± 0.32
23		2.28 ± 0.14	2.80 ± 0.24	2.12 ± 0.27	2.22 ± 0.55
24		2.50 ± 0.29	1.33 ± 0.09	2.07 ± 0.14	2.85 ± 0.46
25		15.12 ± 0.76	10.61 ± 2.14	27.14 ± 2.49	34.57 ± 2.68
26		n.a.	n.a.	n.a.	n.a.

All IC_{50} values are in μmol/L. n.a.: IC_{50} >50 μmol/L.

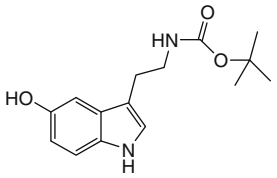
ESI-HRMS were obtained on a Bruker Daltonics APEX II. Reactions involving moisture-sensitive reactants were performed in flame--dried glassware under an atmosphere of argon; reactants were added by using a syringe. Flash column chromatography was performed on silica gel (Acros 60A, 0.035–0.070 mm) and analytical TLC on precoated silica gel plates (Merck 60 F254, 0.25 mm).

Table 3
Inhibition of activity of selected RTK's by derivatives **2** and **7** at three different ATP concentrations

	Compound No.	25 μM ATP	50 μM ATP	250 μM ATP
EGFR	**2**	1.01 ± 0.06	0.98 ± 0.04	1.00 ± 0.29
	7	1.11 ± 0.10	1.12 ± 0.07	1.06 ± 0.08
IGF-1R	**2**	0.93 ± 0.04	0.99 ± 0.10	1.22 ± 0.06
	7	1.07 ± 0.55	1.06 ± 0.35	1.02 ± 0.22
VEGFR-2	**2**	1.06 ± 0.36	1.09 ± 0.08	1.04 ± 0.21
	7	1.56 ± 0.15	1.48 ± 0.19	1.50 ± 0.08
VEGFR-3	**2**	2.17 ± 0.15	2.11 ± 0.10	2.19 ± 0.05
	7	3.01 ± 0.25	2.98 ± 0.19	3.08 ± 0.14

IC_{50} values in μmol/L.

4.2. *tert*-Butyl-2-(5-hydroxy-1*H*-indole-3-yl)ethyl carbamate



To a stirred solution of serotonin hydrochloride **3** (3.00 g, 15.1 mmol) in THF (120 mL) and triethyl amine (2.31 mL, 16.6 mmol) was added di-*tert*-butyl dicarbonate (3.63 g, 16.6 mmol). The solution was stirred at room temperature for 3 h (TLC-control) and all volatiles were removed under reduced pressure. The crude product was purified by column chromatography (*n*-hexane/EtOAc 1:1 v/v) to yield (4.17 g, 15.1 mmol, quantitative) as a yellow solid.

R_f: 0.66 (*n*-hexane/EtOAc 1:1, v/v).

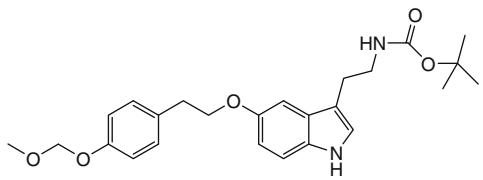
Mp: 54–55 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42 (s, 9H, COO(CH_3)$_3$), 2.86 (t, ^3J = 6.8 Hz, 2H, HN–CH_2–CH_2), 3.41–3.43 (m, 2H, HN–CH_2), 4.66 (br, 1H, O*H*), 6.79 (dd, ^3J = 8.8 Hz, ^4J = 2.4 Hz, 1H, C6$_{indole}$*H*), 6.97–7.01 (m, 2H, C2$_{indole}$*H*, C4$_{indole}$*H*), 7.20 (d, ^3J = 8.8 Hz, 1H, C7$_{indole}$*H*), 7.99 (br, 1H, N$_{indole}$*H*).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.0 (HN–CH_2–CH_2), 28.6 (3C, COOC(CH_3)$_3$), 40.8 (HN–CH_2), 79.5 (COOC(CH_3)$_3$), 103.5 (*C*4$_{indole}$), 111.9, 112.2, 112.5 (*C*3$_{indole}$, *C*6$_{indole}$, *C*7$_{indole}$), 123.3 (*C*2$_{indole}$), 128.2 (*C*3a$_{indole}$), 131.7 (*C*7a$_{indole}$), 149.8 (*C*5$_{indole}$), 156.3 (*C*OOC(CH_3)$_3$).

HRMS: $[M+Na]^+$ $[C_{15}H_{20}N_2O_3Na]^+$, calcd: 299.13661, found: 299.13680.

4.3. *tert*-Butyl-2-{5-[4-(methoxymethoxy)phenylethoxy]-1*H*-indole-3-yl}ethyl carbamate 4



To a stirred solution of *tert*-butyl-2-(5-hydroxy-1*H*-indole-3-yl)-ethylcarbamate (1.32 g, 4.77 mmol) in acetone (100 mL) 4-(methoxymethoxy)phenylethyl-4-methylbenzene sulfonate (1.61 g, 4.77 mmol) and caesium carbonate (3.11 g, 9.55 mmol) were added. The resulting suspension was heated under reflux for 48 h (TLC-control). The solvent was removed under reduced pressure and the crude product was purified by column chromatography (*n*-hexane/EtOAc 2:1 v/v) to yield pure 4 (1.77 g, 4.02 mmol, 84%) as a yellow solid.

R_f: 0.26 (*n*-hexane/EtOAc 2:1, v/v).

Mp: 105–107 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.89 (d, ^3J = 6.6 Hz, 2H, NH–CH_2–CH_2), 3.07 (t, ^3J = 7.2 Hz, 2H, O–CH_2–CH_2), 3.41–3.43 (m, 2H, NH–CH_2), 4.19 (t, ^3J = 7.4 Hz, 2H, O–CH_2), 5.16 (s, 2H, O–CH_2–O), 6.86 (dd, ^3J = 8.4 Hz, ^4J = 2.4 Hz, 1H, C6$_{indole}$*H*), 7.01 (m, 4H, C2$_{Indole}$*H*, C4$_{indole}$*H*, C3$_{phenol}$*H*, C3′$_{phenol}$*H*), 7.22–7.26 (m, 4H, C7$_{indole}$*H*, C2$_{phenol}$*H*, C2′$_{phenol}$*H*, N*H*–CO), 7.90 (br, 1H, N*H*$_{indole}$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.5 (3C, COOC(CH_3)$_3$), 31.8 (NH–CH_2–CH_2), 35.3 (O–CH_2–CH_2), 53.9 (HN–CH_2), 56.0 (O–CH_3), 69.9 (O–CH_2), 94.6 (O–CH_2–O), 102.0 (*C*4$_{indole}$), 104.7 (*C*3$_{indole}$), 111.9, 113.1 (*C*6$_{indole}$, *C*7$_{indole}$), 116.5 (2C, *C*3$_{phenol}$, C3′$_{phenol}$*H*), 122.9 (*C*2$_{indole}$), 130.2 (2C, *C*2$_{phenol}$, C2′$_{phenol}$), 130.3, 131.8, 132.1 (*C*3a$_{indole}$, *C*7a$_{indole}$, *C*1$_{phenol}$), 149.8 (*C*5$_{indole}$), 153.4 (*C*4$_{phenol}$), 156.1 (COO(CH_3)$_3$).

HRMS: $[M+Na]^+$ $[C_{25}H_{32}NO_5Na]^+$, calcd: 463.22034, found: 463.22020.

4.4. General procedure for the preparation of the serotonin derivatives

To stirred solution of **4** (100 mg, 0.227 mol) in DMF (1.0 mL) was added sodium hydride (60% suspension in mineral oil, 13.6 mg, 0.341 mmol) at 0 °C. The mixture was stirred for 15 min when the appropriate alkyl bromide (0.272 mmol) was added and stirring was continued at room temperature for 2–5 h (TLC-control). The reaction mixture was diluted with EtOAc (10 mL), washed with water (4 mL) and brine (4 mL) and the organic phase was dried (Na_2SO_4). All volatiles were removed under reduced pressure, the protected intermediates were purified by column chromatography (*n*-hexane/EtOAc) and dissolved in HCl-solution (0.8 M in MeOH, 3 mL). The solution was stirred at 50 °C for 2–4 h (TLC-control). The solvent was removed under reduced pressure leaving the pure indoles as their hydrochlorides.

4.4.1. 2-[5-(4-Hydroxyphenethoxy)-1-(5-phenylpentyl)-1*H*-indole-3-yl]ethanamine hydrochloride 1

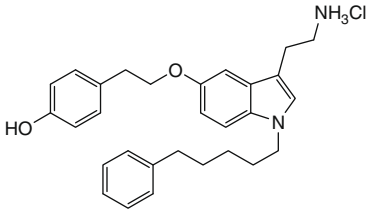


Table 4
IC_{50} values (μM) for the inhibition of proliferation and cytotoxicity against HUVE, MCF-7, HEPM and HT29 cell of selected derivatives

No.	HUVEC		MCF-7		HEPM		HT29	
	Prolif.	Cytotox.	Prolif.	Cytotox.	Prolif.	Cytotox.	Prolif.	Cytotox.
2	1.38 ± 0.3	5.04 ± 1.2	3.85 ± 0.6	4.79 ± 0.7	2.25 ± 0.6	7.76 ± 0.5	3.81 ± 0.2	11.04 ± 0.4
7	1.98 ± 0.3	6.32 ± 0.2	3.57 ± 0.2	3.34 ± 0.2	2.74 ± 0.9	6.64 ± 0.1	4.38 ± 0.2	7.95 ± 0.3
8	0.75 ± 0.2	3.45 ± 2.9	4.79 ± 0.1	4.93 ± 0.5	2.53 ± 0.6	5.70 ± 0.6	4.86 ± 0.1	8.50 ± 0.3

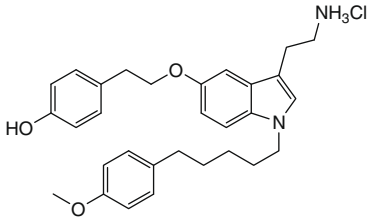
Reaction time:	5 h (coupling), 4 h (deprotection)
Yield:	100 mg (0.21 mmol, 92%) bright yellow solid
Mp:	113 °C

^{1}H NMR (300 MHz, CD_3OD): δ = 1.21–1.25 (m, 2H, N_{indole}–CH_2–CH_2–CH_2), 1.49–1.54 (m, 2H, $N_{indole}CH_2$–CH_2–CH_2–CH_2), 1.69–1.75 (m, 2H, N_{indole}–CH_2–CH_2), 2.46 (t, ^3J = 7.4 Hz, 2H, N_{indole}–CH_2–CH_2–CH_2–CH_2–CH_2), 2.96 (t, ^3J = 7.1 Hz, 2H, O–CH_2–CH_2), 3.04–3.12 (m, 2H, H_3N–CH_2–CH_2), 3.12–3.15 (m, 2H, H_3N–CH_2), 3.96 (t, ^3J = 6.9 Hz, 2H, N_{indole}–CH_2), 4.13 (t, ^3J = 6.9 Hz, 2H, O–CH_2), 6.73–6.81 (m, $C6_{indole}H$, $C3_{phenol}H$, $C3'_{phenol}H$), 7.01–7.19 (m, 10H, $C2_{indole}H$, $C4_{indole}H$, $C7_{indole}H$, $C2_{phenol}H$, $C2'_{phenol}H$, 5 × $C_{phenyl}H$).

^{13}C NMR (75 MHz, CD_3OD): δ = 24.3 (N_{indole}–CH_2–CH_2–CH_2), 27.4, 31.1, 32.1 (N_{indole}–CH_2–CH_2–CH_2–CH_2, H_3N–CH_2–CH_2), 36.1, 36.6 (N_{indole}–CH_2–CH_2–CH_2–CH_2–CH_2, O–CH_2–CH_2), 41.3 (H_3N–CH_2), 47.0 (N_{indole}–CH_2), 71.2 (O–CH_2), 102.8 ($C4_{indole}$), 109.2 ($C3_{indole}$), 111.5 ($C6_{indole}$), 113.5 ($C7_{indole}$), 116.2 (2C, $C3_{phenol}$, $C3'_{phenol}$), 126.6 ($C4_{phenyl}$), 128.3 ($C2_{indole}$), 129.0 ($C3a_{indole}$), 129.2, 129.3 (4C, $C2_{phenyl}$, $C2'_{phenyl}$, $C3_{phenyl}$, $C3'_{phenyl}$), 130.7 ($C7a_{indole}$), 131.0 (2C, $C2_{phenol}$, $C2'_{phenol}$), 133.4 ($C1_{phenol}$), 143.5 ($C1_{phenyl}$), 154.3 ($C5_{indole}$), 156.8 ($C4_{phenol}$).

HRMS: $[M-Cl]^+$ $[C_{29}H_{35}N_2O_2]^+$, calcd: 443.26930, found: 443.26958.

4.4.2. 2-{5-(4-Hydroxyphenethoxy)-1-[5-(4-methoxyphenyl)-pentyl]-1*H*-indole-3-yl}ethanamine hydrochloride 5



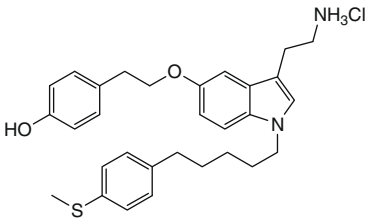
Reaction time:	2 h (coupling), 4 h (deprotection)
Yield:	75 mg (0.15 mmol, 65%) grey solid
Mp:	69–71 °C

^{1}H NMR (400 MHz, CD_3OD): δ = 1.21–1.26 (m, 2H, N_{indole}–CH_2–CH_2–CH_2), 1.49–1.53 (m, 2H, N_{indole}–CH_2–CH_2–CH_2–CH_2), 1.73–1.77 (m, 2H, N_{indole}–CH_2–CH_2), 2.43 (t, ^3J = 7.6 Hz, 2H, N_{indole}–CH_2–CH_2–CH_2–CH_2–CH_2), 2.95 (t, ^3J = 7.0 Hz, 2H, O–CH_2–CH_2), 3.04 (t, ^3J = 7.0 Hz, 2H, H_3N–CH_2–CH_2), 3.14–3.17 (m, 2H, H_3N–CH_2), 3.68 (s, 3H, O–CH_3), 4.00 (t, ^3J = 7.0 Hz, 2H, N_{Indole}–CH_2), 4.14 (t, ^3J = 7.0 Hz, 2H, O–CH_2), 6.71–6.75 (m, 4H, $C3_{phenol}H$, $C3'_{phenol}H$, $C3_{methoxyphenyl}H$, $C3'_{methoxyphenyl}H$), 6.81 (dd, ^3J = 9.0 Hz, ^4J = 1.8 Hz, 1H, $C6_{Indole}H$), 6.94 (d, ^3J = 8.4 Hz, 2H, $C2_{methoxyphenyl}H$, $C2'_{methoxyphenyl}H$), 7.05 (d, ^4J = 2.0 Hz, 1H, $C4_{indole}H$), 7.09–7.11 (m, 3H, $C2_{indole}H$, $C2_{phenol}H$, $C2'_{phenol}H$), 7.18 (d, ^3J = 9.2 Hz, 1H, $C7_{indole}H$).

^{13}C NMR (100 MHz, CD_3OD): δ = 24.4 (N_{indole}–CH_2–CH_2–CH_2), 27.3, 31.2, 32.3 (N_{indole}–CH_2–CH_2–CH_2–CH_2, H_3N–CH_2–CH_2), 35.7, 36.2 (N_{indole}–CH_2–CH_2–CH_2–CH_2–CH_2, O–CH_2–CH_2), 41.2 (H_3N–CH_2), 47.1 (N_{indole}–CH_2), 55.6 (O–CH_3), 71.3 (O–CH_2), 102.8 ($C4_{indole}$), 109.2 ($C3_{indole}$), 111.5 ($C6_{indole}$), 113.5 ($C7_{indole}$), 114.6, 116.2 (4C, $C3_{methoxyphenyl}$, $C3'_{methoxyphenyl}$, $C3_{phenol}$, $C3'_{phenol}$), 128.3 ($C2_{indole}$), 129.1 ($C3a_{indole}$), 130.2, 131.0 (4C, $C2_{methoxyphenyl}$, $C2'_{methoxyphenyl}$, $C2_{phenol}$, $C2'_{phenol}$), 130.8 ($C7a_{indole}$), 133.5 ($C1_{phenol}$), 135.5 ($C1_{methoxyphenyl}$), 154.4 ($C5_{indole}$), 156.9 ($C4_{phenol}$), 159.1 ($C4_{methoxyphenyl}$).

HRMS: $[M-Cl]^+$ $[C_{30}H_{38}N_2O_3]^+$, calcd: 473.27987, found: 473.27978.

4.4.3. 2-{5-(4-Hydroxyphenethoxy)-1-{5-[4-(methylthio)-phenyl]pentyl}-1*H*-indole-3-yl}ethanamine hydrochloride 6



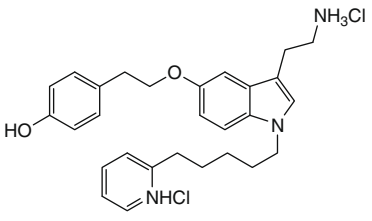
Reaction time:	3 h (coupling), 2 h (deprotection)
Yield:	84 mg (0.16 mmol, 71%) white solid
Mp:	134–136 °C

^{1}H NMR (400 MHz, CD_3OD): δ = 1.19–1.25 (m, 2H, N_{indole}–CH_2–CH_2–CH_2), 1.48–1.51 (m, 2H, N_{indole}–CH_2–CH_2–CH_2–CH_2), 1.71–1.75 (m, 2H, N_{indole}–CH_2–CH_2), 2.36 (s, 3H, S–CH_3), 2.41–2.44 (m, 2H, N_{indole}–CH_2–CH_2–CH_2–CH_2–CH_2), 2.95 (t, ^3J = 6.6 Hz, 2H, O–CH_2–CH_2), 3.04 (t, ^3J = 6.6 Hz, 2H, H_3N–CH_2–CH_2), 3.13–3.15 (m, 2H, H_3N–CH_2), 3.96–3.98 (m, 2H, N_{indole}–CH_2), 4.11–4.15 (m, 2H, O–CH_2), 6.74 (d, ^3J = 8.0 Hz, 2H, $C3_{phenol}H$, $C3'_{phenol}H$), 6.81 (d, ^3J = 8.8 Hz, 1H, $C6_{indole}H$), 6.94 (d, ^3J = 7.6 Hz, 2H, $C2_{phenol}H$, $C2'_{phenol}H$), 7.03–7.10 (m, $C2_{indole}H$, $C4_{indole}H$, $C2_{methylthiophenyl}H$, $C2'_{methylthiophenyl}H$, $C3_{methylthiophenyl}H$, $C3'_{methylthiophenyl}H$), 7.16 (d, ^3J = 8.4 Hz, 1H, $C7_{indole}H$).

^{13}C NMR (100 MHz, CD_3OD): δ = 16.2 (S–CH_3), 24.4 (N_{indole}–CH_2–CH_2–CH_2), 27.3, 31.1, 32.0 (N_{indole}–CH_2–CH_2–CH_2–CH_2,H_3N–CH_2–CH_2), 35.9, 36.2 (N_{indole}–CH_2–CH_2–CH_2–CH_2–CH_2, O–CH_2–CH_2), 41.3 (H_3N–CH_2), 47.0 (N_{indole}–CH_2), 71.3 (O–CH_2), 102.8 ($C4_{indole}$), 109.2 ($C3_{indole}$), 111.5 ($C6_{indole}$), 113.5 ($C7_{indole}$), 116.2 (2C, $C3_{phenol}$, $C3'_{phenol}$), 128.0 (2C, $C2_{methylthiophenyl}$, $C2'_{methylthiophenyl}$), 128.3 ($C2_{indole}$), 129.1 ($C3a_{indole}$), 130.0 (2C, $C3_{methylthiophenyl}$, $C3'_{methylthiophenyl}$), 130.7 ($C7a_{indole}$), 131.0 (2C, $C2_{phenol}$, $C2'_{phenol}$), 133.4 ($C1_{phenol}$), 136.5 ($C4_{methylthiophenyl}$), 140.6 ($C1_{methylthiophenyl}$), 154.4 ($C5_{indole}$), 156.8 ($C4_{phenol}$).

HRMS: $[M-Cl]^+$ $[C_{29}H_{35}N_2O_2S]^+$, calcd: 489.25703, found: 489.25706.

4.4.4. 2-{5-(4-Hydroxyphenethoxy)-1-[5-(pyridine-2-yl)pentyl]-1*H*-indole-3-yl}ethanamine dihydrochloride



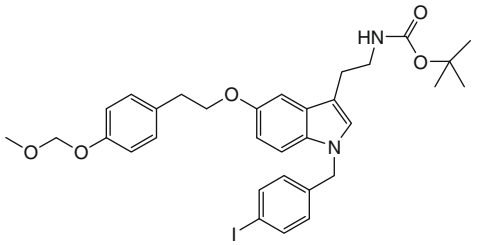
Reaction time:	3 h (coupling), 3 h (deprotection)
Yield:	92 mg (0.18 mmol, 78%) yellow solid
Mp:	35 °C

^{1}H NMR (300 MHz, CD_3OD): δ = 1.27–1.31 (m, 2H, N_{indole}–CH_2–CH_2–CH_2), 1.70–1.75 (m, 2H, N_{indole}–CH_2–CH_2–CH_2–CH_2), 1.80–1.84 (m, 2H, N_{indole}–CH_2–CH_2), 2.93–2.99 (m, 4H, O–CH_2–CH_2, N_{indole}–CH_2–CH_2–CH_2–CH_2–CH_2), 3.03–3.08 (m, 2H, H_3N–CH_2–CH_2), 3.15–3.17 (m, 2H, H_3N–CH_2), 4.06 (t, ^3J = 6.3 Hz, 2H, N_{indole}–CH_2), 4.15 (t, ^3J = 6.8 Hz, 2H, O–CH_2), 6.73 (d, ^3J = 8.1 Hz, 2H, $C3_{phenol}H$, $C3'_{phenol}H$), 6.79 (d, ^3J = 8.7 Hz, 1H, $C6_{indole}H$), 7.05 (d, ^4J = 1.8 Hz, 1H, $C4_{indole}H$), 7.11 (s, 1H, $C2_{indole}H$), 7.11 (d, ^3J = 7.8 Hz, 2H, $C2_{phenol}H$, $C2'_{phenol}H$), 7.22 (d, ^3J = 8.7 Hz, 1H, $C7_{indole}H$), 7.73–7.81 (m, 2H, $C3_{pyridine}H$, $C5_{pyridine}H$), 8.37 (t, ^3J = 7.8 Hz, 1H, $C4_{pyridine}H$), 8.56 (d, ^3J = 8.4 Hz, 1H, $C6_{pyridine}H$).

^{13}C NMR (75 MHz, CD_3OD): δ = 24.3 (H_3N–CH_2–CH_2), 27.1, 29.6, 30.7, (N_{indole}–CH_2–CH_2–CH_2–CH_2), 34.0 (N_{indole}–CH_2–CH_2–CH_2–CH_2–CH_2), 36.1 (O–CH_2–CH_2), 41.2 (H_3N–CH_2), 46.7 (N_{indole}–CH_2), 71.2 (O–CH_2), 102.7 ($C4_{indole}$), 109.4 ($C3_{indole}$), 111.6 ($C6_{indole}$), 113.5 ($C7_{indole}$), 116.2 (2C, $C3_{phenol}$, $C3'_{phenol}$), 125.9 ($C3_{pyridine}$), 128.4 (2C, $C2_{indole}$, $C5_{pyridine}$), 129.0 ($C3a_{indole}$), 130.9 ($C7a_{indole}$), 131.0 (2C, $C2_{phenol}$, $C2'_{phenol}$), 133.3 ($C1_{phenol}$), 141.8 ($C6_{pyridine}$), 147.8 ($C4_{pyridine}$), 154.3 ($C5_{indole}$), 156.9 ($C4_{phenol}$), 158.4 ($C2_{pyridine}$).

HRMS: $[M-HCl-Cl]^+$ $[C_{22}H_{34}N_3O_2]^+$, calcd: 444.26455, found: 444.26419.

4.4.5. *tert*-Butyl-2-{5-[4-(methoxymethoxy)phenethyloxy]-1-(4-iodo-benzyl)-1*H*-indole-3-yl}ethyl carbamate



To a solution of **4** (500 mg, 1.14 mmol) in DMF (5.0 mL) was added sodium hydride (60% suspension in mineral oil, 68 mg, 1.70 mmol) at 0 °C. The mixture was stirred for 15 min at this temperature when 4-iodobenzyl bromide (404 mg, 1.36 mmol) was added. After stirring for 4 h at room temperature (TLC-control) the mixture was diluted with EtOAc (20 mL) and washed with water (8 mL) and brine (8 mL). The organic phase was dried (Na_2SO_4) and all volatiles were removed under reduced pressure. The crude product was purified by column chromatography (*n*-hexane/EtOAc 3:1 v/v) to yield pure product (401 mg, 0.61 mmol, 54%) as a white crystalline solid.

R_f: 0.20 (*n*-hexane/EtOAc 3:1, v/v).

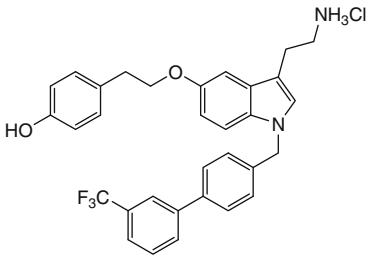
Mp: 100–102 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.35 (s, 9H, $C(CH_3)_3$), 2.75 (t, ^3J = 6.9 Hz, 2H, HN–CH_2–CH_2), 2.97 (t, ^3J = 7.2 Hz, 2H, O–CH_2–CH_2), 3.15–3.17 (m, 2H, HN–CH_2), 3.36 (s, 3H, O–CH_3), 4.13 (t, ^3J = 6.9 Hz, 2H, O–CH_2), 5.15 (s, 2H, N_{indole}–CH_2), 5.25 (s, 2H, O–CH_2–O), 6.72 (dd, ^3J = 8.7 Hz, ^4J = 2.4 Hz, 1H, $C6_{indole}H$), 6.84 (br, 1H, N*H*), 6.92–6.98 (m, 4H, $C3_{phenol}H$, $C3'_{phenol}H$, $C3_{iodophenyl}H$, $C3'_{iodophenyl}H$), 7.05 (s, 1H, $C4_{indole}H$) 7.21–7.27 (m, 4H, $C2_{indole}H$, $C7_{indole}H$, $C2_{phenol}H$, $C2'_{phenol}H$), 7.63 (d, ^3J = 8.1 Hz, 2H, $C2_{iodophenyl}H$, $C2'_{iodophenyl}H$).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 25.4 (HN–CH_2–CH_2), 28.2 ($C(CH_3)_3$), 34.4 (O–CH_2–CH_2), 40.7 (HN–CH_2), 48.5 (N_{indole}–CH_2), 55.4 (O–CH_3), 68.9 (O–CH_2), 77.4 ($C(CH_3)_3$), 93.1, 93.9 (C-1_{I-Ar}, O–CH_2–O), 101.8 ($C4_{indole}$), 110.7 ($C3_{indole}$), 111.6 ($C6_{indole}$), 111.7 ($C7_{indole}$), 116.1 (2C, $C3_{phenol}$, $C3'_{phenol}$), 127.0 ($C2_{indole}$), 128.3 ($C3a_{indole}$), 129.2, 129.9 (4C, $C2_{phenol}$, $C2'_{phenol}$, $C3_{iodophenyl}$, $C3'_{iodophenyl}$), 131.2 ($C7a_{indole}$), 131.7 ($C1_{phenol}$), 137.2 (2C, $C2_{iodophenyl}$, $C2'_{iodophenyl}$), 138.4 (C-$4_{iodophenyl}$), 152.3 ($C4_{phenol}$), 155.3, 155.5 (2C, $COOC(CH_3)_3$, $C5_{indole}$).

HRMS: $[M+Na]^+$ $[C_{32}H_{37}IN_2O_5Na]^+$, calcd: 679.16394, found: 679.16368.

4.4.6. 2-{5-(4-Hydroxyphenethoxy)-1-[4-(3-trifluoromethylphenyl)]benzyl-1*H*-indole-3-yl}ethanamine hydrochloride 7



To a solution of *tert*-butyl-2-{5-[4-(methoxymethoxy)phenethyloxy]-1-(4-iodo-benzyl)-1*H*-indole-3-yl}ethyl carbamate (100 mg, 0.152 mmol) in 1,4-dioxane/water (3:1 v/v, 2.0 mL) were added 3-trifluoromethanephenylboronic acid (29.0 mg, 0.152 mmol), caesium carbonate (356 mg, 0.915 mmol), palladium(II)-acetate (0.67 mg, 3 μmol) and triphenyl phosphine (4.0 mg, 15 μmol). The suspension was heated under reflux for 4 h. After cooling to room temperature the mixture was diluted with EtOAc (10 mL), washed with water (3 mL) and brine (3 mL). The organic phase was dried (Na_2SO_4) and the solvent was removed under reduced pressure. The protected intermediate was purified by column chromatography (*n*-hexane/EtOAc 3:1 v/v, R_f: 0.26) and dissolved in HCl-solution (0.8 M in MeOH, 3 mL). The solution was stirred for 3 h at 50 °C, all volatiles were removed under reduced pressure to leave pure **7** (60.4 mg, 0.106 mmol, 70%) as a white solid.

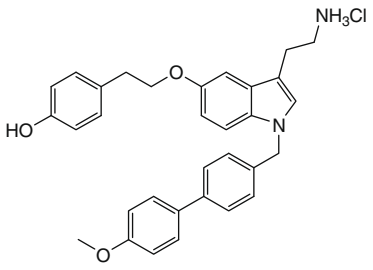
Mp: 161–162 °C.

^{1}H NMR (300 MHz, CD_3OD): δ = 2.96 (t, ^3J = 6.9 Hz, 2H, O–CH_2–CH_2), 3.08 (t, ^3J = 7.1 Hz, 2H, H_3N–CH_2–CH_2), 3.18–3.22 (m, 2H, H_3N–CH_2), 4.14 (t, ^3J = 6.9 Hz, 2H, O–CH_2), 5.33 (s, 2H, N_{Indole}–CH_2), 6.72 (d, ^3J = 8.1 Hz, 2H, $C3_{phenol}H$, $C3'_{phenol}H$), 6.80 (dd, ^3J = 9.0 Hz, ^3J = 2.1 Hz, 1H, $C6_{indole}H$), 7.07–7.13 (m, 4H, $C2_{indole}H$, $C4_{indole}H$, $C2_{phenol}H$, $C2'_{phenol}H$), 7.20–7.26 (m, 4H, $C7_{indole}H$, $C2_{trifluoromethylphenylphenyl}H$, $C2'_{trifluoromethylphenylphenyl}H$, $C5_{trifluoromethylphenyl}H$), 7.53–7.60 (m, 4H, $C3_{trifluoromethylphenylphenyl}H$, $C3'_{trifluoromethylphenylphenyl}H$, $C4_{trifluoromethylphenyl}H$, $C6_{trifluoromethylphenyl}H$), 7.80 (s, 1H, $C2_{trifluoromethylphenyl}H$).

^{13}C NMR (75 MHz, CD_3OD): δ = 24.4 (H_3N–CH_2–CH_2), 36.2 (O–CH_2–CH_2), 41.2 (H_3N–CH_2), 50.5 (N_{indole}–CH_2), 71.3 (O–CH_2), 102.9 ($C4_{indole}$), 110.1 ($C3_{indole}$), 112.0 ($C6_{indole}$), 113.8 ($C7_{indole}$), 116.2 (2C, $C3_{phenol}$, $C3'_{phenol}$), 124.3, 124.4, 124.9 (CF_3, $C2_{trifluoromethylphenyl}$, $C4_{trifluoromethylphenyl}$), 128.4 (2C, $C3_{trifluoromethylphenylphenyl}$, $C3'_{trifluoromethylphenylphenyl}$), 128.8 (2C, $C2_{trifluoromethylphenylphenyl}$, $C2'_{trifluoromethylphenylphenyl}$), 129.5, 130.0 ($C2_{indole}$, $C3a_{indole}$), 130.8 ($C7a_{indole}$), 131.0 (2C, $C2_{phenol}$, $C2'_{phenol}$), 131.6, 131.9 ($C5_{trifluoromethylphenyl}$, $C6_{trifluoromethylphenyl}$) 133.7 ($C1_{phenol}$), 138.8, 139.6, 140.0, 142.9 ($C1_{trifluoromethylphenylphenyl}$, $C4_{trifluoromethylphenylphenyl}$, $C1_{trifluoromethylphenyl}$, $C3_{trifluoromethylphenyl}$), 154.8 ($C5_{indole}$), 156.9 ($C4_{phenol}$).

HRMS: $[M-Cl]^+$ $[C_{32}H_{30}F_3N_2O_2]^+$, calcd: 531.22539, found: 531.22527.

4.4.7. 2-[5-(4–Hydroxyphenethoxy)-1-(4-methoxyphenyl)-benzyl-1*H*-indole-3-yl]ethanamine hydrochloride 8



To a solution of *tert*-butyl-2-{5-[4-(methoxymethoxy)phenethyloxy]-1-(4-iodo-benzyl)-1*H*-indole-3-yl}ethyl carbamate (100 mg, 0.15 mmol) in 1,4-dioxane/water (3:1 v/v, 2.0 mL) were added 4-methoxyphenylboronic acid (23 mg, 0.15 mmol), caesium carbonate (356 mg, 0.92 mmol) and palladium(II)-acetate (0.68 mg, 3.0 μmol). The mixture was heated to reflux for 4 h. After cooling to room temperature the suspension was diluted with EtOAc (10 mL), washed with water (3 mL) and brine (3 mL). The organic phase was dried (Na_2SO_4) and all volatiles were removed under reduced pressure. The protected intermediate was purified by column chromatography (*n*-hexane/EtOAc 1:1 v/v, R_f: 0.70) and dissolved in HCl-solution (0.8 M in MeOH, 3 mL). After stirring for 3 h at 50 °C the solvent was removed under reduced pressure and pure **8** (58 mg, 0.11 mmol, 72%) was obtained as a grey solid.

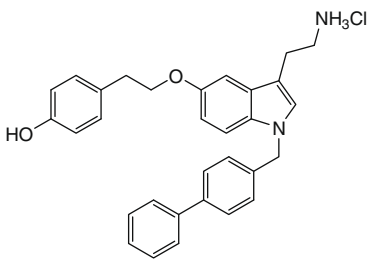
Mp: 203 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.90 (t, ^3J = 6.8 Hz, 2H, O–CH_2–CH_2), 2.96–3.00 (m, 4H, H_3N–CH_2–CH_2), 3.75 (s, 3H, O–CH_3), 4.09 (t, ^3J = 7.2 Hz, 2H, O–CH_2), 5.31 (s, 2H, N_{indole}–CH_2), 6.69 (d, ^3J = 8.4 Hz, 2H, C3$_{phenol}$*H*, C3′$_{phenol}$*H*), 6.74 (dd, ^3J = 8.7 Hz, ^4J = 2.4 Hz, 1H, C6$_{indole}$*H*), 6.95–6.99 (m, 2H, C3$_{methoxyphenyl}$*H*, C3′$_{methoxyphenyl}$*H*), 7.07–7.11 (m, 3H, C4$_{indole}$*H*, C2$_{phenol}$*H*, C2′$_{phenol}$*H*), 7.23 (d, ^3J = 8.1 Hz, 2H, C2$_{methoxyphenylphenyl}$*H*, C2′$_{methoxyphenylphenyl}$*H*, 7.29–7.34 (m, 2H, C2$_{indole}$*H*, C7$_{indole}$*H*), 7.49–7.54 (m, 4H, C3$_{methoxyphenyl}$*H*, C3′$_{methoxyphenyl}$*H*, C2$_{methoxyphenyl}$*H*, C2′$_{methoxyphenyl}$*H*), 8.03 (br, 3H, NH_3), 9.24 (br, 1H, O*H*).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 23.1 (H_3N–CH_2–CH_2), 34.5 (O–CH_2–CH_2), 48.8 (N_{indole}–CH_2), 55.1 (O–CH_3), 69.2 (O–CH_2), 101.6 (*C*4$_{indole}$), 109.1 (*C*3$_{indole}$), 111.0 (*C*6$_{indole}$), 111.9 (*C*7$_{indole}$), 114.3 (2C, *C*2$_{methoxyphenyl}$, *C*2′$_{methoxyphenyl}$), 115.1 (2C, *C*2$_{phenol}$, *C*2′$_{phenol}$*H*), 126.2 (2C, *C*2$_{methoxyphenylphenyl}$, *C*2′$_{methoxyphenylphenyl}$), 127.6 (2C, *C*3$_{methoxyphenylphenyl}$, *C*3′$_{methoxyphenylphenyl}$, 127.6 (*C*2$_{indole}$), 127.7 (2C, *C*3$_{methoxyphenyl}$, *C*3′$_{methoxyphenyl}$), 127.9 (*C*3a$_{indole}$), 128.4 (*C*7a$_{indole}$), 129.8 (2C, *C*3$_{phenol}$, *C*3′$_{phenol}$), 131.4 (*C*4$_{phenol}$), 132.1 (*C*4$_{methoxyphenyl}$), 136.7 (*C*4$_{methoxyphenyl}$), 138.9 (*C*1$_{methoxyphenyl}$), 152.6 (*C*5$_{indole}$), 155.8 (*C*1$_{phenol}$), 158.9 (*C*1$_{methoxyphenyl}$). overlapped: H_3N–CH_2.

HRMS: $[M-Cl]^+$ $[C_{32}H_{33}N_2O_3]^+$, calcd: 493.24912, found: 493.24824.

4.4.8. 2-[5-(4-Hydroxyphenethoxy)-1-(4-phenyl)benzyl-1*H*-indole-3-yl]ethanamine hydrochloride 9



To a solution of *tert*-butyl-2-{5-[4-(methoxymethoxy)phenethyloxy]-1-(4-iodo-benzyl)-1*H*-indole-3-yl}ethyl carbamate (100 mg, 0.15 mmol) in 1,4-dioxane/water (3:1, v/v, 2.0 mL) were added benzene boronic acid (18.5 mg, 0.15 mmol), caesium carbonate (356 mg, 0.92 mmol), bis(triphenylphosphino)palladium(II)-chloride (3.2 mg, 4.6 μmol) and 1,1′-bis(diphenylphosphino)ferrocene (5.0 mg, 9.1 μmol). The mixture was heated to reflux for 12 h. After cooling to room temperature the suspension was diluted with EtOAc (10 mL), washed with water (3 mL) and brine (3 mL). The organic phase was dried (Na_2SO_4) and all volatiles were removed under reduced pressure. The protected intermediate was purified by column chromatography (*n*-hexane/EtOAc 3:1 v/v, R_f: 0.32) and dissolved in HCl-solution (0.8 M in MeOH, 3 mL). The solution was stirred for 3 h at 50 °C, the solvent was removed under reduced pressure and pure **9** (67 mg, 0.114 mmol, 75%) was obtained as a white solid.

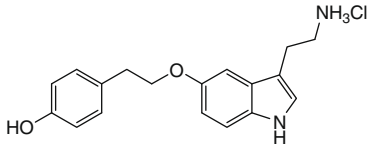
Mp: 214 °C.

^{1}H NMR (400 MHz, CD_3OD/DMSO-d_6): δ = 3.00 (t, ^3J = 7.0 Hz, 2H, O–CH_2–CH_2), 3.09 (t, ^3J = 7.6 Hz, 2H, H_3N–CH_2–CH_2), 3.21 (t, ^3J = 7.4 Hz, 2H, H_3N–CH_2), 4.18 (t, ^3J = 6.4 Hz, 2H, O–CH_2), 5.38 (s, 2H, N_{indole}–CH_2), 6.76 (d, ^3J = 8.8 Hz, 2H, C3$_{phenol}$*H*, C3′$_{phenol}$*H*), 6.85 (dd, ^3J = 9.2 Hz, ^4J = 2.0 Hz, 1H, C6$_{indole}$*H*), 7.13 (d, ^4J = 2.4 Hz, 1H, C4$_{indole}$*H*), 7.17 (d, ^3J = 8.4 Hz, 2H, C2$_{phenol}$*H*, C2′$_{phenol}$*H*), 7.31–7.37 (m, 5H, C2$_{indole}$*H*, C7$_{indole}$*H*, C2$_{phenylphenyl}$*H*, C2′$_{phenylphenyl}$*H*, C4$_{phenyl}$*H*), 7.43–7.47 (m, 2H, C3$_{phenylphenyl}$*H*, C3′$_{phenylphenyl}$*H*), 7.58–7.63 (m, 4H, C2$_{phenyl}$*H*, C2′$_{phenyl}$*H*, C3$_{phenyl}$*H*, C3′$_{phenyl}$*H*).

^{13}C NMR (100 MHz, CD_3OD/DMSO-d_6): δ = 24.4 (H_3N–CH_2–CH_2), 36.1 (O–CH_2–CH_2), 41.1 (H_3N–CH_2), 50.5 (N_{indole}–CH_2), 71.0 (O–CH_2), 102.8 (*C*4$_{indole}$), 110.2 (*C*3$_{indole}$), 112.2 (*C*6$_{indole}$), 113.6 (*C*7$_{indole}$), 116.3 (2C, *C*3$_{phenol}$, *C*3′$_{phenol}$), 127.9, 128.2, 128.9, 130.6 (8C, *C*2$_{phenylphenyl}$, *C*2′$_{phenylphenyl}$, *C*3$_{phenylphenyl}$, *C*3′$_{phenylphenyl}$, *C*2$_{phenyl}$, *C*2′$_{phenyl}$, *C*3$_{phenyl}$, *C*3′$_{phenyl}$) 128.6, 129.0, 129.5, 130.1, (*C*2$_{indole}$, *C*3a$_{indole}$, *C*7a$_{indole}$, *C*4$_{phenyl}$), 131.2 (2C, *C*2$_{phenol}$, *C*2′$_{phenol}$*H*), 133.4 (*C*1$_{phenol}$), 138.7, 141.4, 141.6 (*C*1$_{phenylphenyl}$, *C*4$_{phenylphenyl}$, *C*1$_{phenyl}$), 154.6 (*C*5$_{indole}$), 157.1 (*C*4$_{phenol}$).

HRMS: $[M-Cl]^+$ $[C_{31}H_{31}N_2O_2]^+$, calcd: 463.23800, found: 463.23760.

4.4.9. 2-[5-(4-Hydroxyphenethoxy)-1*H*-indole-3-yl]ethanamine hydrochloride 10



A solution of **4** (150 mg, 0.34 mmol) in HCl (0.8 M in MeOH, 3 mL) was heated to 50 °C for 3 h (TLC-control). The solvent was removed under reduced pressure and the product was obtained as a white solid.

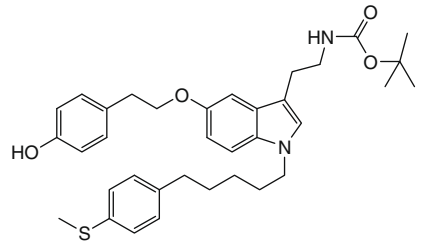
Mp: 115–116 °C.

^{1}H NMR (300 MHz, CD_3OD): δ = 2.93–2.99 (m, 2H, O–CH_2–CH_2), 3.07–3.09 (m, 2H, H_3N–CH_2–CH_2), 3.16–3.18 (m, 2H, H_3N–CH_2), 4.14 (t, ^3J = 7.1 Hz, 2H, O–CH_2), 6.76 (m, 3H, C6$_{indole}$*H*, C3$_{phenol}$*H*, C3′$_{phenol}$*H*), 7.09–7.14 (m, 4H, C2$_{indole}$*H*, C4$_{indole}$*H*, C2$_{phenol}$*H*, C2′$_{phenol}$*H*), 7.26 (t, ^3J = 8.7 Hz, 1H, C7$_{indole}$*H*).

^{13}C NMR (75 MHz, CD_3OD): δ = 24.4 (H_3N–CH_2–CH_2), 36.1 (O–CH_2–CH_2), 41.2 (H_3N–CH_2), 71.3 (O–CH_2), 102.4 (*C*4$_{indole}$), 110.0 (*C*3$_{indole}$), 113.2 (*C*6$_{indole}$), 113.6 (*C*7$_{indole}$), 116.1 (2C, *C*3$_{phenol}$, *C*3′$_{phenol}$), 125.0 (*C*2$_{indole}$), 128.4 (*C*3a$_{indole}$), 130.3 (*C*7a$_{indole}$), 131.0 (2C, *C*2$_{phenol}$, *C*2′$_{phenol}$), 133.5 (*C*1$_{phenol}$), 154.2 (*C*5$_{indole}$), 156.8 (*C*4$_{phenol}$).

HRMS: $[M-Cl]^+$ $[C_{18}H_{21}N_2O_2]^+$, calcd: 297.15975, found: 297.15987.

4.4.10. *tert*-Butyl-2-{5-(4-hydroxyphenethoxy)-1-{5-[4-(methylthio)phenyl]pentyl}-1*H*-indole-3-yl}ethyl carbamate 11



To a solution of **6** (35 mg, 0.067 mmol) and triethyl amine (10.2 μL, 0.073 mmol) in THF (0.5 mL) was added di-*tert*-butyl dicarbonate (16 mg, 0.073 mmol). After stirring at room temperature for 12 h the solvent was removed under reduced pressure and the crude product was purified by column chromatography (*n*-hexane/EtOAc 3:1, v/v) to yield pure product (34 mg, 0.058 mmol, 87%) was as a bright yellow oil.

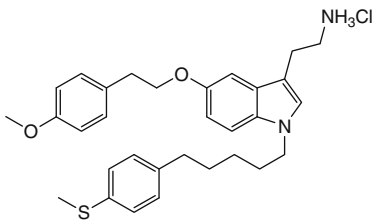
R_f: 0.22 (H/EE 3:1 v/v).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.29–1.33 (m, 2H, N_{indole}–CH_2–CH_2–CH_2), 1.43 (s, 9H, C(CH_3)$_3$) 1.58–1.62 (m, 2H, N_{indole}–CH_2–CH_2–CH_2–CH_2), 1.78–1.82 (m, 2H, N_{indole}–CH_2–CH_2), 2.46 (s, 3H, S–CH_3), 2.53 (t, ^3J = 7.7 Hz, 2H, N_{indole}–CH_2–CH_2–CH_2–CH_2–CH_2), 2.85 (t, ^3J = 6.8 Hz, 2H, NH–CH_2–CH_2), 3.04 (t, ^3J = 7.4 Hz, 2H, O–CH_2–CH_2), 3.36–3.42 (m, 2H, NH–CH_2), 3.99 (t, ^3J = 7.1 Hz, 2H, N_{indole}–CH_2), 4.19 (t, ^3J = 7.1 Hz, 2H, O–CH_2), 6.77–6.82 (m, 2H, C2$_{phenol}H$, C2′$_{phenol}H$), 6.87–6.88 (m, 3H, C6$_{indole}H$, C3$_{phenol}H$, C3′$_{phenol}H$), 6.95 (d, ^4J = 1.5 Hz, 1H, C4$_{indole}H$), 7.04 (d, ^3J = 8.7 Hz, 2H, C3$_{methylthiophenyl}H$, C3′$_{methylthiophenyl}H$), 7.14–7.19 (m, 4H, C2$_{indole}H$, C4$_{indole}H$, C2$_{methylthiophenyl}H$, C2′$_{methylthiophenyl}H$).

^{13}C NMR (100 MHz, CD_3OD): δ = 16.5 (S–CH_3), 25.8 (N_{indole}–CH_2–CH_2–CH_2), 28.6 (3C, C(CH_3)$_3$), 26.6, 30.3, 31.1 (N_{indole}–CH_2–CH_2–CH_2–CH_2), 35.3, 35.4 (N_{indole}–CH_2–CH_2–CH_2–CH_2–CH_2, O–CH_2–CH_2), 41.3 (NH–CH_2), 46.4 (N_{indole}–CH_2), 70.5 (O–CH_2), 79.0 (C(CH_3)$_3$), 102.6 (C4$_{indole}$), 110.2 (C3$_{indole}$), 111.0 (C6$_{indole}$), 112.6 (C7$_{indole}$), 115.5 (2C, C2$_{phenol}$, C2′$_{phenol}$), 126.5 (C2$_{indole}$) 127.3 (2C, C2$_{methylthiophenyl}$, C2′$_{methylthiophenyl}$), 128.2 (C3a$_{indole}$), 129.1 (2C, C3$_{methylthiophenyl}$, C3′$_{methylthiophenyl}$), 130.3 (2C, C3$_{phenol}$, C3′$_{phenol}$), 130.6 (C7a$_{indole}$) 132.0 (C4$_{phenol}$), 135.3 (C4$_{methylthiophenyl}$), 139.6 (C1$_{methylthiophenyl}$), 153.1 (C5$_{indole}$), 154.7 (C1$_{phenol}$), 156.3 (COOC(CH_3)$_3$).

HRMS: $[M+Na]^+$ $[C_{35}H_{44}N_2O_4SNa]^+$, calcd: 611.29140, found: 611.29109.

4.4.11. 2-{5-(4-Methoxyphenethoxy)-1-{5-[4-(methylthio)-phenyl]pentyl}-1*H*-indole-3-yl}ethanamine hydrochloride 12



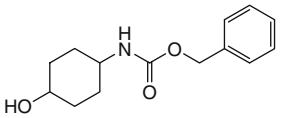
A solution of *tert*-butyl-2-{5-(4-methoxyphenethoxy)-1-{5-[4-(methylthio)phen-yl]pentyl}-1*H*-indole-3-yl}ethylcarbamate (19 mg, 0.032 mmol) in HCl-solution (0.8 M in MeOH, 1.5 mL) was stirred at 40 °C for 3 h. The solvent was removed under reduced pressure and pure **12** (19 mg, 0.032 mmol, quantitative) was obtained as a colourless oil.

^{1}H NMR (400 MHz, CD_3OD): δ = 1.25–1.28 (m, 2H, N_{indole}–CH_2–CH_2–CH_2), 1.56–1.58 (m, 2H, N_{indole}–CH_2–CH_2–CH_2–CH_2), 1.79–1.81 (m, 2H, N_{indole}–CH_2–CH_2), 2.41 (s, 3H, S–CH_3), 2.53 (t, ^3J = 7.4 Hz, 2H, N_{indole}–CH_2–CH_2–CH_2–CH_2–CH_2), 2.99–3.06 (m, 4H, H_3N–CH_2–CH_2, O–CH_2–CH_2), 3.15 (t, ^3J = 7.2 Hz, 2H, H_3N–CH_2), 3.75 (s, 3H, O–CH_3), 4.04–4.07 (m, 2H, N_{Indole}–CH_2), 4.18 (t, ^3J = 6.8 Hz, 2H, O–CH_2), 6.82–6.86 (m, 3H, C6$_{indole}H$, C2$_{methoxyphenyl}H$, C2′$_{methoxyphenyl}H$), 6.98 (d, ^3J = 8.4 Hz, 2H, C3$_{methoxyphenyl}H$, C3′$_{methoxyphenyl}H$), 7.04–7.06 (m, 2H, C2$_{indole}H$, C4$_{indole}H$), 7.10 (d, ^3J = 7.6 Hz, 2H, C3$_{methylthiophenyl}H$, C3$_{methylthiophenyl}H$), 7.21–7.25 (m, 3H, C7$_{indole}H$, C2$_{methylthiophenyl}H$, C2′$_{methylthiophenyl}H$).

^{13}C NMR (100 MHz, CD_3OD): δ = 16.2 (S–CH_3), 24.4 (N_{indole}–CH_2–CH_2–CH_2), 27.3, 31.2, 32.0 (N_{indole}–CH_2–CH_2–CH_2–CH_2, H_3N–CH_2–CH_2), 36.0, 36.2 (N_{indole}–CH_2–CH_2–CH_2–CH_2–CH_2, O–CH_2–CH_2), 41.3 (H_3N–CH_2), 47.1 (N_{indole}–CH_2), 55.7 (O–CH_3), 71.2 (O–CH_2), 102.8 (C4$_{indole}$), 109.2 (C3$_{indole}$), 111.6 (C6$_{indole}$), 113.5 (C7$_{indole}$), 114.8 (2C, C2$_{methoxyphenyl}$, C2′$_{methoxyphenyl}$), 128.0 (2C, C2$_{methylthiophenyl}$, C2′$_{methylthiophenyl}$), 128.3 (C2$_{indole}$), 129.1 (C3a$_{indole}$), 130.0 (2C, C3$_{methylthiophenyl}$, C3′$_{methylthiophenyl}$), 131.0 (2C, C3$_{methoxyphenyl}$, C3′$_{methoxyphenyl}$), 132.1 (C7a$_{indole}$), 133.5 (C4$_{methoxyphenyl}$), 136.6 (C4$_{methylthiophenyl}$), 140.7 (C1$_{methylthiophenyl}$), 154.5 (C5$_{indole}$), 159.7 (C1$_{methoxyphenyl}$).

HRMS: $[M-Cl]^+$ $[C_{31}H_{39}N_2O_2S]^+$, calcd: 503.27268, found: 503.27185.

4.4.12. Benzyl-4-hydroxycyclohexyl carbamate



To a solution of 4-aminocyclohexanol hydrochloride **13** (15.2 g, 0.10 mol) in CH_2Cl_2 (50 mL) was added a saturated solution of sodium carbonate (50 mL), followed by dropwise addition of benzyloxycarbonyl chloride (16.8 mL, 0.12 mol) at 0 °C. The reaction was stirred at this temperature for 30 min, extracted with CH_2Cl_2 (3 × 100 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was washed with *n*-hexane (3 × 50 mL) to afford pure title compound (24.9 g, 0.10 mol, quantitative) as colourless crystals.

R_f: 0.10 (*n*-hexane/EtOAc 2:1 v/v).

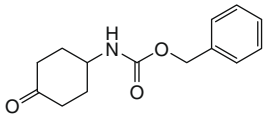
Mp: 163–165 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.14–1.24 (m, 4H, C2$_{cyclohexyl}H$, C3$_{cyclohexyl}H$, C5$_{cyclohexyl}H$, C6$_{cyclohexyl}H$), 1.76–1.78 (m, 4H, C2′$_{cyclohexyl}H$, C3′$_{cyclohexyl}H$, C5′$_{cyclohexyl}H$, C6′$_{cyclohexyl}H$), 3.21–3.29 (m, 2H, C1$_{cyclohexyl}H$, C4$_{cyclohexyl}H$), 4.54 (m, 1H, O*H*), 4.99 (s, 2H, Ph–CH_2), 7.16 (d, ^3J = 7.8 Hz, 1H, N*H*), 7.30–7.39 (m, 5H, 5 × C$_{phenyl}H$).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 30.5 (C2$_{cyclohexyl}$, C6$_{cyclohexyl}$), 33.9 (C3$_{cyclohexyl}$, C5$_{cyclohexyl}$), 49.1 (C1$_{cyclohexyl}$), 65.0 (Ph-CH_2), 68.1 (C4$_{cyclohexyl}$), 127.7 (C2$_{phenyl}$, C2′$_{phenyl}$), 127.8 (C4$_{phenyl}$), 128.3 (C3$_{phenyl}$, C3′$_{phenyl}$), 137.2 (C1$_{phenyl}$), 155.3 (CO).

HRMS: $[M+Na]^+$ $[C_{14}H_{19}NO_3Na]^+$, calcd: 272.12571, found: 272.12570.

4.4.13. Benzyl-4-oxocyclohexylcarbamate 14



To a solution of benzyl-4-hydroxycyclohexyl carbamate (18.4 g, 74 mmol) in EtOAc (500 mL) was added IBX (31.0 g, 0.11 mol) and the suspension was heated to reflux for 4 h. The reaction was cooled, filtered and washed with ice-cold EtOAc (100 mL). The filtrate was pure **14** (16.0 g, 65 mmol, 88%) as a white solid.

R_f: 0.45 (*n*-hexane/EtOAc 1:1, v/v).

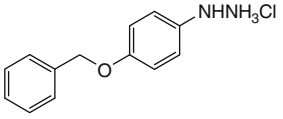
Mp: 83–85 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.60–1.72 (m, 2H, C2$_{cyclohexyl}H$, C6$_{cyclohexyl}H$), 1.98–2.03 (m, 2H, C2′$_{cyclohexyl}H$, C6′$_{cyclohexyl}H$), 2.23–2.30 (m, 2H, C3$_{cyclohexyl}H$, C5$_{cyclohexyl}H$), 2.35–2.45 (m, 2H, C3′$_{cyclohexyl}H$, C5′$_{cyclohexyl}H$), 3.77–3.89 (m, 1H, C1$_{cyclohexyl}H$), 5.03 (s, 2H, CH_2Ph), 7.30–7.42 (m, 6H, 5 × C$_{phenyl}H$, N*H*).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 31.2 (*C*2$_{cyclohexyl}$, *C*6$_{cyclohexyl}$), 38.2 (*C*3$_{cyclohexyl}$, *C*5$_{cyclohexyl}$), 46.9 (*C*1$_{cyclohexyl}$), 65.2 (PhCH_2), 127.7 (*C*2$_{phenyl}$, *C*2′$_{phenyl}$), 127.7 (*C*4$_{phenyl}$), 128.3 (*C*3$_{phenyl}$, *C*3′$_{phenyl}$), 137.0 (*C*1$_{phenyl}$), 155.4 (NHCO), 209.7 (*C*4$_{cyclohexyl}$).

HRMS: [M+Na]$^+$ [$C_{14}H_{17}NO_3Na$]$^+$, calcd: 270.11006, found: 270.10986.

4.4.14. 4-Benzyloxyphenylhydrazine hydrochloride 16



4-Benzyloxyaniline hydrochloride **15** (25.0 g, 0.11 mol) was dissolved in concentrated hydrochloric acid (30.0 mL) and a solution of $NaNO_2$ (8.10 g, 0.12 mol) in water (20 mL) was added dropwise at −5 °C. The mixture was stirred for 1 h (TLC-control) when a solution of $SnCl_2 \times 2H_2O$ (47.9 g, 0.21 mol) in diluted hydrochloric acid (1 N, 200 mL) was added dropwise. The resulting mixture was stirred for an additional 2 h, when it was filtered and washed with Et_2O (3 × 200 mL). The solid was collected and basified by adding NaOH-solution (5 N, 500 mL), filtered again through Celite®, extracted with CH_2Cl_2 (3 × 500 mL). The organic phases were evaporated under reduced pressure, HCl-solution (6 M in MeOH, 500 mL) was added to form the hydrochloride salt and the resulting solid was washed with cold Et_2O (500 mL) to afford pure title compound (20.0 g, 0.083 mmol, 75%) as a violet powder.

R_f: 0.10 (*n*-hexane/EtOAc 1:1, v/v).

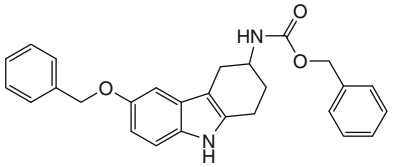
Mp: 181–182 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.03 (s, 2H, CH_2), 6.89 (d, ^3J = 9.0 Hz, 2H, C2$_{NH\text{-}phenyl}H$, C2$_{NH'\text{-}phenyl}H$), 7.01 (d, ^3J = 9.0 Hz, 2H, C3$_{NH\text{-}phenyl}H$, C3$_{NH'\text{-}phenyl}H$), 7.29–7.42 (m, 5H, Cb$_{benzyl}H$ × 5), 10.11 (br, 3H, N*H*NH_2).

^{13}C NMR (50 MHz, DMSO-d_6): δ = 69.5 (*C*H$_2$), 115.4 (*C*2$_{NH\text{-}phenyl}$, *C*2′$_{NH\text{-}phenyl}$), 117.1 (*C*3$_{NH\text{-}phenyl}$, *C*3′$_{NH\text{-}phenyl}$), 127.6 (*C*2$_{benzyl}$, *C*2′$_{benzyl}$), 127.8 (*C*4$_{benzyl}$), 128.4 (*C*3$_{benzyl}$, *C*3′$_{benzyl}$), 137.2 (*C*1$_{benzyl}$), 139.2 (*C*1$_{NH\text{-}phenyl}$), 153.7 (*C*4$_{NH\text{-}phenyl}$).

HRMS: [M+Na−HCl]$^+$ [$C_{13}H_{14}N_2ONa$]$^+$, calcd: 237.09983, found: 237.10004.

4.4.15. Benzyl-6-(benzyloxy)-2,3,4,9-tetrahydro-1*H*-carbazol-3-yl carbamate 17



To a suspension of **16** (10.0 g, 40 mmol) in glacial AcOH (500 mL) was added **14** (10.0 g, 40 mmol) and the mixture was heated to 80 °C for 3 h. The reaction was cooled and all volatiles were removed under reduced pressure. The residue was taken up in EtOAc (500 mL) and washed with saturated sodium bicarbonate-solution (500 mL) and brine (500 mL) and dried (Na_2SO_4). All volatiles were removed under reduced pressure and the crude product was purified by column chromatography (CH_2Cl_2/MeOH = 400:1 to 50:1, v/v) to afford pure title compound (14.0 g, 33 mmol, 83%) as a yellow oil.

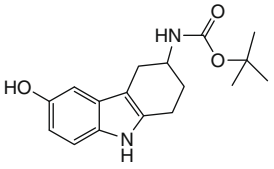
R_f: 0.58 (CH_2Cl_2/MeOH 50:1 v/v).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.61–1.81 (m, 1H, C2$_{tetrahydrocarbazole}H$), 1.95–2.05 (m, 1H, C2′$_{tetrahydrocarbazole}H$), 2.36–2.44 (m, 1H, C4$_{tetrahydrocarbazole}H$), 2.71–2.91 (m, 3H, C1$_{tetrahydrocarbazole}H$, C1′$_{tetrahydrocarbazole}H$, C4′$_{tetrahydrocarbazole}H$), 3.64–3.83 (m, 1H, C3$_{tetrahydrocarbazole}H$), 5.02 (s, 2H, NHCOOCH_2Ph), 5.03 (s, 2H, OCH_2Ph), 6.68 (dd, ^3J = 8.4 Hz, ^4J = 2.2 Hz, 1H, C7$_{tetrahydrocarbazole}H$), 6.90 (d, ^4J = 2.2 Hz, 1H, C5$_{tetrahydrocarbazole}H$), 7.09 (d, ^3J = 8.6 Hz, 1H, C8$_{tetrahydrocarbazole}H$), 7.29–7.45 (m, 10H, 10 × C$_{phenyl}H$), 8.30 (s, 1H, CON*H*), 10.51 (br, 1H, C9$_{tetrahydrocarbazole}H$).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 21.5 (*C*1$_{tetrahydrocarbazole}$), 27.5 (*C*4$_{tetrahydrocarbazole}$), 29.0 (*C*2$_{tetrahydrocarbazole}$), 47.4 (*C*3$_{tetrahydrocarbazole}$), 65.0 (NHCOOCH_2), 69.7 (OCH_2), 101.1 (*C*5$_{tetrahydrocarbazole}$), 106.3 (*C*4a$_{tetrahydrocarbazole}$), 110.3 (*C*8$_{tetrahydrocarbazole}$), 111.0 (*C*7$_{tetrahydrocarbazole}$), 127.3, 127.4 (*C*2$_{O\text{-}benzyl}$, *C*2$_{COO\text{-}benzyl}$, *C*6$_{O\text{-}benzyl}$, *C*6$_{COO\text{-}benzyl}$), 127.4 (*C*4$_{O\text{-}benzyl}$, *C*4$_{COO\text{-}benzyl}$), 127.7 (*C*8a$_{tetrahydrocarbazole}$), 128.2 (*C*3$_{O\text{-}benzyl}$, *C*3$_{COO\text{-}benzyl}$, *C*5$_{O\text{-}benzyl}$, *C*5$_{COO\text{-}benzyl}$), 131.3 (*C*5a$_{tetrahydrocarbazole}$), 134.2 (*C*1a$_{tetrahydrocarbazole}$), 137.2 (*C*1$_{COO\text{-}benzyl}$), 137.9 (*C*1$_{O\text{-}benzyl}$), 151.8 (*C*6$_{tetrahydrocarbazole}$), 155.5 (*C*O).

HRMS: [M+Na]$^+$ [$C_{27}H_{26}N_2O_3Na$]$^+$, calcd: 449.18356, found: 449.18378.

4.4.16. *tert*-Butyl-6-hydroxy-2,3,4,9-tetrahydro-1*H*-carbazol-3-yl carbamate 18



To a solution of **17** (10.0 g, 23.4 mmol) in MeOH (200 mL) was added $Pd(OH)_2$ (5%, 1.0 g, 50% wet on carbon) and di-*tert*-butyl dicarbonate (6.10 g, 28.1 mmol) and the mixture was stirred over night under an atmosphere of hydrogen (ambivalent pressure). The solution was filtered and the solvent was removed under reduced pressure. The crude product that was purified by column chromatography (CH_2Cl_2/MeOH = 20:1, v/v) to yield pure **18** (6.0 g, 19.9 mmol, 85%) as a white solid.

R_f: 0.31 (CH_2Cl_2/MeOH 10:1, v/v).

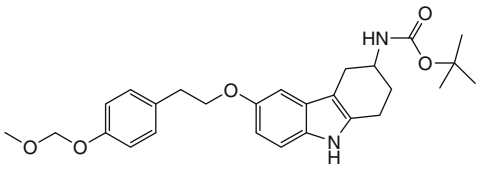
Mp: 196–198 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.41 (s, 9H, C(CH_3)$_3$), 1.66–1.72 (m, 1H, C2$_{tetrahydrocarbazole}H$), 1.96–1.99 (m, 1H, C2′$_{tetrahydrocarbazole}H$), 2.35–2.41 (m, 1H, C4$_{tetrahydrocarbazole}H$), 2.71–2.79 (m, 3H, C1$_{tetrahydrocarbazole}H$, C1′$_{tetrahydrocarbazole}H$, C4′$_{tetrahydrocarbazole}H$), 3.62–3.78 (m, 1H, C3$_{tetrahydrocarbazole}H$), 6.48 (dd, ^3J = 8.4 Hz, ^4J = 2.4 Hz, 1H, C7$_{tetrahydrocarbazole}H$), 6.61 (d, ^4J = 2.4 Hz, 1H, C5$_{tetrahydrocarbazole}H$), 6.91 (m, 1H, CON*H*), 7.00 (d, ^3J = 8.8 Hz, 1H, C8$_{tetrahydrocarbazole}H$), 8.47 (s, 1H, O*H*), 10.30 (br, 1H, C9$_{tetrahydrocarbazole}H$).

^{13}C NMR (50 MHz, DMSO-d_6): δ = 21.7 (*C*1$_{tetrahydrocarbazole}$), 27.5 (*C*4$_{tetrahydrocarbazole}$), 28.3 (C(*C*H$_3$)$_3$), 29.3 (*C*2$_{tetrahydrocarbazole}$), 47.0 (*C*3$_{tetrahydrocarbazole}$), 77.4 (*C*(CH$_3$)$_3$), 101.5 (*C*5$_{tetrahydrocarbazole}$), 105.9 (*C*4a$_{tetrahydrocarbazole}$), 109.9 (*C*8$_{tetrahydrocarbazole}$), 110.7 (*C*7$_{tetrahydrocarbazole}$), 127.8 (*C*5a$_{tetrahydrocarbazole}$), 130.6 (*C*8a$_{tetrahydrocarbazole}$), 133.9 (*C*1a$_{tetrahydrocarbazole}$), 150.1 (*C*6$_{tetrahydrocarbazole}$), 155.0 (*C*O).

HRMS: $[M+H]^+$ $[C_{17}H_{23}N_2O_3]^+$, calcd: 303.17032, found: 303.17076.

4.4.17. *tert*-Butyl-6-(4-(methoxymethoxy)phenethoxy)-2,3,4,9-tetrahydro-1*H*-carbazol-3-yl carbamate 19



To a stirred solution of **18** (1.45 g, 4.77 mmol) in acetone (100 mL) 4-(methoxymethoxy)phenylethyl-4-methylbenzene sulfonate (1.61 g, 4.77 mmol) and caesium carbonate (3.11 g, 9.55 mmol) were added. The resulting suspension was heated to reflux for 48 h (TLC-control) and afterwards the solvent was removed under reduced pressure. The crude product was purified by column chromatography (*n*-hexane/EtOAc 2:1, v/v) to give pure **19** (1.51 g, 3.11 mmol, 65%) as colourless crystals.

R_f: 0.45 (*n*-hexane/EtOAc 1:1, v/v).

Mp: 102–103 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 (s, 9H, C(CH_3)$_3$), 1.95–2.01 (m, 1H, C2$_{tetrahydrocarbazole}$*H*), 2.07–2.15 (m, 1H, C2′$_{tetrahydrocarbazole}$*H*), 2.47–2.59 (m, 1H, C4$_{tetrahydrocarbazole}$*H*), 2.78–2.82 (m, 2H, C1$_{tetrahydrocarbazole}$*H*, C4′$_{tetrahydrocarbazole}$*H*), 2.98–3.02 (m, 1H, C1′$_{tetrahydrocarbazole}$*H*), 3.06 (t, ^3J = 7.2 Hz, 2H, PhCH_2CH$_2$O), 3.48 (s, 3H, OCH_3), 4.17 (t, ^3J = 7.2 Hz, 2H, PhCH$_2$CH_2O), 4.62–4.70 (m, 1H, C3$_{tetrahydrocarbazole}$*H*), 5.16 (s, 2H, OCH_2Ph), 6.78 (dd, ^3J = 8.4 Hz, ^4J = 2.4 Hz, 1H, C7$_{tetrahydrocarbazole}$*H*), 6.89 (d, ^4J = 2.4 Hz, 1H, C5$_{tetrahydrocarbazole}$*H*), 6.99 (d, ^3J = 8.7 Hz, 2H, C3$_{phenol}$*H*, C3′$_{phenol}$*H*), 7.16 (d, ^3J = 8.7 Hz, 1H, C8$_{tetrahydrocarbazole}$*H*), 7.22 (d, ^3J = 6.6 Hz, 2H, C2$_{phenol}$*H*, C2′$_{phenol}$*H*), 7.62 (br, 1H, CON*H*), 10.31 (br, 1H, C9$_{tetrahydrocarbazole}$*H*).

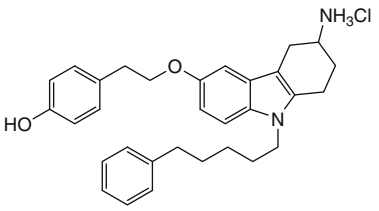
^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.9 (*C*1$_{tetrahydrocarbazole}$), 28.2 (*C*4$_{tetrahydrocarbazole}$), 28.6 (C(*C*H$_3$)$_3$), 28.7 (*C*2$_{tetrahydrocarbazole}$), 35.3 (Ph*C*H$_2$CH$_2$O), 46.2 (*C*3$_{tetrahydrocarbazole}$), 56.0 (O*C*H$_3$), 70.0 (PhCH$_2$*C*H$_2$O), 79.5 (*C*(CH$_3$)$_3$), 94.7 (O*C*H$_2$O), 101.7 (*C*5$_{tetrahydrocarbazole}$), 107.5 (*C*4a$_{tetrahydrocarbazole}$), 111.2 (*C*8$_{tetrahydrocarbazole}$), 111.7 (*C*7$_{tetrahydrocarbazole}$), 116.4 (*C*3$_{phenol}$, *C*3′$_{phenol}$), 128.2 (*C*8a$_{tetrahydrocarbazole}$), 130.0 (*C*2$_{phenol}$, *C*2′$_{phenol}$), 131.5 (*C*1$_{phenol}$), 132.1 (*C*5a$_{tetrahydrocarbazole}$), 133.8 (*C*1a$_{tetrahydrocarbazole}$), 153.2 (*C*6$_{tetrahydrocarbazole}$), 155.7 (*C*O), 155.9 (*C*4$_{phenol}$).

HRMS: $[M+Na]^+$ $[C_{27}H_{34}N_2O_5Na]^+$, calcd: 489.23599, found: 489.23567.

4.5. General procedure for the preparation of the tetrahydrocarbazole derivatives

To a stirred solution of **19** (100 mg, 0.215 mol) in DMF (1.0 mL) was added sodium hydride (60% suspension in mineral oil, 12.9 mg, 0.323 mmol) at 0 °C. The solution was stirred at this temperature for 15 min when the appropriate alkyl bromide (0.258 mmol) was added and stirring was continued at room temperature for 2–18 h (TLC-control). Afterwards the suspension was diluted with EtOAc (10 mL), washed with water (4 mL) and brine (4 mL) and the organic phase was dried (Na_2SO_4). All volatiles were removed under reduced pressure, the protected intermediate was purified by column chromatography (*n*-hexane/EtOAc) and redissolved in HCl-solution (0.8 M in MeOH, 3.0 mL). The solution was stirred at 50 °C for 1–3 h (TLC-control) and the solvent was removed under reduced pressure to afford the pure tetrahydrocarbazoles as their hydrochlorides.

4.5.1. 4-(2-(3-Amino-9-(5-phenylpentyl)-2,3,4,9-tetrahydro-1*H*-tetrahydrocarbazole-6-yloxy)ethyl)phenol hydrochloride 2



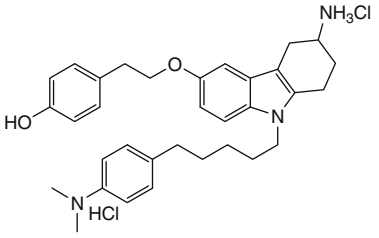
Reaction time:	4 h (coupling), 1 h (deprotection)
Yield:	91 mg (0.18 mmol, 82%)
Mp:	112–113 °C

^{1}H NMR (300 MHz, CD_3OD): δ = 1.22–1.35 (m, 2H, N$_{tetrahydrocarbazole}$–CH$_2$–CH$_2$–CH_2), 1.51–1.61 (m, 2H, N$_{tetrahydrocarbazole}$–CH$_2$–CH$_2$–CH$_2$–CH_2), 1.62–1.75 (m, 2H, N$_{tetrahydrocarbazole}$–CH$_2$–CH_2), 1.95–2.07 (m, 1H, C2$_{tetrahydrocarbazole}$*H*), 2.22–2.37 (m, 1H, C2′$_{tetrahydrocarbazole}$*H*), 2.52 (t, J = 10.5 Hz, 2H, N$_{tetrahydrocarbazole}$–CH$_2$–CH$_2$–CH$_2$–CH$_2$–CH_2), 2.63–2.78 (m, 1H, C4$_{tetrahydrocarbazole}$*H*), 2.78–2.87 (m, 2H, C1$_{tetrahydrocarbazole}$*H*, C4′$_{tetrahydrocarbazole}$*H*), 2.95 (t, ^3J = 7.2 Hz, 2H, O–CH$_2$–CH_2), 3.08–3.19 (m, 1H, C1′$_{tetrahydrocarbazole}$*H*), 3.51–3.61 (m, 1H, C3$_{tetrahydrocarbazole}$*H*), 3.96 (t, ^3J = 7.8 Hz, 2H, N$_{tetrahydrocarbazole}$–CH_2), 4.11 (t, ^3J = 7.2 Hz, 2H, O–CH_2–CH$_2$), 6.71–6.77 (m, 3H, C3$_{phenol}$*H*, C3′$_{phenol}$*H*, C7$_{tetrahydrocarbazole}$*H*), 6.88 (d, ^4J = 2.4 Hz, 1H, C5$_{tetrahydrocarbazole}$*H*), 7.04–7.19 (m, 8H, C2$_{phenol}$*H*, C2′$_{phenol}$*H*, C8$_{tetrahydrocarbazole}$*H*, 5 × C$_{phenyl}$*H*).

^{13}C NMR (75 MHz, CD_3OD): δ = 21.0 (*C*1$_{tetrahydrocarbazole}$), 27.2 (N$_{tetrahydrocarbazole}$–CH$_2$–CH$_2$–*C*H$_2$), 27.4 (*C*4$_{tetrahydrocarbazole}$), 28.4 (N$_{tetrahydrocarbazole}$–CH$_2$–*C*H$_2$), 31.1 (N$_{tetrahydrocarbazole}$–CH$_2$–CH$_2$–CH$_2$–*C*H$_2$), 32.3 (*C*2$_{tetrahydrocarbazole}$), 36.2 (O–CH$_2$–*C*H$_2$), 36.7 (N$_{tetrahydrocarbazole}$–CH$_2$–CH$_2$–CH$_2$–*C*H$_2$–CH$_2$), 43.9 (N$_{tetrahydrocarbazole}$–*C*H$_2$), 49.1 (*C*3$_{tetrahydrocarbazole}$), 71.3 (O–*C*H$_2$–CH$_2$), 102.5 (*C*5$_{tetrahydrocarbazole}$), 105.2 (*C*4a$_{tetrahydrocarbazole}$), 110.9 (*C*8$_{tetrahydrocarbazole}$), 112.4 (*C*7$_{tetrahydrocarbazole}$), 116.2 (*C*3$_{phenol}$, *C*3′$_{phenol}$), 126.7 (*C*8a$_{tetrahydrocarbazole}$), 128.3 (*C*4$_{phenyl}$), 129.2 (*C*2$_{phenyl}$, *C*2′$_{phenyl}$), 129.4 (*C*3$_{phenyl}$, *C*3′$_{phenyl}$), 130.8 (*C*5a$_{tetrahydrocarbazole}$), 131.0 (*C*2$_{phenol}$, *C*2′$_{phenol}$), 133.6 (*C*1$_{phenol}$), 135.1 (*C*1a$_{tetrahydrocarbazole}$), 143.5 (*C*1$_{phenyl}$), 154.3 (*C*6$_{tetrahydrocarbazole}$), 156.9 (*C*4$_{phenol}$).

HRMS: $[M+H-HCl]^+$ $[C_{31}H_{37}N_2O_2]^+$, calcd: 469.28495, found: 469.28522.

4.5.2. 4-(2-(3-Amino-9-(5-(4-(dimethylamino)phenyl)pentyl)-2,3,4,9-tetrahydro-1*H*-tetrahydrocarbazole-6-yloxy)ethyl)-phenol dihydrochloride 20



Reaction time:	3 h (coupling), 1 h (deprotection)
Yield:	95 mg (0.16 mmol, 75%)
Mp:	154–156 °C

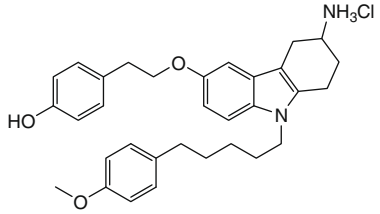
^{1}H NMR (400 MHz, CD_3OD): δ = 1.28–1.36 (m, 2H, N$_{tetrahydrocarbazole}$–CH$_2$–CH$_2$–CH_2), 1.58–1.64 (m, 2H, N$_{tetrahydrocarbazole}$–CH$_2$–CH$_2$–CH$_2$–

CH_2), 1.70–1.78 (m, 2H, $N_{tetrahydrocarbazole}$–CH_2–CH_2), 1.95–2.08 (m, 1H, $C2_{tetrahydrocarbazole}H$), 2.28–2.36 (m, 1H, $C2'_{tetrahydrocarbazole}H$), 2.63 (t, ^3J = 7.6 Hz, 2H, $N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2–CH_2), 2.69–2.75 (m, 1H, $C4_{tetrahydrocarbazole}H$), 2.87–2.94 (m, 2H, $C1_{tetrahydrocarbazole}H$, $C4'_{tetrahydrocarbazole}H$), 2.97 (t, ^3J = 7.2 Hz, 2H, O–CH_2–CH_2), 3.13–3.18 (m, 1H, $C1'_{tetrahydrocarbazole}H$), 3.22 (s, 6H, $N(CH_3)_2$), 3.57–3.64 (m, 1H, $C3_{tetrahydrocarbazole}H$), 3.98–4.09 (m, 2H, $N_{tetrahydrocarbazole}$–CH_2), 4.13 (t, ^3J = 7.2 Hz, 2H, O–CH_2–CH_2), 6.73 (d, ^3J = 8.4 Hz, 2H, $C3_{phenol}H$, $C3'_{phenol}H$), 6.77 (dd, ^3J = 8.4 Hz, ^4J = 2.0 Hz, 1H, $C7_{tetrahydrocarbazole}H$), 6.89 (d, ^4J = 2.4 Hz, 1H, $C5_{tetrahydrocarbazole}H$), 7.12 (d, ^3J = 8.0 Hz, 2H, $C3_{dimethylaminophenyl}H$, $C3'_{dimethylaminophenyl}H$), 7.17 (d, ^3J = 8.8 Hz, 1H, $C8_{tetrahydrocarbazole}H$), 7.31 (d, ^3J = 8.4 Hz, 2H, $C2_{phenol}H$, $C2'_{phenol}H$), 7.49 (d, ^3J = 8.4 Hz, 2H, $C2_{dimethylaminophenyl}H$, $C2'_{dimethylaminophenyl}H$).

^{13}C NMR (75 MHz, CD_3OD): δ = 21.1 ($C1_{tetrahydrocarbazole}$), 27.2 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2), 27.2 ($C4_{tetrahydrocarbazole}$), 28.3 ($N_{tetrahydrocarbazole}$–CH_2–CH_2), 31.0 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2), 31.9 ($C2_{tetrahydrocarbazole}$), 35.8 (O–CH_2–CH_2), 36.1 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2–CH_2), 43.8 ($N_{tetrahydrocarbazole}$–CH_2), 47.2 ($N(CH_3)_2$), 49.2 ($C3_{tetrahydrocarbazole}$), 71.2 (O–CH_2–CH_2), 102.4 ($C5_{tetrahydrocarbazole}$), 105.3 ($C4a_{tetrahydrocarbazole}$), 110.9 ($C8_{tetrahydrocarbazole}$), 112.4 ($C7_{tetrahydrocarbazole}$), 116.2 ($C3_{phenol}$, $C3'_{phenol}$), 121.3 ($C3_{dimethylaminophenyl}$, $C3'_{dimethylaminophenyl}$), 128.2 ($C8a_{tetrahydrocarbazole}$), 130.8 ($C5a_{tetrahydrocarbazole}$), 131.0 ($C2_{phenol}$, $C2'_{phenol}$), 131.5 ($C2_{dimethylaminophenyl}$, $C2'_{dimethylaminophenyl}$), 133.6 ($C1_{phenol}$), 135.2 ($C1a_{tetrahydrocarbazole}$), 141.6 ($C1_{dimethylaminophenyl}$), 146.4 ($C4_{dimethylaminophenyl}$), 154.2 ($C6_{tetrahydrocarbazole}$), 156.8 ($C4_{phenol}$).

HRMS: $[M+H-2HCl]^+$ $[C_{33}H_{42}N_3O_2]^+$, calcd: 512.32715, found: 512.32730.

4.5.3. 4-(2-(3-Amino-9-(5-(4-methoxyphenyl)pentyl)-2,3,4,9-tetrahydro-1*H*-tetrahydrocarbazole-6-yloxy)ethyl)phenol hydrochloride 21



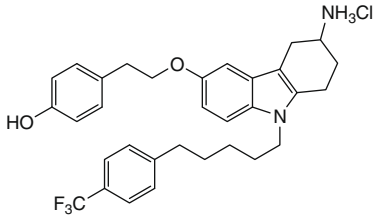
Reaction time:	18 h (coupling), 3 h (deprotection)
Yield:	74 mg (0.14 mmol, 64%)
Mp:	107–111 °C

^{1}H NMR (400 MHz, CD_3OD): δ = 1.27–1.33 (m, 2H, $N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2), 1.46–1.59 (m, 2H, $N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2), 1.67–1.76 (m, 2H, $N_{tetrahydrocarbazole}$–CH_2–CH_2), 1.96–2.07 (m, 1H, $C2_{tetrahydrocarbazole}H$), 2.24–2.33 (m, 1H, $C2'_{tetrahydrocarbazole}H$), 2.48 (t, J = 7.6 Hz, 2H, $N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2–CH_2), 2.68–2.74 (m, 1H, $C4_{tetrahydrocarbazole}H$), 2.82–2.89 (m, 2H, $C1_{tetrahydrocarbazole}H$, $C4'_{tetrahydrocarbazole}H$), 2.96 (t, ^3J = 7.6 Hz, 2H, O–CH_2–CH_2), 3.11–3.16 (m, 1H, $C1'_{tetrahydrocarbazole}H$), 3.54–3.63 (m, 1H, $C3_{tetrahydrocarbazole}H$), 3.73 (s, 3H, OCH_3), 3.93–4.04 (m, 2H, $N_{tetrahydrocarbazole}$–CH_2), 4.13 (t, ^3J = 7.2 Hz, 2H, O–CH_2–CH_2), 6.71–6.78 (m, 5H, $C3_{methoxyphenyl}H$, $C3'_{methoxyphenyl}H$, $C7_{carbazol}H$, $C3_{phenol}H$, $C3'_{phenol}H$), 6.88 (d, ^4J = 2.4 Hz, 1H, $C5_{tetrahydrocarbazole}H$), 6.97 (d, ^3J = 8.8 Hz, 2H, $C2_{methoxyphenyl}H$, $C2'_{methoxyphenyl}H$), 7.11 (d, J = 8.4 Hz, 2H, $C2_{phenol}H$, $C2'_{phenol}H$), 7.17 (d, ^3J = 8.8 Hz, 1H, $C8c_{Tetrahydrocarbazole}H$).

^{13}C NMR (100 MHz, CD_3OD): δ = 21.0 ($C1_{tetrahydrocarbazole}$), 27.2 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2), 27.3 ($C4_{tetrahydrocarbazole}$), 28.4 ($N_{tetrahydrocarbazole}$–CH_2–CH_2), 31.1 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2), 32.4 ($C2_{tetrahydrocarbazole}$), 35.7 (O–CH_2–CH_2), 36.1 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2–CH_2), 43.9 ($N_{tetrahydrocarbazole}$–CH_2), 49.1 ($C3_{tetrahydrocarbazole}$), 55.7 (OCH_3), 71.3 (O–CH_2–CH_2), 102.5 ($C5_{tetrahydrocarbazole}$), 105.2 ($C4a_{tetrahydrocarbazole}$), 110.9 ($C8_{tetrahydrocarbazole}$), 112.4 ($C7_{tetrahydrocarbazole}$), 114.6 ($C3_{methoxyphenyl}$, $C3'_{methoxyphenyl}$), 116.2 ($C3_{phenol}$, $C3'_{phenol}$), 128.3 ($C8a_{tetrahydrocarbazole}$), 130.3 ($C2_{methoxyphenyl}$, $C2'_{methoxyphenyl}$), 130.8 ($C5a_{carbazol}$), 130.9 ($C2_{phenol}$, $C2'_{phenol}$), 133.6 ($C1_{phenol}$), 135.2 ($C1a_{tetrahydrocarbazole}$), 135.5 ($C1_{methoxyphenyl}$), 154.2 ($C6_{tetrahydrocarbazole}$), 156.8 ($C4_{phenol}$), 159.1 ($C4_{methoxyphenyl}$).

HRMS: $[M+H-HCl]^+$ $[C_{32}H_{39}N_2O_3]^+$, calcd: 499.29552, found: 499.29514.

4.5.4. 4-(2-(3-Amino-9-(5-(4-(trifluoromethyl)phenyl)pentyl)-2,3,4,9-tetrahydro-1*H*-tetrahydrocarbazole-6-yloxy)ethyl)-phenol hydrochloride 22



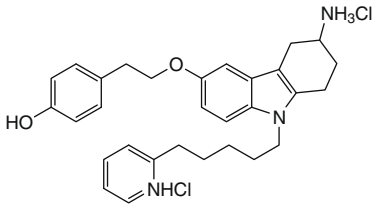
Reaction time:	10 h (coupling), 2 h (deprotection)
Yield:	75 mg, (0.13 mmol, 60%)
Mp:	125–128 °C

^{1}H NMR (200 MHz, CD_3OD): δ = 1.21–1.38 (m, 2H, $N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2), 1.42–1.81 (m, 4H, $N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2), 1.90–2.17 (m, 1H, $C2_{tetrahydrocarbazole}H$), 2.20–2.38 (m, 1H, $C2'_{tetrahydrocarbazole}H$), 2.58 (t, ^3J = 7.6 Hz, 2H, $N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2–CH_2), 2.67–2.76 (m, 1H, $C4_{tetrahydrocarbazole}H$), 2.79–2.88 (m, 2H, $C1_{tetrahydrocarbazole}H$, $C4'_{tetrahydrocarbazole}H$), 2.93 (t, ^3J = 7.0 Hz, 2H, O–CH_2–CH_2), 3.07–3.17 (m, 1H, $C1'_{tetrahydrocarbazole}H$), 3.42–3.63 (m, 1H, $C3_{tetrahydrocarbazole}H$), 3.97 (t, ^3J = 6.8 Hz, 2H, $N_{tetrahydrocarbazole}$–CH_2), 4.10 (t, ^3J = 7.0 Hz, 2H, O–CH_2–CH_2), 6.68–6.77 (m, 3H, $C3_{phenol}H$, $C3'_{phenol}H$, $C7_{tetrahydrocarbazole}H$), 6.87 (d, ^4J = 2.2 Hz, 1H, $C5_{tetrahydrocarbazole}H$), 7.08 (d, J = 8.6 Hz, 2H, $C2_{phenol}H$, $C2'_{phenol}H$), 7.14 (d, J = 8.8 Hz, 1H, $C8_{tetrahydrocarbazole}H$), 7.21 (d, J = 8.0 Hz, 2H, $C2_{trifluoromethylphenyl}H$, $C2'_{trifluoromethylphenyl}H$), 7.46 (d, J = 8.2 Hz, 2H, $C3_{trifluoromethylphenyl}H$, $C3'_{trifluoromethylphenyl}H$).

^{13}C NMR (50 MHz, CD_3OD): δ = 21.0 ($C1_{tetrahydrocarbazole}$), 27.2 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2), 27.4 ($C4_{tetrahydrocarbazole}$), 28.4 ($N_{tetrahydrocarbazole}$–CH_2–CH_2), 31.0 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2), 31.9 ($C2_{tetrahydrocarbazole}$), 36.2 (O–CH_2–CH_2), 36.3 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2–CH_2), 43.9 ($N_{tetrahydrocarbazole}$–CH_2), 49.1 ($C3_{tetrahydrocarbazole}$), 71.4 (O–CH_2–CH_2), 102.5 ($C5_{tetrahydrocarbazole}$), 105.2 ($C4a_{tetrahydrocarbazole}$), 111.0 ($C8_{tetrahydrocarbazole}$), 112.5 ($C7_{tetrahydrocarbazole}$), 116.2 ($C3_{phenol}$, $C3'_{phenol}$), 125.7–126.8 (m, $C3_{trifluoromethylphenyl}$, $C3'_{trifluoromethylphenyl}$), 128.3 ($C8a_{tetrahydrocarbazole}$), 128.6–128.7 (m, CF_3), 129.3 ($C4_{trifluoromethylphenyl}$), 130.0 ($C2_{trifluoromethylphenyl}$, $C2'_{trifluoromethylphenyl}$), 130.8 ($C5a_{tetrahydrocarbazole}$), 131.0 ($C2_{phenol}$, $C2'_{phenol}$), 133.7 ($C1_{phenol}$), 135.2 ($C1a_{tetrahydrocarbazole}$), 148.3 ($C1_{trifluoromethylphenyl}$), 154.3 ($C6_{tetrahydrocarbazole}$), 156.9 ($C4_{phenol}$).

HRMS: $[M+H-HCl]^+$ $[C_{32}H_{36}N_2O_2F_3]^+$, calcd: 537.27234, found: 537.27201.

4.5.5. 4-(2-(3-Amino-9-(5-(pyridine-2-yl)pentyl)-2,3,4,9-tetrahydro-1*H*-tetrahydrocarbazole-6-yloxy)ethyl) phenol dihydrochloride 23



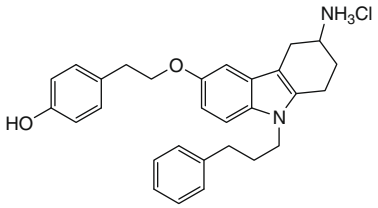
Reaction time:	3 h (coupling), 1 h (deprotection)
Yield:	80 mg (0.15 mmol, 68%)
Mp:	125–128 °C

^{1}H NMR (400 MHz, CD_3OD): δ = 1.29–1.37 (m, 2H, $N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2), 1.70–1.79 (m, 4H, $N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2), 1.98–2.09 (m, 1H, $C2_{tetrahydrocarbazole}H$), 2.28–2.36 (m, 1H, $C2'_{tetrahydrocarbazole}H$), 2.69–2.75 (m, 1H, $C4_{tetrahydrocarbazole}H$), 2.84–2.92 (m, 2H, $C1_{tetrahydrocarbazole}H$, $C4'_{tetrahydrocarbazole}H$), 2.93–2.99 (m, 4H, $N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2, O–CH_2–CH_2), 3.12–3.17 (m, 1H, $C1'_{tetrahydrocarbazole}H$), 3.56–3.66 (m, 1H, $C3_{tetrahydrocarbazole}H$), 3.98–4.04 (m, 2H, $N_{tetrahydrocarbazole}$–CH_2), 4.12 (t, ^3J = 7.2 Hz, 2H, O–CH_2–CH_2), 6.71–6.75 (m, 3H, $C3_{phenol}H$, $C3'_{phenol}H$, $C7_{tetrahydrocarbazole}H$), 6.86 (d, J = 2.4 Hz, 1H, $C5_{tetrahydrocarbazole}H$), 7.11 (d, ^3J = 8.0 Hz, 2H, $C2_{phenol}H$, $C2'_{phenol}H$), 7.16 (d, J = 8.4 Hz, 1H, $C8_{tetrahydrocarbazole}H$), 7.75–7.82 (m, 2H, $C3_{pyridine}H$, $C5_{pyridine}H$), 8.38 (td, ^3J = 8.0 Hz, ^4J = 1.2 Hz, 1H, $C4_{pyridine}H$), 8.58 (d, ^3J = 8.0 Hz, 1H, $C6_{pyridine}H$).

^{13}C NMR (100 MHz, CD_3OD): δ = 21.1 ($C1_{tetrahydrocarbazole}$), 27.2 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2), 27.2 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2), 28.4 ($C4_{tetrahydrocarbazole}$), 29.7 ($N_{tetrahydrocarbazole}$–CH_2–CH_2), 30.8 ($C2_{tetrahydrocarbazole}$), 34.1 (O–CH_2–CH_2), 36.2 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2–CH_2–CH_2), 43.7 ($N_{tetrahydrocarbazole}$–CH_2), 49.2 ($C3_{tetrahydrocarbazole}$), 71.3 (O–CH_2–CH_2), 102.4 ($C5_{tetrahydrocarbazole}$), 105.4 ($C4a_{tetrahydrocarbazole}$), 111.0 ($C8_{tetrahydrocarbazole}$), 112.5 ($C7_{tetrahydrocarbazole}$), 116.2 ($C3_{phenol}$, $C3'_{phenol}$), 125.9 ($C5_{pyridine}$), 128.3 ($C3_{pyridine}$), 128.4 ($C8a_{tetrahydrocarbazole}$), 130.9 ($C5a_{tetrahydrocarbazole}$), 131.0 ($C2_{phenol}$, $C2'_{phenol}$), 133.6 ($C1_{phenol}$), 135.3 ($C1a_{tetrahydrocarbazole}$), 142.0 ($C4_{pyridine}$), 147.9 ($C6_{pyridine}$), 154.3 ($C6_{tetrahydrocarbazole}$), 156.9 ($C4_{phenol}$), 158.5 ($C2_{pyridine}$).

HRMS: $[M+H-2HCl]^+$ $[C_{30}H_{35}N_3O_2]^+$, calcd: 470.28020, found: 470.27981.

4.5.6. 4-(2-(3-Amino-9-(3-phenylpropyl)-2,3,4,9-tetrahydro-1*H*-tetrahydrocarbazole-6-yloxy)ethyl)phenol hydrochloride 24



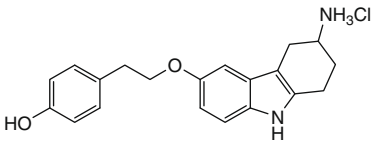
Reaction time:	2 h (coupling), 1 h (deprotection)
Yield:	73 mg, (0.15 mmol, 71%)
Mp:	92–94 °C

^{1}H NMR (200 MHz, CD_3OD): δ = 1.88–2.04 (m, 3H, $N_{tetrahydrocarbazole}$–CH_2–CH_2, $C2_{tetrahydrocarbazole}H$), 2.22–2.37 (m, 1H, $C2'_{tetrahydrocarbazole}H$), 2.59 (t, ^3J = 7.6 Hz, 2H, $N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2), 2.68–2.80 (m, 3H, $C4_{tetrahydrocarbazole}H$, $C4'_{tetrahydrocarbazole}H$, $C1_{tetrahydrocarbazole}H$), 2.94 (t, ^3J = 7.0 Hz, 2H, O–CH_2–CH_2), 3.02–3.18 (m, 1H, $C1'_{tetrahydrocarbazole}H$), 3.52–3.62 (m, 1H, $C3_{tetrahydrocarbazole}H$), 3.98 (t, ^3J = 7.2 Hz, 2H, $N_{tetrahydrocarbazole}$–CH_2), 4.10 (t, ^3J = 7.0 Hz, 2H, O–CH_2–CH_2), 6.70–6.75 (m, 3H, $C3_{phenol}H$, $C3'_{phenol}H$, $C7_{tetrahydrocarbazole}H$), 6.86 (s, 1H, $C5_{tetrahydrocarbazole}H$), 7.05–7.27 (m, 8H, $C2_{phenol}H$, $C2'_{phenol}H$, $C8_{tetrahydrocarbazole}H$, 5 × $C_{phenyl}H$).

^{13}C NMR (100 MHz, CD_3OD): δ = 20.9 ($C1_{tetrahydrocarbazole}$), 27.2 ($C4_{tetrahydrocarbazole}$), 28.3 ($N_{tetrahydrocarbazole}$–CH_2–CH_2), 32.8 ($C2_{tetrahydrocarbazole}$), 33.9 ($N_{tetrahydrocarbazole}$–CH_2–CH_2–CH_2), 36.2 (O–CH_2–CH_2), 43.4 ($N_{tetrahydrocarbazole}$–CH_2), 49.2 ($C3_{tetrahydrocarbazole}$), 71.3 (O–CH_2–CH_2), 102.5 ($C5_{tetrahydrocarbazole}$), 105.2 ($C4a_{tetrahydrocarbazole}$), 110.8 ($C8_{tetrahydrocarbazole}$), 112.5 ($C7_{tetrahydrocarbazole}$), 116.2 ($C3_{phenol}$, $C3'_{phenol}$), 127.0 ($C8a_{tetrahydrocarbazole}$), 128.3 ($C4_{phenyl}$), 129.4 ($C2_{phenyl}$, $C2'_{phenyl}$), 129.4 ($C3_{phenyl}$, $C3'_{phenyl}$), 130.8 ($C5a_{tetrahydrocarbazole}$), 130.9 ($C2_{phenol}$, $C2'_{phenol}$), 133.6 ($C1_{phenol}$), 135.1 ($C1a_{tetrahydrocarbazole}$), 142.6 ($C1_{phenyl}$), 154.3 ($C6_{tetrahydrocarbazole}$), 156.9 ($C4_{phenol}$).

HRMS: $[M+H-HCl]^+$ $[C_{29}H_{33}N_2O_2]^+$, calcd: 441.25365, found: 441.25346

4.5.7. 4-(2-(3-Amino-2,3,4,9-tetrahydro-1*H*-tetrahydrocarbazole-6-yloxy)ethyl)phenol hydrochloride 25



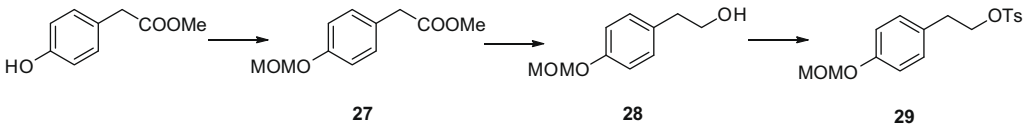
A solution of *tert*-butyl-6-(4-(methoxymethoxy)phenethoxy)-2,3,4,9-tetrahydro-1*H*-tetrahydrocarbazole-3-yl carbamate (47.0 mg, 0.10 mmol) in HCl (0.8 M in MeOH, 0.1 mL) was heated to 50 °C for 2 h (TLC-control). The solvent was removed under reduced pressure to afford pure **25** (32.0 mg, 0.09 mmol, 90%) as a yellow solid.

Mp: 249 °C (decomposition).

^{1}H NMR (200 MHz, CD_3OD): δ = 1.95–2.13 (m, 1H, $C2_{tetrahydrocarbazole}H$), 2.20–2.32 (m, 1H, $C2'_{tetrahydrocarbazole}H$), 2.64–2.76 (m, 1H, $C4_{tetrahydrocarbazole}H$), 2.87–2.98 (m, 4H, $C1_{tetrahydrocarbazole}H$, $C4'_{tetrahydrocarbazole}H$, O–$CH_2$–$CH_2$), 3.07–3.18 (m, 1H, $C1'_{tetrahydrocarbazole}H$), 3.55–3.70 (m, 1H, $C3_{tetrahydrocarbazole}H$), 4.10 (t, ^3J = 7.0 Hz, 2H, O–CH_2–CH_2), 6.68–6.75 (m, 3H, $C3_{phenol}H$, $C3'_{phenol}H$, $C7_{tetrahydrocarbazole}H$), 6.85 (d, J = 2.6 Hz, 1H, $C5_{tetrahydrocarbazole}H$), 7.08–7.16 (m, 3H, $C8_{tetrahydrocarbazole}H$, $C2_{phenol}H$, $C2'_{phenol}H$).

^{13}C NMR (100 MHz, CD_3OD): δ = 21.1 ($C1_{tetrahydrocarbazole}$), 26.7 ($C4_{tetrahydrocarbazole}$), 27.8 ($C2_{tetrahydrocarbazole}$), 35.7 (O–CH_2–CH_2), 48.8 ($C3_{tetrahydrocarbazole}$), 70.9 (O–CH_2–CH_2), 104.9 ($C5_{tetrahydrocarbazole}$), 111.7 ($C4a_{tetrahydrocarbazole}$), 111.8 ($C8_{tetrahydrocarbazole}$), 112.0 ($C7_{tetrahydrocarbazole}$), 115.7 ($C3_{phenol}$, $C3'_{phenol}$), 128.2 ($C8a_{tetrahydrocarbazole}$), 130.4 ($C1_{phenol}$), 130.5 ($C2_{phenol}$, $C2'_{phenol}$), 133.0 ($C5a_{tetrahydrocarbazole}$), 133.9 ($C1a_{tetrahydrocarbazole}$), 153.6 ($C6_{tetrahydrocarbazole}$), 156.4 ($C4_{phenol}$).

HRMS: $[M+H-HCl]^+$ $[C_{20}H_{23}N_2O_2]^+$, calcd: 323.17540, found: 323.17521.



27 28 29

4.5.8. Methyl-2-(4-methoxymethoxy)phenyl acetate 27

A stirred solution of methyl-2-(4-hydroxyphenyl) acetate (10.0 g, 60.2 mmol) in CH_2Cl_2 (100 mL) was cooled to 0 °C and diisopropyl amine (15.6 g, 120.0 mmol) was added over 5 min. Subsequently, bromo(methoxy) methane (9.02 g, 72.2 mmol) was added dropwise and stirring was continued for 12 h at room temperature (TLC-control). The reaction mixture was washed with water (50 mL) and dried (Na_2SO_4). All volatiles were removed under reduced pressure and the crude product was purified by chromatography (*n*-hexane/EtOAc 4:1, v:v) to yield pure **27** (8.29 g, 39.4 mmol, 66%) as a colourless oil.

R_f: 0.37 (*n*-hexane/EtOAc, 4:1, v:v).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.51 (s, 3H, CH_2–O–CH_3), 3.61 (s, 2H, CH_2–$COOCH_3$), 3.72 (s, 3H, $COOCH_3$), 5.19 (s, 2H, O–CH_2–O), 7.03 (d, ^3J = 8.8 Hz, 2H, C3–*H*, C3′–*H*), 7.27 (d, ^3J = 8.8 Hz, 2H, C2–*H*, C2′–*H*).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 40.5 (CH_2–$COOCH_3$), 52.1 ($COOCH_3$), 56.1 (CH_2–O–CH_3), 94.6 (O–CH_2–O), 116.5 (2C, *C*3, *C*3′), 127.5 (*C*1), 130.4 (2C, *C*2, *C*2′), 156.5 (*C*4), 172.4 ($COOCH_3$).

HRMS: [M+Na]$^+$ [$C_{11}H_{14}O_4Na$]$^+$, calcd: 233.07843, found: 233.07833.

4.5.9. 2-[4-(Methoxymethoxy)phenyl] ethanol 28

A stirred solution of **27** (8.23 g, 39.1 mmol) in THF (100 mL) was cooled to 0 °C and lithium aluminium hydride (5.20 g, 137.0 mmol) was added in portions over a period of 30 min. After stirring for 15 h at room temperature (TLC-control) the reaction mixture was again cooled to 0 °C and aqueous NaOH-solution (2 M, 100 mL) was added carefully. The phases were separated, the aqueous phase was extracted with EtOAc (2 × 60 mL) and the combined organic phases were dried (Na_2SO_4). All volatiles were removed under reduced pressure and the crude product was purified by chromatography (*n*-hexane/EtOAc 1:1, v:v) to yield pure **28** (6.20 g, 34.1 mmol, 87%) as a colourless oil.

R_f: 0.41 (*n*-hexane/EtOAc, 1:1, v/v).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.55 (br, 1H, OH), 2.81 (t, ^3J = 6.6 Hz, 2H, C1–CH_2), 3.47 (s, 3H, O–CH_3), 3.82 (t, ^3J = 6.6 Hz, 2H, C1–CH_2–CH_2), 5.15 (s, 2H, O–CH_2–O), 6.99 (d, ^3J = 8.5 Hz, 2H, C3–*H*, C3′–*H*), 7.15 (d, ^3J = 8.5 Hz, 2H, C2–*H*, C2′–*H*).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 38.5 (C1–CH_2), 56.1 (O–CH_3), 63.9 (C1–CH_2–CH_2), 94.7 (O–CH_2–O), 116.6 (2C, *C*3, *C*3′), 130.1 (2C, *C*2, *C*2′), 131.9 (*C*1), 156.0 (*C*4).

HRMS: [M+Na]$^+$ [$C_{10}H_{14}O_3Na$]$^+$, calcd: 205.08352, found: 205.08346.

4.5.10. 4-(Methoxymethoxy)phenylethyl-4-methylbenzene sulfonate 29

A stirred solution of **28** (6.15 g, 33.8 mmol) in CH_2Cl_2 (350 mL) was cooled to 0 °C and triethyl amine (5.12 g, 50.6 mmol), *p*-tolylsulfonyl chloride (9.01 g, 47.3 mmol) and 4-*N*,*N*-dimethylamino pyridine (250 mg, 0.21 mmol) were added successively. After stirring for 22 h at room temperature the reaction mixture was washed with water (200 mL), aqueous NH_4Cl-solution (half-saturated, 200 mL) and brine (saturated, 200 mL). The combined aqueous extracts were reextracted with CH_2Cl_2 (200 mL), the combined organic phases were dried (Na_2SO_4) and all volatiles were removed under reduced pressure. The crude product was purified by chromatography (*n*-hexane/EtOAc, 3:1, v/v) to yield pure **29** (10.3 g, 30.6 mmol, 91%) as a colourless oil.

R_f: 0.63 (*n*-hexane/EtOAc, 3:1, v/v).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.43 (s, 3H, $C4_{tosyl}$–CH_3), 2.89 (t, ^3J = 7.2 Hz, 2H, $C1_{phenyl}$–CH_2), 3.47 (s, 3H, O–CH_3), 4.16 (t, ^3J = 7.2 Hz, 2H, $C1_{phenyl}$–CH_2–CH_2), 5.14 (s, 2H, O–CH_2–O), 6.92 (d, ^3J = 8.9 Hz, $C3_{phenyl}H$, $C3'_{phenyl}H$), 7.02 (d, ^3J = 8.9 Hz, $C2_{phenyl}H$, $C2'_{phenyl}H$), 7.29 (d, ^3J = 8.3 Hz, $C3_{tosyl}H$, $C3'_{tosyl}H$), 7.70 (d, ^3J = 8.9 Hz, $C2_{tosyl}H$, $C2'_{tosyl}H$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.8 ($C1_{tosyl}$–CH_3), 34.7 ($C1_{phenyl}$–CH_2), 56.1 (O–CH_3), 70.9 ($C1_{phenyl}$–CH_2–CH_2), 94.6 (O–CH_2–O), 116.6 (2C, $C3_{phenyl}$, $C3'_{phenyl}$), 128.0 (2C, $C2_{phenyl}$, $C2'_{phenyl}$), 129.7 ($C1_{phenyl}$), 129.9 (2C, $C2_{tosyl}$, $C2'_{tosyl}$), 130.1 (2C, $C3_{tosyl}$, $C3'_{tosyl}$), 133.2 ($C1_{tosyl}$), 144.8 ($C4_{tosyl}$), 156.3 ($C4_{phenyl}$).

HRMS: [M+Na]$^+$ [$C_{17}H_{20}O_5SNa$]$^+$, calcd: 359.09237, found: 359.09255.

4.6. Biology

4.6.1. RTK-ELISA-assay

The following solutions were prepared for the RTK assay:

(1) PBS (10×): 1.0 g KCl, 1.0 g KH_2PO_4, 40.0 g NaCl, 13.2 g Na_2HPO_4 (for 1.0 L)
(2) PBST: (PBS 10× + 0.5% Tween 20) diluted 1:10
(3) substrate: stock solution:poly-Glu-Tyr 4:1, 10.0 mg/mL in PBS, diluted with PBS to 100 μg/mL
(4) kinase buffer: 100 mM HEPES, 100 mM NaCl, 0.10 mM Na_3VO_4
(5) kinase solutions: kinase buffer, kinase stock solution (fusion proteins, N-terminally fused to GST, Biomol GmbH) diluted to reach the concentration as defined in Table 5.
(6) ATP solution: 100 μM ATP (200 μM and 1000 μM for checking dependence on ATP-concentration) in 40.0 mM $MnCl_2$; a solution of 40.0 mM $MnCl_2$ as negative control.
(7) inhibitor solutions: defined concentrations in 5% aqueous DMSO; 5% aqueous DMSO as positive control and negative control.
(8) antibody solution: PBST + 0.2% BSA, anti-phosphotyrosine-antibody, peroxidase coupled (PY20 Calbiochem) in PBST diluted.
(9) luminescence reagent: BM chemiluminescence ELISA substrate (Roche Diagnostics GmbH) solution A (luminol and 4-iodophenol) and B stabilized H_2O_2) 100:1, mixed 15 min before use.

A 96-well microtiter plate (Greiner bio-one, Lumitrac 600, flat bottom white) was incubated with substrate solution (100 μL/well) overnight at 4 °C. The solution was removed and the plate washed with PBST (2 × 2 min). Afterwards the kinase solution (50.0 μL/well) and the inhibitor solution (25.0 μL/well) were

Table 5
Preparation of kinase solution

Kinase stock solution (ng/μL)	Concentration per well (ng)	Amount in 5 mL (μL)
VEGFR-2: 145	5.0	3.44
VEGFR-3: 149	20.0	13.42
IGF-IR: 94.0	15.0	15.96
EGFR: 108	5.0	4.63

added. As positive and as negative control 5% aqueous DMSO (25.0 μL/well) was used instead of inhibitor solution. Addition of the ATP solution (25.0 μL/well) started the reaction, whereas a 40 mM $MnCl_2$ solution (25.0 μL/well) was added instead of ATP solution. The plate was incubated for 30 min on a microplate shaker, followed by washing steps with PBST (3 × 2 min). Subsequently, antibody solution (100 μL/well) was added and the plate was incubated again for 1 h on a microplate shaker. Finally the plate was washed with PBST (3 × 5 min) and the chemiluminescence substrate (50.0 μL/well) was added. After 3 min the emitted light was measured using a luminometer (Orion Microplate Luminometer, Berthold).

4.6.2. Cell proliferation and toxicity

Cell proliferation was measured using the Cell Proliferation ELISA, BrdU Assay Kit (Cat. No. 11669915001) from Roche. The assay was performed according to the kit instruction.

In brief, HUVECs (PromoCell, HUVEC-c C-12200) were cultured in 25 cm^2 flasks at 37 °C in a humid atmosphere with 5% CO_2. MCF-7 cells, HEPM cells and HT29 cells were obtained from ATCC and cultured in 75 cm^2 flasks at 37 °C in a humid atmosphere with 5% CO_2. To perform the assay, 5000 cells were seeded into each well of a 96-well microplate (greiner bio-one, flat bottom white, μclear) and cultured in a final volume of 100 μL/well. After 24 h, the medium was replaced and the cells were incubated with 100 μL/well of the compounds for 24 h, whereas the DMSO concentration was lower than 0.5% (v/v).

Still containing the compounds, the cells were incubated with 5-bromo-2′-deoxyuridine (BrdU) labelling solution, leading to an incorporation of BrdU into only mitotic active cells for 4 h. After the removal of the solution, the cells were fixed and incubated with an anti-BrdU-POD solution. Finally, the cells were washed several times and incubated with the substrate solution. The bottom of the microplate was sealed with white cover foil to measure the chemiluminescence using an Orion Microplate Luminometer (Berthold).

For determination of IC_{50} values, the relative luminescence units per second (RLU/s) were blotted against the inhibitor concentrations.

Cytotoxicity was measured using the Cell Proliferation Reagent WST-1 (Cat. No. 13396300) from Roche. The assay was performed according to the instructions. The cells were cultured as described above.

Seven thousand and five hundred cells per well were exposed to different concentrations of the inhibitors for 24 h before adding the WST-1 reagent. After 4 h and again after 24 h the absorbance was measured using Optimax UV–vis spectrometer (wavelengths: 440 nm and 650 nm). For determination of toxicity the differences in absorbance (650 nm–440 nM) were blotted against inhibitor concentration.

Acknowledgement

We gratefully acknowledge VW-Foundation for financial support.

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