Ex-chiral pool synthesis and pharmacological aspects of 3-pyrrolidinyl-isoxazoles

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Employing the dopamine autoreceptor agonist (−)-3-PPP (3) as well as the cholinergic receptor ligands 4 and 5 as lead compounds the 3-pyrrolidinylisoxazoles 2a,b as well as its optical antipodes ent-2a,b were synthesized from (R)-aspartic acid (6) and (S)-aspartic acid (ent-6), respectively. Pharmacological properties of the target compounds were evaluated employing dopamine D2 receptor binding studies and functional experiments on muscarinic M2 receptors.

1. Introduction

EPC-syntheses of β-amino acids [1] and their applications for bioorganic and medicinal chemistry have become research topics of rapidly increasing interest. Thus, it could be shown that β-peptides can adopt remarkably well ordered conformations [2, 3]. Furthermore β-amino acids can serve as useful building blocks for the construction of bioactive compounds including β-lactams [4, 5], peptidomimetics [6, 7], anti-tumor agents (e.g. taxol) [8] and dopamine autoreceptor agonists [9, 10]. In recent work, we have described a practical synthesis of the glycine receptor agonist β-proline (1) in enantiomerically pure form [11–13]. Employing the carboxylate function as a synthetic precursor for the construction of an isoxazole heterocycle, β-proline (1) should give access to the 3-pyrrolidinylisoxazoles 2. The N-propyl derivative 2b can be regarded as a heterocyclic surrogate for the dopamine autoreceptor agonist (−)-3-PPP (3) [1]. Furthermore, the structural similarity of the methylpyrrolidine 2a with the aza-analog 4, which is known as a highly active agonist for cerebrocortical muscarinic receptors [14], and the regioisomer ABT-418 (5), a potent neuronal nicotinic acetylcholine receptor activator [15–18] intrigued us to investigate the synthesis as well as the pharmacological properties of 2a,b and the optical antipodes ent-2a,b.

2. Investigations, result and discussion

2.1. Synthesis

For the preparation of the (R)-configured target compounds 2a,b the N-benzyl protected β-proline ester 9 should serve as a central intermediate. According to our previously reported protocol the synthesis of 9 was performed in 76% overall yield [11, 12]. In practice, unnatural (R)-aspartic acid (6) was perbenzylated and reduced to afford the diol 7. Subsequent activation by methanesulfonyl chloride, rearrangement and hydrogenolysis gave the pyrrolidine mesylate 8 which could be readily transformed into the ester 9 by displacement with NaCN/Bu4NCN and methanolysis. For an exchange of the N-benzyl protecting group by a methyl substituent 9 was hydrogenated using Pearlman’s catalyst followed by reductive methylation (formaldehyde, NaCN/Bu4NCN). Thus, the N-methylpyrrolidine 10a could be obtained. For the synthesis of the N-propyl analog 10b slightly modified reaction conditions using Zn(BH4)2 instead of NaCN/Bu4NCN turned out to be advantageous [19, 20]. The construction of the isoxazole subunit was performed by employing the methodology developed by Hauser et al. [21]. Thus, the methyl esters 10a,b were reacted with the diion derived from acetone oxime and

Scheme 1

Scheme 2

ent-6 → ent-9 → ent-10a,b → ent-2a,b

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BuLi resulting in formation of the β-keto oximes 11a and 11b, respectively. Finally, cyclization was accomplished by treatment of crude 11a,b with methanesulfonyl chloride and triethylamine. Under these conditions, the target compounds 2a and 2b could be synthesized in pure form. Analogously, the optical antipodes ent-2a, b were prepared from natural (S)-aspartic acid (ent-6) through the intermediates ent-9a,b, ent-10a,b and ent-11a,b.

2.2. Pharmacological investigations

Due to the structural analogy between the isoxazoles 2 and the classical dopamine D2 autoreceptor agonist (−)-3-PPP (3), D2 receptor binding studies were performed. Employing bovine striatal membranes the ability of the test compounds to displace the specific radioligand (3H)-pramipexole was evaluated [22]. It turned out that, the isoxazoles 2a, 2b, ent-2a and ent-2b revealed significant but only modest affinity at D2 receptors labelled with (3H)-pramipexole (Table). The (R)-configured substrates 2a,b with an isoxazole substituent identically positioned to the 3-hydroxyphenyl moiety of (S)-(−)-3-PPP (3) displayed a higher activity than the optical antipodes ent-2a,b.

Table: Dopamine D2 receptor binding, [3H]-ligand: pramipexole

<table>
<thead>
<tr>
<th>Compound</th>
<th>2a</th>
<th>2b</th>
<th>ent-2a</th>
<th>ent-2b</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kᵢ (μM)</td>
<td>32</td>
<td>34</td>
<td>120</td>
<td>65</td>
<td>0.052</td>
</tr>
</tbody>
</table>

The structural similarity of 2a with the muscarinic agonist 4 prompted us to check for an interaction of 2a with muscarinic receptors in a functional test system. It is evident that the reaction mixture was filtered (Celite), the solvent evaporated, and the residue was purified by bulb to bulb distillation (20-30 °C, 0.01 mmbar) to give (R)-pyrrolidine-3-carboxylic acid methyl ester (4). The solvent was evaporated and the methanol (23 ml) and CH₂O (36% in H₂O, 4.5 ml). Subsequently, NaBH₃CN (0.65 g, 10.2 mmol) was added at −20 °C. The reaction mixture was slowly warmed to RT (1 h), stirred for 20 h, and centrifuged with HCI (10%, 45 ml), and extracted with Et₂O (2 × 60 ml). The aqueous layer was basified (K₂CO₃, pH = 11-12) and extracted with CHCl₃ (4 × 60 ml). The organic layer was dried (MgSO₄), evaporated, and the residue was purified by flash chromatography (CH₂Cl₂/Methanol, 9:1) to give 10b (4.02 g, 62.9%) as a colorless oil: [α]D = −8.5°, c = 3.0 in CHCl₃. IR (NaCl, cm⁻¹): 2950-2935 (C-H), 2875 (C-H, OCH₃), 2795 (C-H, NCH₂), 1735 (C=O), 1515-1445 (C-H), 1195-1170 (C-O). 1H NMR (CDCl₃), 6 ppm: 3.63 (s, 3 H, OCH₃), 3.63 (s, 3 H, OCH₃), 1.96 (s, 3 H, NCH₂), 1.75 (s, 3 H, OCH₃), 1.45 (s, 3 H, NCH₂), 0.85 (s, 3 H, NCH₂). 13C NMR (CDCl₃), δ ppm: 25.0 (C₃H₇), 43.0 (C₃H₇), 41.9 (C₆H₅), 112.0 (M-COOCH₃), 84.0 (CH₃NC(CH₃)), 142.0 (M-COOCH₃), 140.0 (M-CH₂CH₃), 128.0 (M-H), 156.0 (M-CH₂), 144.5 (M-CH₂). MS: m/z (%) = 120 (100), 105 (91), 104 (51), 89 (80), 88 (40), 73 (45), 65 (13), 43 (50), 35 (55), 31 (100), 29 (83), 27 (13), 21 (22). 2.3. (R)-1-Methylpyrrolidine-3-carboxylic acid methyl ester (10a)

A solution of 9 (4.43 g, 19.8 mmol) in methanol (5 ml) was hydrogenated in a Parr hydrogenation apparatus (14 h, 1010 mbar, 210 ml H₂) using 20% Pd(OH₂/C (10.6 g) as a catalyst. The reaction mixture was filtered (Celite), stirred after addition of freshly distilled propionylaldehyde (0.715 ml, 9.88 mmol) for 0.5 h, and cooled to 0 °C. Then, Zn/H₂SO₄ (prepared from 0.44 g ZnCl₂, 2.5 g Et₂O and 0.116 g NaBH₃CN as described before) was added to the reaction mixture. The solution was warmed to RT, stirred for 4 h, and separated by aqueous solution of NaOH (15%, 5 ml), and extracted with Et₂O (5 × 5 ml). The aqueous layer was basified (K₂CO₃, pH = 10-11) and extracted with CHCl₃ (5 × 5 ml). The organic layer was washed with CHCl₃ (5 × 5 ml). The organic layer was dried (MgSO₄), evaporated, and the residue was purified by flash chromatography (CH₂Cl₂/MeOH, 9:1) to give 11b (2.25 g, 78%) as a colorless oil: [α]D = −8.5°, c = 3.0 in CHCl₃. IR (NaCl, cm⁻¹): 2875 (C-H), 2795 (C-H, NCH₂), 1735 (C=O), 1515-1445 (C-H), 1195-1170 (C-O). 1H NMR (CDCl₃), 6 ppm: 1.96 (s, 3 H, NCH₂), 1.75 (s, 3 H, OCH₃), 1.45 (s, 3 H, NCH₂), 0.85 (s, 3 H, NCH₂). 13C NMR (CDCl₃), δ ppm: 25.0 (C₃H₇), 43.0 (C₃H₇), 41.9 (C₆H₅), 112.0 (M-COOCH₃), 84.0 (CH₃NC(CH₃)), 142.0 (M-COOCH₃), 140.0 (M-CH₂CH₃), 128.0 (M-H), 156.0 (M-CH₂), 140.0 (M-CH₂), 128.0 (M-CH₂CH₃), 112.0 (M-COOCH₃), 84.0 (CH₃NC(CH₃)), 70.0 (CH₃COOCH₃), 43.0 (C₃H₇), 41.9 (C₆H₅). IR (NaCl, cm⁻¹): 2950-2935 (C-H), 2875 (C-H, OCH₃), 2795 (C-H, NCH₂), 1735 (C=O), 1515-1445 (C-H), 1195-1170 (C-O). MS: m/z (%) = 120 (100), 105 (91), 104 (51), 85 (80), 84 (41), 73 (100), 65 (100), 43 (50), 31 (100), 29 (83), 27 (13), 21 (22).
3.7. Interaction with muscarinic receptors in guinea pig atria
The procedure has been described previously [23]. In short, isolated guinea pig atria were suspended in modified Tyrode's solution, electrically stimulated at a frequency of 3 Hz, and the force of contraction was recorded isovolumetrically. After an equilibration period of 60 min, a concentration-effect curve of oxotremorine M was measured. Subsequently, oxotremorine M was washed out in drug free Tyrode's solution for 60 min. Thereafter, to test for an agonist action, the test compounds were added in a cumulative fashion starting at 1 nM with an exposure time of 10 min for each concentration. To test for an antagonist action, 100 nM of a test compound were applied for 60 min before the oxotremorine concentration-effect curve was measured in the presence of the compound.

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References

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