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Gabapentin hybrid peptides and bioconjugates

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ABSTRACT

Synthetic approaches to gabapentin bioconjugates that overcome the tendency of gabapentin to cyclize into its γ-lactam are studied. Gabapentin was converted by N-acylation at its N-terminus into di-, tri-, and tetrapeptides (L-Ala-Gbp, L-Val-Gbp, L-Ala-L-Phe-Gbp, Gly-L-Ala-β-Ala-Gbp). Carboxyl-activated Boc-protected gabapentin was used to *N*-, *O*-, and *S*-acylate small peptides and hormones to give conjugates that could also provide prodrugs containing conformationally constrained gabapentin units.

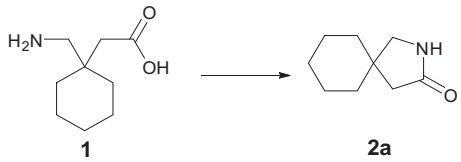
1. Introduction

Gabapentin 1-(aminomethyl)cyclohexane acetic acid, Gbp **1**, (trade name Neurontin), a structural analog of γ-aminobutyric acid (GABA), is utilized for the treatment of epilepsy,[1] neuropathic pain,[2–4] restless legs syndrome,[5] anxiety disorders,[6] hot flushes[7] and numerous other conditions.[8,9] Anhydrous gabapentin exists in three polymorphic forms.[10,11] The mechanism of action of gabapentin remains elusive.[12] Apparently, it does not directly interact with GABA receptors.[13–15] The absorption of gabapentin and clearance of the drug depends on the dosage and the patient.

Gabapentin **1** easily undergoes facile intramolecular cyclization to form the five-membered cyclic lactam 2-aza-spiro[4,5]decan-3-one **2a** (Scheme 1). While gabapentin has low toxicity (DL_{50}, tested in mice) at more than 8000 mg/kg, lactam **2a** has a much higher toxicity rating of 300 mg/kg.[16] The presence of γ-lactam **2a** as an impurity or the potential formation of **2a** during storage in any active ingredient must therefore be minimized both in solution and solid states.[16,17]

To improve Gbp bioavailability, much attention has been given to: (i) Gbp conformational properties in peptide sequences,[18–25] (ii) Gbp conjugated systems,[26–30] (iii) intermolecular interactions of Gbp,[31] its hydrates,[32] and derivatives,[33] (iv) Gbp multicomponent co-crystals with various carboxylic acids,[11,34] and (v) the formation of N-terminus bioconjugates of gabapentin, for example, with bile[29,35] acid or gabapentin enacarbil **3** (Fig. 1) (Horizant, XP-13512).[36,37]

The preparation of the Gbp bioconjugate **3** indicated that chemical instability, insufficient oral absorption, rapid pre-systemic metabolism, and toxicity of Gbp could be overcome by formulation of Gbp prodrugs.[38] The development of gabapentin peptides or acylated at N-, O-, S-termini derivatives that could transform in vivo to release the active drug could thus be a feasible strategy to improve the physicochemical, biopharmaceutical and/or pharmacokinetic, and pharmacological properties of Gbp, and thereby increase its usefulness.[39]



Scheme 1. Intramolecular cyclization of gabapentin (Gbp) into 2-aza-spiro[4,5]decan-3-one **2a**.

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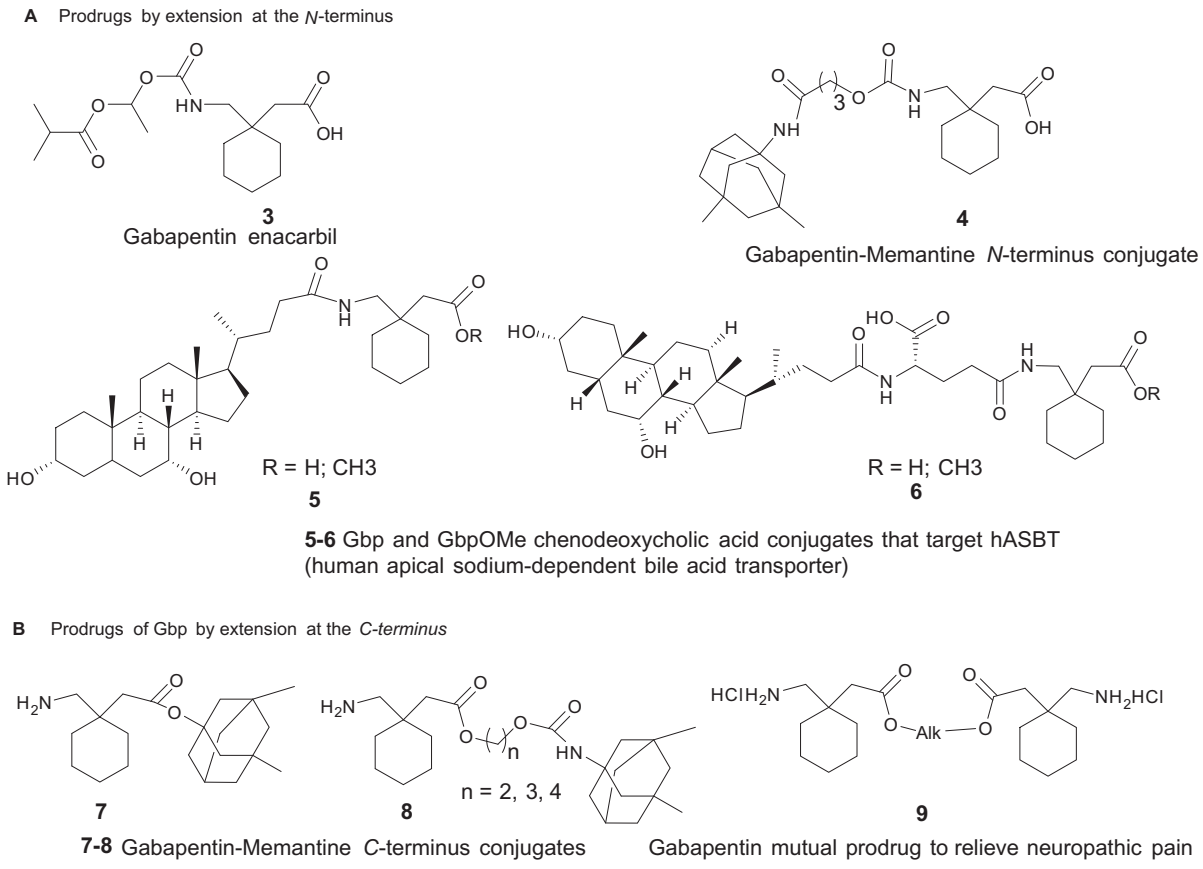

Figure 1. Reported Gbp bioconjugates.

Gabapentin enacarbil (**3**) was designed to improve physicochemical, biopharmaceutical and pharmacokinetic properties of the pharmacologically active gabapentin molecule.[40] The oral bioavailability of gabapentin was increased from 25% to 84% by use of the gabapentin enacarbil prodrug in monkeys, which showed dose-proportional gabapentin exposure in humans.[41] With these promising results in mind, we developed gabapentin conjugates that might increase bioavailability of the drug in similar ways to the gabapentin enacarbil prodrug **3**. An additional search revealed reports on both C- and N-terminus gabapentin conjugated systems (**4**–**9**, Fig. 1). In the development of bile acid prodrugs of Gbp the authors' strategy was to couple the Gbp drug to a natural substrate to create a molecule that mimics the three-dimensional structure of natural human (apical) sodium-dependent bile acid transporter.[29] Thus, the synthesis of glu-chenodeoxycholic acid and chenodeoxycholic acid Gbp and its methyl ester conjugates **5** and **6** helped overcome the limitations that gabapentin faces in its zwitterionic form and improve gabapentin oral bioavailability. For the purpose of treating neurological diseases, novel Gbp-adamantine (e.g., memantine) combinatorial N-terminus (**4**) and C-terminus (**7** and **8**) compositions were reported.[30] Gabapentin hydrochloride dimeric esters linked via a short C2–C6 aliphatic chain **9** were also synthesized and evaluated as mutual prodrugs to relieve neuropathic pain.[26]

The limited literature describing gabapentin N- and C-terminus bioconjugates is a testimony to the ease of intramolecular cyclization of **1** to form γ-lactam **2a**[10] (also encountered during our work with gabapentin **1**, see later). Coupling Gbp **1** at its N-terminus with other amino[42,33,22,20] or bile acids was reported using DCC/HOBt reagents and *N*-Boc or *N*-Cbz protection.[43] Moderate yields (50–73%) were reported with DCC/HOSu in THF.[44]

2. Results and discussion

We now report (i) benzotriazole-mediated acylations of Gbp **1** with amino acids, di- and tri-peptides; together with (ii) a series of N-protected carbonyl-activated gabapentin conjugates with biologically important *S*-, *O*-, *N*-nucleophiles.

2.1. Synthesis of Gbp-conjugated at the N-terminus with amino acids and peptides

Our first strategy to stabilize conformationally constrained Gabapentin **1** involved the construction of small α-,γ-peptidomimetics by N-acylation of **1** with carbonyl activated N-protected amino acids and small peptides. Benzotriazolyl precursors were constructed by solution-phase step-wise coupling of intermediate benzotriazolides with unprotected α-and β-amino acids.[45–47] This method allowed us to build up two- and three-amino acid units that were further activated at the C-terminus carbonyl group as benzotriazolides. For the synthesis of **14a**,**b**, only one CO-activation step was required. The conjugation of benzotriazolyl amino acids and peptides with Gbp employed known methodology (MeCN, 1.5 equiv TEA, 6 h, rt)[47] to give compounds **14a**–**d** (77–86%); formation of **2** was not detected. Gabapentin **1** was acylated with COBt-activated *N*-Cbz protected amino acid segments (Cbz-L-Val-OH, Cbz-L-Ala-OH) to form **14a** (77%), **14b** (86%) *N*-Boc dipeptide (Boc-L-Ala-L-Phe-OH) **14c** (81%), and **14d** *N*-Cbz-tripeptide (Cbz-Gly-L-Ala-β-Ala-OH) (83%) (Scheme 2). *N*-Cbz-protected aminoacids L-alanine and L-valine used for acylation of **1** at its N-terminus are structurally similar to gabapentin enacarbil **3**. For the same reason—Cbz-Gly-L-Ala-β-Ala-Bt **12** was employed as acylation reagent. Such substituents should not restrict the