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Original article

Synthesis, characterization and *in vitro* pharmacology of novel pregabalin derivatives

Štefica Horvat [a], Zdenko Hameršak [a,*], Irena Stipetić [a], Thierry Jolas [b]

[a] Department of Organic Chemistry and Biochemistry, Rudjer Bošković Institute, P.O. Box 180, 10002 Zagreb, Croatia
[b] Cerep, Le Bois l'Evêque, B.P. 30001, Celle l'Evescault 86600, France

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ABSTRACT

A series of novel pregabalin derivatives were synthesized starting from N-protected pregabalin, different amino sugars, adamantylamine, serotonin and tryptamine. New compounds were spectroscopically characterized and *in vitro* tested on gabapentin receptor binding assay. The serotonin-pregabalin adduct showed significant binding effect and its IC_{50} value was determined.

1. Introduction

Pregabalin (PGB; *S*-(+)-3-isobutylgaba) and gabapentin (GBP) are efficacious drugs in the treatment of epilepsy, neuropathic pain, and anxiety states [1,2]. Both PGB and GBP are considered to bind to the $\alpha_2\delta$ subunit of voltage-gated Ca^{2+} channels (VGCC) with nanomolar affinity. This interaction appears to diminish the amount of Ca^{2+} entering the presynaptic terminal following depolarization, leading to a decrease in neurotransmitter release [3]. Previous studies on structure–activity relationships of pregabalin focused on the incorporation of substituents at positions along the hexanoic acid backbone [4] or to synthesis of the carboxylate bioisostere analogs in which carboxyl group has been replaced by tetrazole moieties [5].

The goal of this study was to design and generate novel pregabalin analogs and to evaluate their binding affinity for the $\alpha_2\delta$ subunit of voltage-gated Ca^{2+} channels. We thereby aim to optimize the binding affinity of these analogs through structure–activity relationships focusing mainly on C-terminal modifications of PGB. Our manipulations involved amidation of PGB with different amino sugars (2-amino-2-deoxy-D-glucopyranose, 2-amino-2-deoxy-D-galactopyranose, 2-amino-2-deoxy-D-mannopyranose, β-D-glucopyranosylamine), with bioactive antiviral agent 1-adamantylamine (Symmetrel), or with neuromodulators such as tryptamine or serotonin (Scheme 1).

Among the different strategies developed for improving properties of different drugs, the "sugar approach" has recently emerged as a new attractive and versatile tool. [for review see Ref. [6]]. Due to the poly-hydroxylated nature of sugars and the large array of sugars that are available, attachment of sugar to drugs can (i) utilize active transport systems, (ii) modify the physico-chemical properties of the construct, and (iii) target the compound. The clinical use of a highly active carbohydrate-based carbonic anhydrase inhibitor, i.e., topiramate, constitutes an interesting demonstration of the validity of this approach [7]. The carbohydrate moieties can also serve as recognition sites for carbohydrate-binding proteins – lectins. Studies with sugar modified catanionic vesicles demonstrated the ability of this method to control the glycoconjugate concentration in the membrane and to explore relationship between ligand separation distance and multivalent lectin binding at the bilayer interface [8]. In addition, glycopolymers could be used to control interspecies transmission and epidemic in specific host. Thus, *in vitro* and *in vivo* infection experiments on influenza viruses using glycopolymers that were carrying terminal 2,6-sialic acid residues on lactosamine repeats demonstrated marked differences in inhibitory activity against different species of viruses [9].

The rationale to conjugate 1-adamantylamine to PGB originates from our previous results that modifications of small bioactive compounds with hydrophobic adamantane moiety may be of particular benefit for passive cellular absorption by membrane penetration or attachment [10,11]. Multivalent ligands can function as inhibitors or effectors of biological processes [12,13]. We envisioned that, by amidation of PGB with tryptamine or serotonin,

* Corresponding author. Tel.: +385 1 4571 300; fax: +3851 4680108.
E-mail address: hamer@irb.hr (Z. Hameršak).

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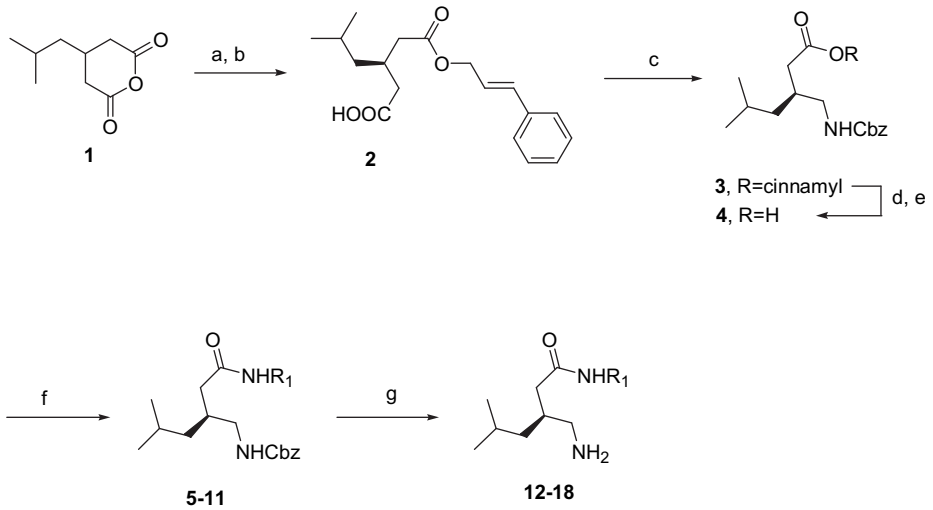
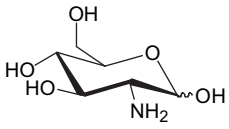
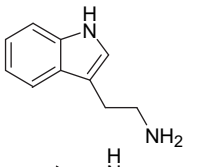
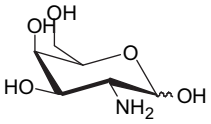
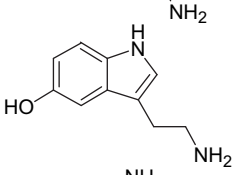
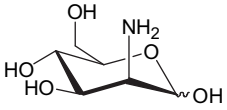
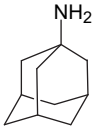
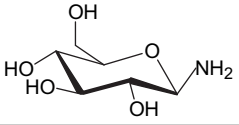


Compound	R_1NH_2	Compound	R_1NH_2
5, 12		**9, 16**	
6, 13		**10, 17**	
7, 14		**11, 18**	
8, 15			

Scheme 1. Synthesis of pregabalin derivatives **12**–**18**. Reactions and conditions: (a) quinine, toluene, cinnamyl alc. −30 °C, 24 h; (b) resol. *S*-phenylethylamine; (c) $(PhO)_2PON_3$, Et_3N, benzyl alc.; (d) $Pd(OAc)_2$, PPh_3, morpholine, EtOH, reflux, 3 h; (e) (1) 1-adamantylamine, MTBE/2-PrOH (2) HCl; (f) (1) *N*-ethylmorpholine, $ClCO_2iBu$, THF, −15 °C, 2 min; (2) RNH_2, room temperature, 24 h; (g) H_2, Pd/C, EtOH/H_2O/HCl, room temperature, 2 h.

potent bioactivity can arise from the high functional affinities of multivalent ligand–receptor interactions.

2. Results and discussion

2.1. Chemistry

Pregabalin derivatives **12**–**18** were synthesized in two steps from N-protected pregabalin (Z-PGB, **4**) (Scheme 1). Z-PGB **4** of high enantiomeric purity (>98% ee) was obtained by a modification of procedure we have already reported earlier [14]. Instead of cinnamyl alcohol, benzyl alcohol was used in the Curtius rearrangement. Cinnamyl protection of **3** was cleaved by palladium acetate-triphenyl phosphine, affording amino-protected acid **4**, which was purified *via* its adamantylamine salt. The coupling of **4** with the corresponding amine was achieved by using mixed anhydride method in the presence of isobutyl chloroformate to yield N-terminally protected compounds **5**–**11**. Hydrogenation of **5**–**11** and subsequent purification by RP HPLC gave the desired pregabalin amides **12**–**18**.

The proposed structures of compounds **12**–**18** were confirmed by NMR spectroscopy (Tables 1–3). ^1H and ^{13}C resonances were

Table 1
Chemical shifts of anomeric forms (C-1, H-1) and tautomeric composition of carbohydrate–pregabalin derivatives **12**–**15** in DMSO-d_6 solution estimated from the NMR data.

Compound	Parent amino sugar[a]	Tautomeric composition	DMSO-d_6		
			$\delta_{C\text{-}1}$ (ppm)	$\delta_{H\text{-}1}$ (ppm)	%
12	2-GlcN	α-pyranose	90.61	4.93	70
		β-pyranose	95.34	4.45	30
13	2-GalN	α-pyranose	90.97	4.94	38
		β-pyranose	95.82	4.40	26
		α-furanose	93.79	5.06	6
		β-furanose	99.99	4.95	8
	2-GlcN	α-pyranose	90.56	4.93	13
		β-pyranose	95.35	4.45	9
14	2-ManN	α-pyranose	92.59	4.85	86
		β-pyranose	93.19	4.71	14
15	1-GlcN	β-pyranose	79.48	4.71	100

[a] 2-GlcN, 2-amino-2-deoxy-D-glucose; 2-GalN, 2-amino-2-deoxy-D-galactose; 2-ManN, 2-amino-2-deoxy-D-mannose; 1-GlcN, β-D-glucopyranosylamine.

Table 2
NMR chemical shift data (ppm) of the major tautomer in DMSO-d_6 solution of glycoconjugates **12**–**15** derived from pregabalin.

Residue	Atom	**12** (α-pyranose)		**13** (α-pyranose)		**14** (α-pyranose)		**15** (β-pyranose)	
		δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Sugar[a]	1	4.93	90.61	4.94	90.97	4.85	92.59	4.71	79.48
	2	3.62	54.23	4.01	50.00	3.97	53.86	3.05	72.38
	3	3.51	70.48	3.65	67.31	3.77	68.21	3.18	77.48
	4	3.13	71.15	3.72	68.18	3.41	67.45	3.07	70.00
	5	3.60	72.08	3.80	70.43	3.58	72.89	3.09	78.51
	6	3.48/3.63	61.07	3.41/3.54	60.55	3.50/3.62	61.51	3.41/3.62	60.95
	NH	7.87	–	7.79	–	7.65	–	8.47	–
Pregabalin	1	–	171.39	–	171.57	–	171.75	–	171.98
	2	2.22	37.79	2.22	37.85	2.30	37.51	2.11/2.19	38.23
	3	2.06	31.63	2.07	31.68	2.05	31.64	1.97	33.01
	4	1.16	40.58	1.17	40.53	1.17	40.48	1.08/1.16	40.85
	5	1.64	24.38	1.64	24.39	1.63	24.39	1.61	24.53
	6/6′	0.84/0.86	22.15/22.75	0.82/0.88	22.74/22.80	0.86/0.87	22.29/22.80	0.84/0.85	22.59/22.68
	7	2.78	42.77	2.78	42.60	2.77	42.59	2.62	43.47

[a] 2-amino-2-deoxy-D-glucose for **12**; 2-amino-2-deoxy-D-galactose for **13**; 2-amino-2-deoxy-D-mannose for **14**; D-glucopyranosylamine for **15**.

assigned by homonuclear COSY experiments combined with ^1H–^{13}C correlation techniques through one bond (HMQC) and multiple bonds (HMBC). The population of isomers in equilibrated DMSO-d_6 solutions of amino sugar-derived pregabalin compounds **12**–**15** was estimated by integration of the signal intensities of the anomeric carbon atom (C-1) in the 80–96 ppm region of the ^{13}C NMR spectra (Table 1).

The NMR spectra of 2-GlcN and 2-ManN derivatives **12** and **14** showed two sets of sugar resonances attributable to the sugar moiety in its α- and β-pyranose forms. The α-pyranose forms were the major tautomers. NMR analysis of compound **13** provided evidence that amidation of pregabalin with 2-amino-2-deoxy-D-galactose resulted in four sets of signals consistent with the presence of *galacto*-sugar residue in α- and β-pyranose as well as α- and β-furanose forms in the solution. The NMR spectra of **13** also revealed the presence of α- and β-pyranose forms of compound **12** (22%) in solution (Table 1), most probably formed by epimerization of 2-amino-2-deoxy-D-galactose under applied reaction conditions. As can be seen from the data in Table 1, the glucosylamine unit of **15** resided only in β-pyranose form.

The ^1H and ^{13}C chemical shifts data for compounds **12**–**18** are summarized in Tables 2 and 3.

2.2. Pharmacology

Pregabalin analogs **12**–**18** were screened for their ability to displace [^{3}H]gabapentin from $\alpha_2\delta$ subunit-containing calcium channels in cell membrane homogenates from rat cerebral cortex. Although C-terminally modified pregabalin analogs in which the carboxylate has been replaced by tetrazole moiety showed binding to $\alpha_2\delta$ protein [5], contrary to our expectation that conjugation with amino sugar moieties will improve the targeting of pregabalin molecule towards cell membrane, carbohydrate–pregabalin conjugates **12**–**15** did not express ability to compete with [^{3}H]gabapentin for binding to the $\alpha_2\delta$ subunit of voltage-dependent Ca^{2+} channels. From the compounds **12**–**18** tested at 10 μM, and besides pregabalin, only derivative **17** with a serotonin (5-hydroxytryptamine) moiety showed significant inhibition of specific [^{3}H]gabapentin binding (Table 4). Noteworthy is that derivative **16** with a tryptamine moiety showed minor inhibition at

Table 3
NMR chemical shift data (ppm) of the pregabalin conjugates **16**–**18** in DMSO-d_6 solution.

Residue	Atom	**16**		**17**		**18**	
		δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
R-NH[a]	1-NH	10.86	–	10.50	–	7.56	–
	2	7.15	122.61	7.03	123.03	–	50.89
	3	–	111.68	–	110.67	1.92	40.88
	3′	2.82	25.17	2.75	25.30		
	3″	3.35	39.46	3.29	39.37		
	4	7.52	118.18[b]	6.81	102.15	2.00	28.74
	5	6.97	118.16[b]	–	150.16	1.61	35.99
	6	7.06	120.87	6.59	111.26		
	7	7.34	111.37	7.12	111.64		
	8	–	136.24	–	130.80		
	9	–	127.16	–	127.82		
	3″-NH	8.24	–	8.19	–		
Pregabalin	1	–	171.16	–	171.07	–	170.63
	2	2.13/2.23	37.94	2.19	37.04	2.11/2.18	38.43
	3	2.05	31.97	2.07	31.45	2.02	31.61
	4	1.08/1.16	40.60	1.13	40.55	1.12	40.44
	5	1.62	24.46	1.63	24.43	1.63	24.38
	6/6′	0.82/0.84	22.32/22.69	0.85/0.86	22.23/22.80	0.84/0.86	22.22/22.78
	7	2.71	42.87	2.75	42.70	2.75	42.72
	NH_2	NO	–	7.89	–	7.95	–

[a] Tryptamine for **16**; serotonin for **17**; 1-adamantylamine for **18**.
[b] Assignments of signals can be interchangeable. NO = not observed.

Table 4
Inhibition of [^{3}H]gabapentin binding in cell membrane homogenates from rat cerebral cortex. Each inhibition represents the mean of duplicate determinations.

Compound	Test concentration (μM)	% Inhibition of control specific [^{3}H]gabapentin binding[a]
Pregabalin	10	**92**
12	10	18
13	10	9
14	10	14
15	10	17
16	10	18
17	10	**51**
18	10	3

[a] Compounds showing significant inhibition are indicated in bold.

the same testing concentration indicating that the presence of an hydroxyl on the tryptamine's indole group improved binding of the pregabalin analog. IC_{50} values obtained for derivative **17** and gabapentin were respectively 13 μM and 43 nM and K_i values 11 μM and 35 nM (Fig. 1).

3. Conclusion

Seven new pregabalin derivatives were synthesized and spectroscopically characterized. Gabapentin receptor binding assay study showed the one among them, the serotonin-pregabalin adduct **17**, possesses a promising activity (K_i = 11 μM). Further manipulation of the serotonin moiety may provide analogs with higher affinity for this target. In addition, characterization of the activity of the serotonin-pregabalin adduct at serotonergic targets may provide interesting information about the therapeutic interest of such a compound.

4. Experimental

4.1. Chemistry

Melting points were determined on a Tottoli (Büchi) apparatus and are uncorrected. Optical rotations were measured at 25 °C using an Optical Activity LTD automatic AA-10 polarimeter. NMR spectra were recorded on a Bruker AV 600 spectrometer, operating at 150.91 MHz for ^{13}C and 600.13 MHz for ^{1}H nuclei. The spectra were measured in DMSO-d_6 solutions at 25 °C. Chemical shifts in parts per million were referenced to TMS. Spectra were assigned

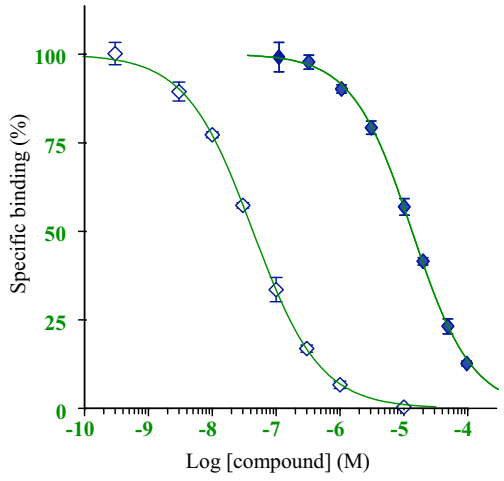


Fig. 1. Inhibition of [^{3}H]gabapentin binding in cell membrane homogenates from rat cerebral cortex. The graph shows curves for gabapentin (open diamond) and **17** (filled diamond). Each point represents the mean of duplicate determinations.

based on 2D homonuclear (COSY, NOESY) and heteronuclear (HMQC, HMBC) experiments. High resolution mass spectrometry (HRMS) was performed on 4800 *Plus* MALDI TOF/TOF™ Analyzer. Reversed-phase high-performance liquid chromatography (RP HPLC) was performed on a Varian Pro Star 230 HPLC system using a Eurospher 100 reversed-phase C-18 semipreparative (250 × 8 mm ID, 5 μm) (flow rate: 1.0 ml/min) or analytical (250 × 4 mm ID, 5 μm) (flow rate: 0.5 ml/min) column under isocratic conditions using different concentrations of MeOH in 0.1% aqueous trifluoroacetic acid (TFA). UV detection was performed at 215 nm using a Varian Pro Star 335 photodiode-array detector.

Enantiomeric purity of **4** was determined on Chiralpak AS using *n*-hexane/EtOH/TFA (100:5:0.1) at 206 nm.

4.2. Synthesis

4.2.1. (−)-(S)-3-(Benzyloxycarbonylamino-methyl)-5-methyl-hexanoic acid (*4*)

The solution of crude carbamate **3** [14] (10.2 g, 35 mmol, 90% ee) in abs. EtOH (50 ml) was refluxed for 10 min, and then cooled to 70 °C. Morpholine (5.1 ml, 70 mmol) was added followed by triphenylphosphine (80 mg, 0.3 mmol) and $Pd(OAc)_2$ (2 mg, 0.009 mmol). The reaction mixture was refluxed for 3 h. Most of the solvent was evaporated at reduced pressure, 200 ml of 3% Na_2CO_3 and 80 ml of EtOAc were added. The mixture was stirred for 15 min, aqueous layer was separated and washed again with EtOAc. Upon acidification to pH 1.5 with conc. HCl, extraction with CH_2Cl_2 afforded 7.2 g of oily benzyl-carbamate **4** which was dissolved in 130 ml of MTBE and 40 ml of 2-propanol and treated with 1-adamantylamine (3.4 g, 22 mmol). Crystals were collected on filter, dissolved in dichloromethane and the solution was treated with 2% HCl to liberate free acid. The organic solution was washed with H_2O and dried to obtain upon evaporation 5.6 g of pure **4** as yellowish oil (>98% ee).

$[\alpha]_D^{25}$ = −4.4 (*c* = 25, EtOH). ^{1}H NMR (DMSO-d_6), δ/ppm: 0.81–0.85 (m, 6H, $2CH_3$), 1.00–1.16 (m, 2H, CH–**CH_2**–CH), 1.55–1.66 (m, 1H, CH_3–CH–CH_3), 1.91–2.27 (m, 3H, HOOC–**CH_2**, CH_2–CH–CH_2), 2.84–3.09 (m, 2H, NH–**CH_2**), 5.01 (s, 2H, Ph-**CH_2**), 7.26–7.28 (m, 5H, Ar-H) 12.01 (brs, 1H, COOH).

^{13}C NMR (DMSO-d_6), δ/ppm: 22.9 (CH_3), 23.2 (CH_3), 25.1 (CH), 33.6 (CH), 37.6 (CH_2), 41.4 (CH_2), 44.3 (CH_2), 65.6 (CH_2), 128.1 (2 × Ar-CH), 128.2 (Ar-CH), 128.7 (2 × Ar-CH), 137.8 (Ar-C), 156.8 (NH–C=O), 174.4 (COOH). IR (film on KBr): 3340 (O=CO–H), 2956, 2929, 2871, 1708 (C=O), 1534, 1258 (CO–O), 697 cm^{-1}.

Anal. Calcd. for $C_{16}H_{23}NO_4$ (293.36): C, 65.51; H, 7.90; N, 4.77. Found: C, 65.70; H, 8.15; N, 4.41.

4.2.2. Synthesis of pregabalin derivatives *5*–*11*

To a chilled solution (−15 °C) of *N*-benzyloxycarbonyl-pregabalin (**4**) (147 mg, 0.5 mmol) in THF (4 ml), *N*-ethylmorpholine (NEM, 63 μl, 0.5 mmol) and isobutyl chloroformate (65 μl, 0.5 mmol) were added. The resulting mixture was stirred for 2 min at the same temperature and a precooled solution of the corresponding amine (0.5 mmol) in THF–water mixture (6:4, 10 ml) was then added. The reaction mixture was stirred for 10 min at −15 °C and then overnight at room temperature. The solvent was evaporated *in vacuo* and the residue was purified by semipreparative RP HPLC using different concentrations of MeOH/0.1% TFA.

Molecular structures of compounds **5**–**11** were confirmed by NMR spectroscopy (see Supplementary data).

Compound **5** was prepared from Z-PGB (**4**) and 2-amino-2-deoxy-D-glucopyranose hydrochloride. Purification with RP HPLC by using 62.75% MeOH/0.1% TFA afforded 132 mg (58%) of **5**. M.p. 122–125 °C; $[\alpha]_D^{25}$ = +30 (*c* = 1.0, MeOH).

Compound **6** was prepared from Z-PGB (**4**) and 2-amino-2-deoxy-D-galactopyranose hydrochloride. Purification with RP HPLC by using 62.75% MeOH/0.1% TFA afforded 136 mg (60%) of **6**. M.p. 103–105 °C; $[\alpha]_D^{25} = +34$ ($c = 1.0$, MeOH).

Compound **7** was prepared from Z-PGB (**4**) and 2-amino-2-deoxy-D-mannopyranose hydrochloride. Purification with RP HPLC by using 62.75% MeOH/0.1% TFA afforded 129 mg (57%) of **7**. M.p. 76–78 °C; $[\alpha]_D^{25} = -10$ ($c = 1.0$, MeOH).

Compound **8** was prepared from Z-PGB (**4**) and β-D-glucopyranosylamine. Purification with RP HPLC by using 62.75% MeOH/0.1% TFA afforded 107 mg (47%) of **8**. M.p. 83–85 °C; $[\alpha]_D^{25} = 0$ ($c = 1.0$, MeOH).

Compound **9** was prepared from Z-PGB (**4**) and tryptamine. Purification with RP HPLC by using 64.5% MeOH/0.1% TFA afforded 135 mg (62%) of **9**. Solid foam; $[\alpha]_D^{25} = 0$ ($c = 1.0$, MeOH).

Compound **10** was prepared from Z-PGB (**4**) and serotonin. Purification with RP HPLC by using 64.5% MeOH/0.1% TFA afforded 126 mg (56%) of **10**. Solid foam; $[\alpha]_D^{25} = 0$ ($c = 1.0$, MeOH).

Compound **11** was prepared from Z-PGB (**4**) and 1-adamantylamine. Purification with RP HPLC by using 71.5% MeOH/0.1% TFA afforded 115 mg (54%) of **11**. Solid foam; $[\alpha]_D^{25} = -1$ ($c = 1.0$, MeOH).

4.2.3. Synthesis of pregabalin derivatives **12–18**

The solution of the protected pregabalin conjugate (0.11 mmol) in ethanol (5 ml), containing water (0.2 ml) and 1 M HCl (0.1 ml) was hydrogenated in the presence of 10% Pd/C (50 mg) for 2 h. The catalyst was filtered off, the filtrate was evaporated *in vacuo* and the residue was then purified by semi-preparative RP HPLC using different concentrations of MeOH/0.1% TFA. The trifluoroacetate ion present after preparative HPLC, was removed using an SPE cartridge. The cartridge was first eluted with water and then with MeOH to recover the pregabalin conjugates. The effluent was evaporated and the residue dissolved in water and lyophilized. Compounds **12–18** were at least 95% pure as assessed by analytical RP HPLC. Molecular structures were confirmed by mass spectrometry and NMR spectroscopy (see Tables 1–3).

*4.2.3.1. 2-(Pregabalylamino)-2-deoxy-D-glucopyranose (**12**).* Compound **12** was prepared from **5**. Purification with RP HPLC by using 10% MeOH/0.1% TFA afforded 28 mg (80%) of **12**, which was 97.0% pure by HPLC. M.p. 125–128 °C; $[\alpha]_D^{25} = +34$ ($c = 1.0$, MeOH). HRMS calcd for $C_{14}H_{28}N_2O_6$ (M^+) 321.2020, found 321.2034.

*4.2.3.2. 2-(Pregabalylamino)-2-deoxy-D-galactopyranose (**13**).* Compound **13** was prepared from **6**. Purification with RP HPLC by using 10% MeOH/0.1% TFA afforded 23 mg (65%) of **13**, which was 95.3% pure by HPLC. M.p. 148–145 °C; $[\alpha]_D^{25} = +26$ ($c = 1.0$, MeOH). HRMS calcd for $C_{14}H_{28}N_2O_6$ (M^+) 321.2020, found 321.2031.

*4.2.3.3. 2-(Pregabalylamino)-2-deoxy-D-mannopyranose (**14**).* Compound **14** was prepared from **7**. Purification with RP HPLC by using 10% MeOH/0.1% TFA afforded 26 mg (76%) of **14**, which was 95.7% pure by HPLC. M.p. 115–120 °C; $[\alpha]_D^{25} = -10$ ($c = 1.0$, MeOH). HRMS calcd for $C_{14}H_{28}N_2O_6$ (M^+) 321.2020, found 321.2007.

*4.2.3.4. N-Pregabalyl-β-D-glucopyranosylamine (**15**).* Compound **15** was prepared from **8**. Purification with RP HPLC by using 10% MeOH/0.1% TFA afforded 31 mg (90%) of **15**, which was 98.5% pure by HPLC. M.p. 142–145 °C; $[\alpha]_D^{25} = +12$ ($c = 1.0$, MeOH). HRMS calcd for $C_{14}H_{28}N_2O_6$ (M^+) 321.2020, found 321.2029.

*4.2.3.5. $N^{3''}$-Pregabalyl-tryptamine (**16**).* Compound **16** was prepared from **9**. Purification with RP HPLC by using 50.5% MeOH/0.1% TFA afforded 26 mg (79%) of **16**, which was 99.0% pure by HPLC. Oil; $[\alpha]_D^{25} = +6$ ($c = 1.0$, MeOH). HRMS calcd for $C_{18}H_{27}N_3O$ (M^+) 302.2227, found 302.2233.

*4.2.3.6. $N^{3''}$-Pregabalyl-serotonin (**17**).* Compound **17** was prepared from **10**. Purification with RP HPLC by using 57.5% MeOH/0.1% TFA afforded 28 mg (80%) of **17**, which was 98.0% pure by HPLC; M.p. 142–145 °C (violet crystals). HRMS calcd for $C_{18}H_{27}N_3O_2$ (M^+) 318.2176, found 318.2164.

*4.2.3.7. N-Pregabalyl-(1-adamantyl)amine (**18**).* Compound **18** was prepared from **11**. Purification with RP HPLC by using 68% MeOH/0.1% TFA afforded 8 mg (24%) of **18**, which was 97.7% pure by HPLC. M.p. 142–148 °C; $[\alpha]_D^{25} = +6$ ($c = 1.0$, MeOH). HRMS calcd for $C_{18}H_{32}N_2O$ (M^+) 293.2587, found 293.2599.

4.3. Pharmacology

Cell membrane homogenates from rat cerebral cortex (50 μg protein), prepared as described previously [15], were incubated 60 min at 22 °C with 10 nM [^{3}H]gabapentin in the absence or presence of the test compounds in a buffer containing 10 mM Hepes/Tris (pH 7.4). Nonspecific binding was determined in the presence of 100 μM gabapentin. Following incubation, the samples were filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI and rinsed several times with ice-cold 10 mM Hepes/Tris, 100 mM NaCl using a 96-sample cell harvester (Unifilter, Packard). The filters were dried, then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard).

Compounds were prepared as 10 mM stock solutions in pure DMSO. All intermediate dilutions before addition to the assay well were made in pure DMSO. A last 100-fold dilution was made in the assay well to reach the final testing concentration.

The specific ligand binding to the receptors was defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabelled ligand. The results were expressed as a percent of control specific binding ((measured specific binding/control specific binding)×100) and as a percent inhibition of control specific binding (100−((measured specific binding/control specific binding)×100)) obtained in the presence of the test compounds.

The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (nH) were determined by non-linear regression analysis of the competition curves generated with mean replicate values using Hill equation curve fitting ($Y = D + [(A - D)/(1 + (C/C_{50})^{nH})]$, where Y = specific binding, D = minimum specific binding, A = maximum specific binding, C = compound concentration, $C_{50} = IC_{50}$, and nH = slope factor). This analysis was performed using a software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot® 4.0 for Windows® (© 1997 by SPSS Inc.). The inhibition constants (K_i) were calculated using the Cheng Prusoff equation ($K_i = IC_{50}/(1+(L/K_D))$, where L = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor). A scatchard plot was used during assay development to determine the K_D value for gabapentin. All experiments were done in duplicate. Gabapentin was used as a standard reference compound and tested in each experiment at several concentrations to obtain a competition curve from which its IC_{50} is calculated.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejmech.2009.12.049.

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