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Research paper

Systematic variation of the benzenesulfonamide part of the GluN2A selective NMDA receptor antagonist TCN-201

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ABSTRACT

GluN2A subunit containing *N*-methyl-D-aspartate receptors (NMDARs) are highly involved in various physiological processes in the central nervous system, but also in some diseases, such as anxiety, depression and schizophrenia. However, the role of GluN2A subunit containing NMDARs in pathological processes is not exactly elucidated. In order to obtain potent and selective inhibitors of GluN2A subunit containing NMDARs, the selective negative allosteric modulator **2** was systematically modified at the benzenesulfonamide part. The activity of the test compounds was recorded in two electrode voltage clamp experiments using *Xenopus laevis oocytes* expressing exclusively NMDARs with GluN1a and GluN2A subunits. It was found that halogen atoms in 3-position of the benzenesulfonamide part result in high GluN2A antagonistic activity. With an IC_{50} value of 204 nM the 3-bromo derivative **5i** (*N*-{4-[(2-benzoylhydrazino)carbonyl]benzyl}-3-bromobenzenesulfonamide) has 2.5-fold higher antagonistic activity than the lead compound **2** and represents our new lead compound.

1. Introduction

N-Methyl-D-aspartate receptors (NMDARs) are glutamate and glycine gated ion channels, which are highly expressed in the central nervous system (CNS). The heterotetrameric receptor can be comprised of seven different subunits, which are subclassified into three subfamilies GluN1, GluN2 and GluN3 [1]. Whereas eight different splice variants of the GluN1 subunit (GluN1a-h) are encoded by a single gene, four different types of GluN2 subunits (GluN2A-D) and two different types of GluN3 subunits (GluN3A, B) are encoded by different genes [2]. A functional NMDAR is comprised of two GluN1 subunits containing the binding site for the co-agonist glycine and two additional subunits from the GluN2 or GluN3 family. Whilst GluN3 subunits also have a binding site for glycine, the GluN2 subunits are responsible for glutamate binding. Both endogenous agonists glycine and (*S*)-glutamate are required to activate the NMDA receptor [1,2]. With these three subtype families diheteromeric receptors, containing GluN1 and one type of GluN2 subunits can be formed. Additionally, triheteromeric receptors, containing two different GluN2 subunits or one GluN2 and one GluN3 subunit and, furthermore, glycine activated NMDARs containing two GluN1 and two GluN3 subunits are known. The high number of possible combinations of GluN subunits and the influence of the different subunits on the properties of the NMDAR lead to highly diverse functional activity of the NMDA system in the CNS [1,3].

The subunit composition differs depending on the developmental stage of the CNS, especially for the GluN2A subunit. Whereas the neonatal GluN2A subunit expression level is quite low in rat brain, in the adult brain the GluN2A subunit represents one of the predominant GluN2 subunits [4]. Furthermore, GluN2A containing NMDARs are involved in synaptic plasticity and learning processes [5]. It was found that GluN2A knockout mice show decreased anxiety-like behavior across multiple tests and less depressant-like behavior in the forced swimming test, which implicates a possible involvement of GluN2A subunit containing

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NMDARs in the pathophysiology of these diseases [6]. Different studies demonstrated the involvement of GluN2A subunit hypofunction in the pathophysiology of chronic schizophrenia [7,8]. Moreover, GluN2A subunit containing NMDARs are also correlated to other neurological diseases like cerebral ischemia, seizure disorder, Huntington's, Parkinson's and Alzheimer's disease. However, the role of the GluN2A subunit is not fully understood so far, due to contrary results in different studies [9]. Thus, GluN2A subunit selective ligands are highly needed as tools to investigate the role of GluN2A subunits on physiological and pathophysiological processes.

5-Phosphonomethylquinoxalinedione PEAQX and its active diastereomer NVP-AAM077 (**1**) belong to the first described compounds selectively targeting GluN2A containing NMDARs (Fig. 1). Both compounds compete with glutamate for its binding site at the GluN2A subunit [10]. However, the selectivity towards NMDARs with other GluN2 subunits, in particular the GluN2B subunit, is rather low [11].

In 2010, Bettini et al. reported the most promising selective GluN2A negative allosteric modulator (NAM) TCN-201 (**2**, Fig. 1), which shows moderate antagonistic activity at GluN2A containing NMDARs (IC_{50} = 109 nM, HEK cells) and high selectivity over all other GluN2 subunits [12–14]. Based on biological tests, a new binding site was postulated for **2**, which was confirmed in 2012 by mutagenesis of GluN2A containing NMDARs. The new binding site of **2** is located at the interface of the GluN1 and GluN2A subunits. Hansen *et* al. also showed that binding of **2** resulted in a noncompetitive potency reduction of glycine at the GluN1 subunit [14]. Recently this hypothesis of the mechanism was confirmed by crystallization of **2** with the ligand binding domain of the GluN1/GluN2A containing receptor [15].

In 2016 Hackos *et* al. described some positive allosteric modulators (PAMs) binding at the same or overlapping binding site as the NAM **2**. One of the most active compounds with sufficient selectivity for GluN2A over other GluN2 subunits was the thiazolopyrimidine GNE-0723 (**3**, Fig. 1) with an EC_{50}-value of 21 nM. NAMs (e.g. **2**) and PAMs (e.g. **3**) would represent valuable tools for the analysis of the effects resulting from inhibition or overactivation of GluN2A containing NMDARs [16]. However, the activity of the NAM **2** (IC_{50} = 109 nM) is still too low to serve as versatile tool compound.

Therefore, we aim at the development of novel NAMs for GluN2A subunit containing NMDARs with high activity. An approach to increase the activity is the systematic variation of the substitution pattern of **2**. Since **2** was the result of a high-throughput screening [12–14]. systematic structure activity relationships are not yet reported. Very recently, MPX-007 (**4**, Fig. 1), which is derived from **2**, was reported to have 13-fold higher GluN2A activity and better solubility in H_2O than **2** [17]. The main structural differences between **2** and **4** are the replacement of the central benzene ring by a pyrazine ring and the exchange of the hydrazide moiety by a *N*-methylcarboxamido group [18].

In order to identify the structural elements, which are responsible for high GluN2A antagonistic activity, single point variations of **2** were performed systematically herein and the blockade of GluN2A subunit containing NMDARs was recorded. In this work, we focused on the systematic variation of the 3-chloro-4-fluorophenyl moiety. The substitution pattern of the phenyl ring should be modified (compounds **5**) and the phenyl ring should be replaced either by other aromatic (compounds **6**) or aliphatic substituents (compounds **7**). (Fig. 2).

2. Synthesis

For the synthesis of novel NAMs **5**–**7** three reaction paths were pursued, which used the common educt 4-(aminomethyl)benzoic acid (**8**) (Scheme 1). Path A was preferred, since it allowed the introduction of diverse substituents in the last reaction step of the synthesis (late stage diversification strategy). Therefore, most of the compounds were synthesized according to Path A.

Path A started with the introduction of the Boc-protective group by reaction of **8** with di-*tert*-butyl dicarbonate [19]. The resulting BOC-protected amino acid **9** was treated with benzoylhydrazine and COMU® as coupling agent [20] to form the diacylhydrazine **10** in 90% yield. Subsequent deprotection of **10** with trifluoroacetic acid resulted in the building block **11** as trifluoroacetate salt, which was transformed into the free amine by treatment with $NaHCO_3$. The free amine **11** and also the trifluoroacetate salt were used in the last step of the synthesis to obtain diverse sulfonamides **5**–**7**.

The amine **11** was reacted with the corresponding sulfonyl chloride in H_2O under similar reaction conditions as reported by Deng et al. [21]. According to this report, the pH value was constantly kept at 8 by addition of Na_2CO_3 using a syringe pump equipped with a pH-meter. The careful control of the pH value is essential, as hydrolysis of sulfonyl chlorides occurs at pH > 10 and protonation (i.e. deactivation) of the amine at pH < 7. To simplify the method a $Na_2B_4O_7$ buffer keeping the pH value constant at 9 was used instead of pH-meter and syringe pump. Most of the

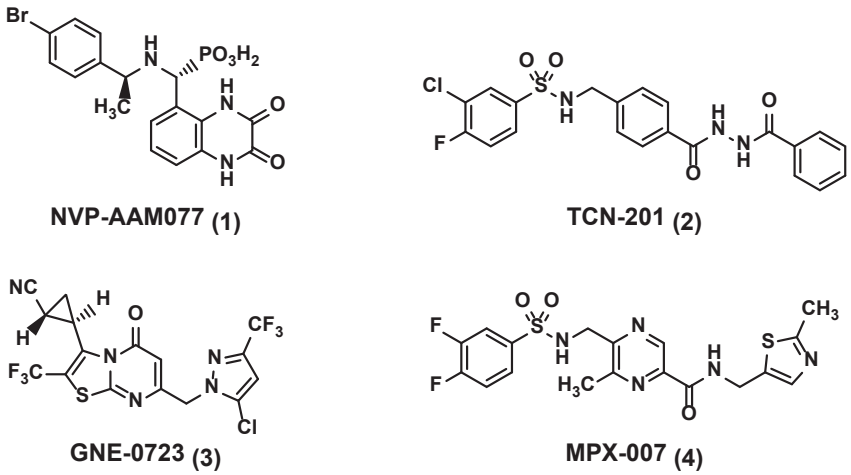

Fig. 1. GluN2A selective NMDAR ligands.