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Original article

Synthesis and biological evaluation of new GABA-uptake inhibitors derived from proline and from pyrrolidine-2-acetic acid

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Abstract

Several synthetic approaches to *N*-alkylated derivatives of 4-hydroxypyrrolidine-2-carboxylic acid and 4-hydroxypyrrolidine-2-acetic acid are described. The final compounds have been evaluated as potential inhibitors of the GABA transport proteins GAT-1 and GAT-3. The biological assays used were based on bovine material or porcine brain. As compared to the corresponding 4-unsubstituted compounds, the 4-hydroxypyrrolidine-2-carboxylic acid and 4-hydroxypyrrolidine-2-acetic acid derivatives showed a significant decrease in the inhibitory potency at both GAT-1 and GAT-3 with only four compounds having reasonable affinity to GAT-1 (IC_{50}: 5.1, 6.6 and 9.4 μM) or GAT-3 (IC_{50}: 19.9 μM), respectively. The biological data of the 4-hydroxypyrrolidine-2-acetic acid derivatives indicates that (2*S*)-configuration at the C-2 position for potent inhibition of GAT-1 and (4*R*)-configuration at the C-4 position for potent inhibition of GAT-3 may be crucial.

Keywords: GABA-uptake inhibitors; Antiepileptic; GAT-1; GAT-3; Pyrrolidines

1. Introduction

Dysfunctioning of GABAergic synapses resulting in a decrease of GABAergic transmission has been invoked for diseases such as epilepsy [1], Huntington's chorea [2], and Parkinson's disease [3]. Sodium-dependent GABA-uptake systems have been found to be the principal means by which GABA in the synaptic cleft is inactivated. In contrast to the direct enhancement of GABA neurotransmission by $GABA_A$ agonists and benzodiazepines, inhibition of the GABA transport system palliates GABA deficiency in vivo without giving rise to the development of tolerance [4]. Four different GABA transporters have been identified thus far (GAT-1 [5], GAT-2 [5b], GAT-3 [6] and BGT-1 [7]) differing in their regional distribution in the brain and the body and in their sensitivity to pharmacological agents [8]. GAT-1 and GAT-3 are high affinity transporters for GABA expressed specifically in the CNS thereby being valid targets to modulate GABA-uptake. Potent inhibitors of GAT-1 such as SK&F 89976-A (**1**), (±)-*cis*-SK&F 100591-A (**2**) [9] and tiagabine (**3**) [10] (Fig. 1) have been synthesized and their pharmacology was intensively investigated. Dhar succeeded in the synthesis of the first GAT-3 selective, highly active inhibitor (*S*)-SNAP-5114 (**4**) (Fig. 1) exhibiting an IC_{50} value of 5 μM and a selectivity of 78:1 (GAT-3:GAT-1) [11]. However, for drug design and further pharmacological studies of GABA neurotransmission, it is still highly desirable to find GABA-uptake inhibitors having high potency and selectivity.

Previously, we reported the synthesis of the pyrrolidine derivatives **5** and **6** exhibiting potent inhibition of GAT-1 and GAT-3 and high selectivity (Fig. 2) [12].

Herein, we present the synthesis and biological evaluation of new pyrrolidine analogues having further structural modifications. The affinity of (±)-*cis*-SK&F 100591-A [9] (**2**) (Fig. 1) to GAT-1 implies that a hydroxy group in the *N*-heterocycle is likely to be acceptable for GABA-uptake transporters. Thus, we introduced a hydroxy group into the C-4 position of pyrrolidine-2-carboxylic acid and pyrrolidine-

Abbreviations: Bn, benzyl; Cbz, benzyl carboxylate; CC, column chromatography; DEAD, diethyl azodicarboxylate; DMAP, (4-dimethylamino)-pyridine; GAT, GABA transport protein; LDA, lithium diisopropylamide; prep., preparative; r.t., room temperature; TBAF, tetrabutylammonium fluoride; TBDMSCl, (*tert*-butyl)dimethlylsilyl chloride; TEA, triethylamine; TMSCl, trimethylsilyl chloride.

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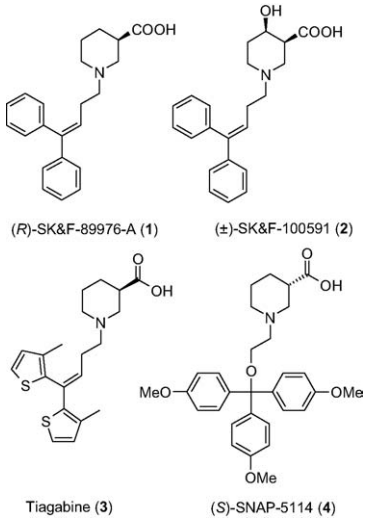

Fig. 1. Structures of representative known GABA-uptake inhibitors.

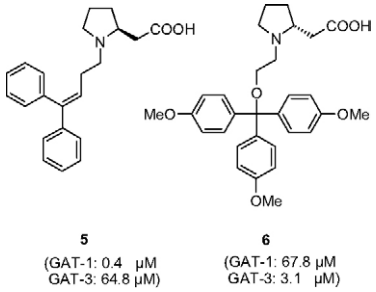

Fig. 2. Structures of GABA-uptake inhibitors having a pyrrolidine core structure.

2-acetic acid. The nitrogen atom of potent GABA-uptake inhibitors is generally substituted by appropriate bulky lipophilic groups. Therefore, four typical *N*-substituents **a**–**d** were chosen and two different series of pyrrolidine derivatives **7** and **8** were prepared in enantiomerically pure form as potential GABA-uptake inhibitors. In addition, the four stereoisomers of 4-hydroxypyrrolidine-2-acetic acid **9** were synthesized (Fig. 3). Compounds **7**–**9** were evaluated for their selectivity and inhibitory potency at GAT-1 and GAT-3. The results are expected to contribute to the optimization of structure–activity relationships.

2. Chemistry

L-*trans*-4-Hydroxypyrrolidine [(2*S*,4*R*)-**10**] was chosen as a precursor for the synthesis of the four key intermediates (2*S*,4*R*)-**11**, (2*R*,4*R*)-**11**, (2*S*,4*R*)-**12** and (2*R*,4*R*)-**13** (Fig. 4)

Compounds (2*S*,4*R*)-**11** [13] and (2*R*,4*R*)-**11** [13,14] were prepared from (2*S*,4*R*)-**10** according to literature procedures.

The synthesis of the intermediate (2*S*,4*R*)-**12** is illustrated in Fig. 5. The amino and the hydroxy functionality of (2*S*,4*R*)-**10** were protected with a Cbz and a Bn group, respectively, according to literature procedures [15]. Following transformation into the diazoketone (2*S*,4*R*)-**16** employing $(COCl)_2$ and diazomethane at 0 °C (yield: 80%), Wolff rearrangement initiated by AcOAg-TEA in MeOH afforded (2*S*,4*R*)-**17** in 81% yield. A simultaneous N,O-deprotection of (2*S*,4*R*)-**17** led to (2*S*,4*R*)-**12** in 90% yield (47% overall yield).

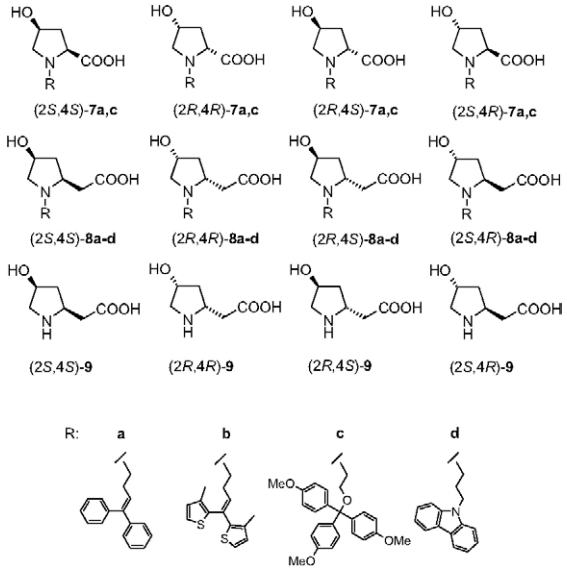

Fig. 3. The structures of the target compounds.

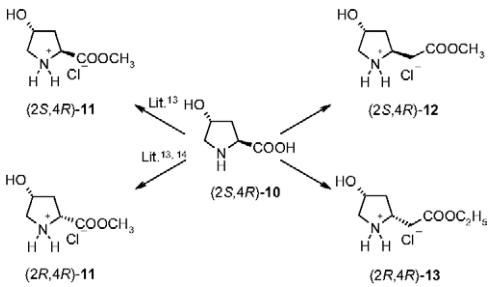

Fig. 4. The structures of the four key intermediates prepared from (2*S*,4*R*)-**10**.

Compound (2*R*,4*R*)-**13** was prepared as depicted in Fig. 6. The nitrogen atom of (2*S*,4*R*)-**10** was protected according to Ref. [15a]. Subsequent anodic oxidation in methanol gave the α-methoxy pyrrolidine derivative **18** as a mixture of diastereomers in 97% yield (*ds* = 41/59) [16]. Protection of the hydroxy group using TBDMSCl in the presence of imidazole (yield: 85%) followed by the nucleophilic addition of 1-ethoxy-1-(trimethylsilyloxy)ethene afforded (2*R*,4*R*)-**20** as the major product (yield: (2*R*,4*R*)-**20**: 79%, (2*S*,4*R*)-**20**: 9%) [17]. O-deprotection of (2*R*,4*R*)-**20** was accomplished in 88% yield by means of TBAF in THF [18]. Finally, the resulting product (2*R*,4*R*)-**21** was subjected to hydrogenation over 10% Pd-C in conc. HCl/EtOH to give (2*R*,4*R*)-**13** in 89% yield (46% overall yield).

To synthesize the lipophilic target structures shown in Fig. 7, the key intermediates (2*S*,4*R*)-**11** and (2*R*,4*R*)-**11** were *N*-alkylated with the respective halides of **a** and **c** (see Fig. 7). Subsequent saponification led to the target compounds (2*S*,4*R*)-**7a**, **c** and (2*R*,4*R*)-**7a**, **c** in moderate yields. Inversion of the stereocenter at C-4 of the pyrrolidine cycle was achieved via an intramolecular Mitsunobu reaction of (2*S*,4*R*)-