Bioorganic & Medicinal Chemistry Letters 26 (2016) 3866–3869

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis, structure–activity relationships and biological evaluation of 4,5,6,7-tetrahydropyrazolopyrazines as metabotropic glutamate receptor 5 negative allosteric modulators

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ARTICLE INFO

Article history:
Received 17 May 2016
Revised 5 July 2016
Accepted 7 July 2016
Available online 9 July 2016

Keywords:
mGluR5
Negative allosteric modulator
Hit to lead studies
CNS drug discovery

ABSTRACT

The design, synthesis and SAR studies of novel 4,5,6,7-tetrahydropyrazolopyrazines as metabotropic glutamate receptor 5 (mGluR5) negative allosteric modulators (NAMs) are presented in this letter. Starting from a HTS hit compound (**1**, IC_{50} = 477 nM), optimization of various groups led to the synthesis of a potent mGluR5 NAM (**32**, IC_{50} = 75 nM) with excellent rat PK profile and good brain penetration. This compound produced oral antidepressant-like effect in a mouse tale suspension model (MED: 30 mg/kg).

Metabotropic glutamate receptors (mGluRs) are members of the G protein-coupled receptor (GPCR) class C and are divided into eight subtypes based on their sequence homology, downstream signal transduction and pharmacological output.[1,2] As the mGluR5 is post-synaptically expressed in the central nervous system (CNS) and periphery,[3] modulation of mGluR5 would be useful for the treatment of both central and peripheral nervous systems disorders. Whereas mGluR5 orthosteric antagonists often have poor selectivity for mGluR subtypes and limited ability to penetrate biological membranes due to their hydrophilicity,[4] mGluR5 negative allosteric modulators (NAMs) are favorable small molecule drug candidates due to their good selectivity and appropriate pharmacokinetic properties.[5] Several preclinical and clinical evidence supports the therapeutic potential of mGluR5 NAMs in the treatment of CNS disorders, including anxiety, depression, Parkinson's disease levodopa-induced dyskinesia (PD-LID), and fragile X syndrome (FXS).[6] In addition, a number of phase II or III clinical trials with mGluR5 NAMs for different indications, including Mavoglurant (AFQ-056, Novartis), Dipraglurant (ADX-48621, Addex), Basimglurant (RG-7090, Roche) and STX-107 (Seaside Therapeutics, Roche) are completed or currently underway.[7] However, no mGluR5 NAM drug has so far been launched.

Most of the compounds advanced in clinical trials (Fig. 1) are characterized by a di-substituted alkyne as a common structure that can potentially be influenced by metabolic activation, leading to unacceptable toxicity.[8] In addition, the intellectual space around the disubstituted alkyne chemotypes is quite crowded. These concerns continuously urged medicinal chemists to seek novel mGluR5 NAMs without the alkyne structure.[9] Our research program has also been directed toward the development of novel non-alkyne mGluR5 NAMs. We describe herein the design, synthesis, structure–activity relationship (SAR) and biological profiles of a novel class of mGluR5 NAMs possessing 4,5,6,7-tetrahydropyrazolopyrazine as a core structure.

In our drug discovery program for novel mGluR5 NAMs, 4,5,6,7-tetrahydropyrazolopyrazine derivative **1** (Fig. 1) was selected through a high-throughput screening using our corporate library as a hit compound, which has a moderate inhibitory activity for human mGluR5 (IC_{50}: 477 nM). Our initial study was an investigation of the SAR in order to identify a lead compound and develop its optimization strategy. We first determined functional IC_{50} values of the synthesized compounds for inhibition of human mGluR5 using calcium mobilization assay with HEK293 cells expressing recombinant human receptor and EC_{90} concentration of L-glutamate.

We developed two versatile synthetic routes that allow easy access to tetrahydropyrazolopyrazine derivatives with various substituents (Scheme 1). The required pyrazole derivatives **3** were

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http://dx.doi.org/10.1016/j.bmcl.2016.07.019
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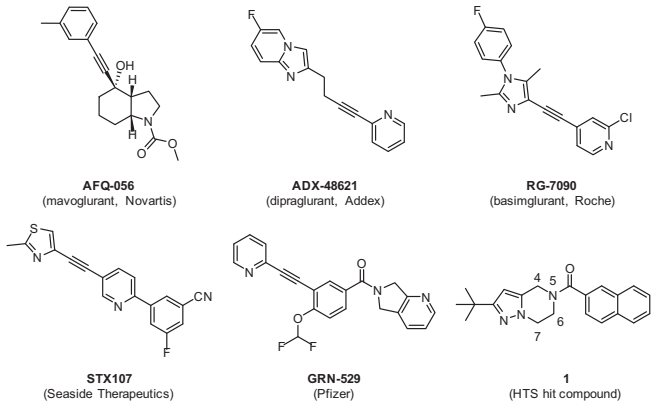


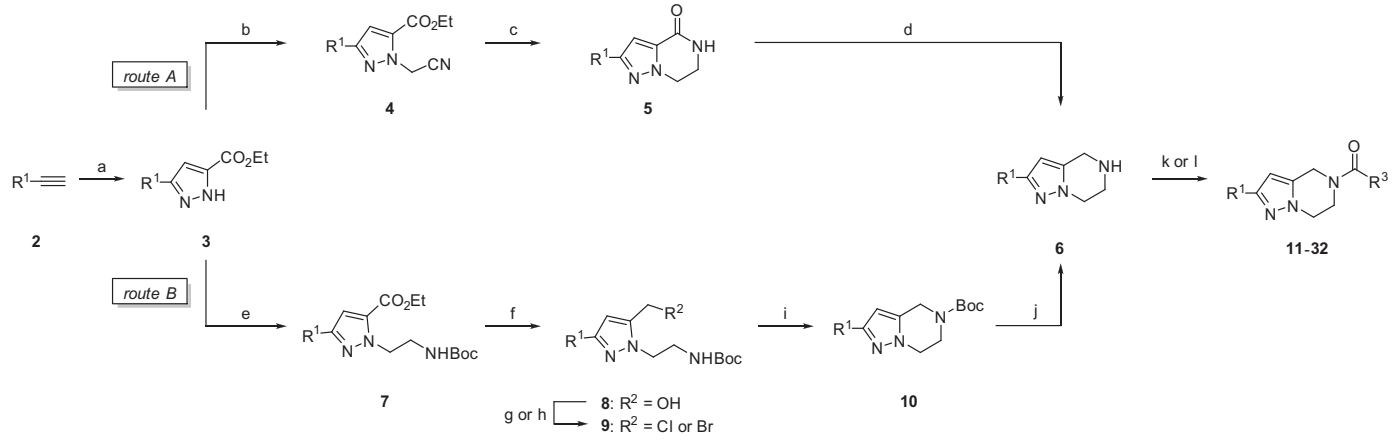
Figure 1. Structures of representative mGluR5 NAMs and the alkyne-lacking HTS hit compound **1**.

purchased or prepared via 1,3-dipolar cycloaddition reaction with the commercially available alkynes **2** and ethyl diazoacetate. In route A, alkylation of **3** afforded the nitrile derivatives **4**. Reduction of the cyano group of **4** with $NaBH_4$ in the presence of $CoCl_2$ followed by cyclization generated the tetrahydropyrazolopyrazinones **5** whose lactam moiety was reduced by $LiAlH_4$ to give the precursor amines **6**. In route B, Mitsunobu reaction of **3** and *tert*-butyl (2-hydroxyethyl)carbamate afforded **7**. Reduction of the ester of **7** with $LiAlH_4$ produced the corresponding alcohols **8**. Halogenation of **8** with MsCl or *N*-bromosuccinimide generated **9**,[10] which were then cyclized using NaH to give **10**. Deprotection of the Boc group in **10** with hydrochloric acid gave **6**. Finally, amidation of **6** with appropriate carboxylic acids or acyl halides provided compounds **11**–**32**. The precursor amines **6** of compound **11**–**18** (R^1 = Ph, 4-pyridyl, 3-pyridyl or 2-pyridyl) were synthesized via route A, and the others (R^1 = monosubstituted 2-pyridyl) were synthesized via route B.

In order to identify the minimum pharmacophore, we initially replaced the left-hand and the right-hand partial structures of compound **1** with a smaller substituent, respectively. Replacement of ^{t}Bu group of **1** by methyl or a lower alkyl resulted in severe decrease of the inhibitory activity. Conversion of 2-naphthyl group of **1** to a monocyclic substituent group, such as phenyl, pyridyl, or pyrrolyl, also led to decrease of inhibitory activity (data not shown). Various combinations of substitutions were then explored as shown in Table 1, leading to compound **11** (R^1 and R^3 = Ph) with improved mGluR5 inhibitory activity (IC_{50}: 127 nM). However, this compound showed poor aqueous solubility (2 μg/mL at pH 7.4, 4 μg/mL at 1.2). To overcome this drawback, a nitrogen atom was introduced into the left phenyl ring (**12**–**14**) affording only **14** (R^1 = 2-pyridyl and R^3 = Ph) as a compound with moderate mGluR5 inhibitory activity (IC_{50}: 235 nM) and sufficiently improved aqueous solubility (>100 μg/mL at both pH 7.4 and 1.2). Additionally, compound **14** showed good liver microsomal stability (human: 92%, rat: 95%), and low IC_{50} value for CYP enzymes (IC_{50}: >50 μM for CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6 and 3A4 isoforms). These good physicochemical and metabolic properties seemed to be appropriate for selection of **14** as lead compound.

We next turned our attention to the effects of various substituents on the left-hand 2-pyridyl group and the right-hand phenyl group of compound **14** (Table 2). Substitution of a chloride at the *ortho*-, *meta*- or *para*-position of the phenyl group (**15**–**17**) resulted in only the *meta*-substitution being tolerated. Di-substitution of the chloride in the 3,5-position (**18**) was also tolerated. Next, the effects of a substitution at the 3-, 4-, 5- or 6-position of the left-hand 2-pyridyl group (**19**–**24**) were investigated. The 3-Me (**19**), 4-Me (**20**) and 6-Me (**22**) substituted compounds had weaker mGluR5 inhibitory activity than the parent lead compound **14**, whereas the 5-Me (**21**), 5-F (**23**) or 5-Cl (**24**) substituted compounds had an inhibitory activity comparable to that of compound **14** (IC_{50}: 244, 150 and 201 nM, respectively). Fixing the 5-F group as the most suitable R^4 substituent for good mGluR5 inhibitory activity, several R^5 substituents at the *meta*-position of the right-hand phenyl group were examined. As shown in Table 2, small substituents, such as a chloro (**25**), fluoro (**26**), and methyl (**27**) groups were tolerable (IC_{50}: 150, 257 and 150 nM, respectively), while a methoxy (**28**), cyano (**29**) and trifluoromethyl (**30**) groups decreased mGluR5 inhibitory activity (IC_{50}: 482, >10,000 and 383 nM, respectively). A marked improvement in the activity was seen with compound **32** (R^4 = 5-F and R^5 = 3-Cl, 5-F), which showed 6-fold increased in inhibitory activity (IC_{50}: 75 nM) compared to the HTS hit **1.** Compound **32** also showed moderate aqueous solubility (7 μg/mL at pH 7.4, 79 μg/mL at pH 1.2) and appropriate liver microsomal stability (human: >97%, rat: >97%). Other physicochemical properties as well as in vitro ADMET profile of **32** are shown in Table 3. There seems to be no serious concern regarding compound **32** CYP enzyme inhibition (IC_{50}: >40 μM for CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6 and 3A4 isoforms) or induction (human AhR and human PXR: negative).[11]

In addition, compound **32** showed sufficient cell permeability and low potential as P-glycoprotein substrate, which suggest good



Scheme 1. Reagents and conditions: (a) ethyl diazoacetate, toluene, reflux; (b) chloroacetonitril, NaH, DMF, 0 °C to rt; (c) $NaBH_4$, $CoCl_2$, MeOH, rt, then K_2CO_3; (d) $LiALH_4$, THF, reflux; (e) *tert*-butyl (2-hydroxyethyl)carbamate, diisopropyl azodicarboxylate, Ph_3P, THF, 0 °C to rt; (f) $LiAlH_4$, THF, 0 °C; (g) MsCl, iPr_2NEt, CH_2Cl_2, 0 °C; (h) NBS, Ph_3P, CH_2Cl_2, 0 °C; (i) NaH, DMF, 50 °C; (j) 4 M HCl in dioxane or AcOEt, rt; (k) R^3COOH, EDCI, HOBt, DMF, rt; (l) R^3COCl, Et_3N, CH_2Cl_2, 0 °C.