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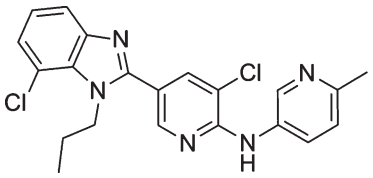
Benzimidazoles as Potent and Orally Active mGlu5 Receptor Antagonists with an Improved PK Profile

David Carcache,[†] Ivo Vranesic,[‡] Joachim Blanz,[†] Sandrine Desrayaud,[†] Markus Fendt,[‡] and Ralf Glatthar*,[†]

[†]Global Discovery Chemistry and [‡]Neuroscience, Novartis Institutes for BioMedical Research, Basel CH-4002 Switzerland

ABSTRACT A focused chemical optimization effort of compound **1** based on metabolite elucidation is described, resulting in **15i**, a highly potent and selective mGlu5 receptor antagonist with an improved pharmacokinetic profile compared to **1**. Characterization of **15i** *in vivo* in the fear-potentiated startle (FPS) paradigm revealed a robust reduction of conditioned fear behavior. This effect nicely correlates with the rat brain pharmacokinetics.

KEYWORDS mGlu5, benzimidazoles, pharmacokinetics



L-Glutamate serves as the neurotransmitter at the majority of excitatory synapses in the mammalian central nervous system (CNS) and as such is involved in a variety of physiological and pathophysiological functions. The existence of neuromodulatory glutamate receptors, called metabotropic glutamate receptors (mGluRs), provides a mechanism by which glutamate can modulate cell excitability and synaptic transmission. Metabotropic glutamate receptors are G-protein-coupled receptors linked to multiple second messenger systems modulating for example ion channel functions in the neurons.[1−3] Eight different types of metabotropic glutamate receptors are known (mGluR1−mGluR8), which are divided into three groups according to their sequence homology, effector coupling and pharmacology. Group I mGlu receptors (mGluR1 and mGluR5) are positively coupled to phospholipase C; group II mGlu receptors (mGluR2 and mGluR3) and group III mGlu receptors (mGluR4, mGluR6, mGluR7 and mGluR8) are negatively coupled to adenylate cyclase. mGluRs are broadly distributed throughout the CNS and are specifically localized at discrete synaptic and extrasynaptic sites.

mGlu5 receptor expression is especially high in brain regions thought to mediate and modulate emotions like fear and anxiety (amygdala, prefrontal cortex, hippocampus and basal ganglia).[4−6] mGlu5 receptors are predominantly postsynaptically located and have been shown to play a role in regulating glutamatergic transmission *via* potentiation of NMDA receptor activity. Excessive glutamatergic transmission has been proposed to play a role in psychiatric diseases like anxiety disorders and depression.[7,8] Additionally, mGlu5 receptor antagonism has been proposed as a potential target for the treatment of pain,[9] obesity,[10] Parkinson's disease,[11] drug abuse,[12] migraine,[13] gastroesophageal reflux disease (GERD)[14] and fragile X syndrome (FXS).[15] Therefore, the development of potent and selective mGlu5 receptor antagonists as potential therapeutic agents has been the focus of significant research in our laboratories.

We recently disclosed the piperidyl amide-based selective mGluR5 antagonist **1** (Figure 1) that shows robust anxiolytic-like activity in different animal models of fear and anxiety.[16]

The moderate metabolic stability of compound **1**, based on *in vivo* plasma clearance in rat and *in vitro* microsomal clearance data in rat and human required further optimization.

In the present letter, the chemical derivation effort of **1** to an improved compound with a superior pharmacokinetic profile and increased potency *in vivo* will be discussed.

As a result of the moderate metabolic stability of **1**, the biotransformation of this compound was studied in human and rat liver microsomes as well as *in vivo* in rat after intravenous or oral administration (Scheme 1).

In microsomal incubations, six phase I metabolites were found and characterized by means of mass spectrometry. Metabolites **m1**, **m2** and **m3** were oxidation products of the 2-ethylpiperidin-1-yl moiety. Further oxidation and ring-opening led to metabolite **m4**, whereas oxidation of the methyl group in the 6-methylpyridin-3-ylamino moiety led to metabolite **m5**. H/D exchange indicated N-oxidation at the 5-chloro-6-(6-methylpyridin-3-ylamino)pyridin-3-yl moiety in the case of metabolite **m6**. *In vivo*, the metabolites **m1**−**m3** were also detected in plasma besides the parent compound.

The formation of phase II metabolites was not observed in any of the *in vitro* or *in vivo* samples. The abundances of **1** and its metabolites in plasma suggested that the elimination of **1** may predominantly occur via oxidation of the 2-ethylpiperidin-1-yl moiety (**m1**−**m3**), with major metabolite being **m1** *in vivo* and **m5** *in vitro*.[18]

With the piperidyl moiety identified as the major site of metabolism in compound **1**, the initial derivation efforts

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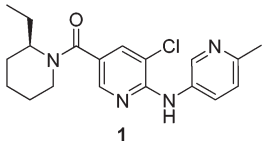
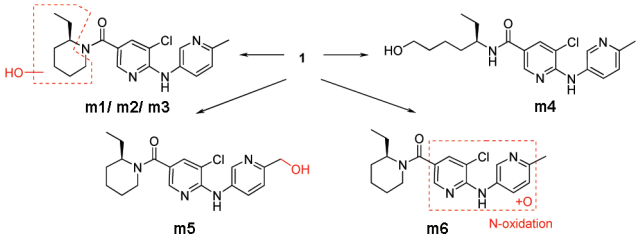


IC_{50} mGluR5a (Ca^{2+} flux / PI): 32 / 36 nM
Solubility[b] (pH 6.8): 58 mg/L
HLM/RLM[c]: 89 / 87 μL/min/mg protein
In vivo CL (rat)[d]: 37.2 mL/min/kg
MRT[e] (rat)[d]: 0.9 h
F (rat)[d]: 54%

Figure 1. mGluR5 antagonist. [a]hmGluR5: Ca^{2+} flux using glutamate and phosphoinositol turnover using quisqualate as agonist;[17] data are geometric means of $n \geq 2$, IC_{50} (nM). [b]Solubility (mg/L) measured in a dissolution template potentiometric titration approach. [c]HLM/RLM: human and rat liver microsomal intrinsic clearance (μL/min/mg microsomal protein). [d]$n = 3$ Sprague−Dawley rats/group. [e]MRT = mean residence time (h).

Scheme 1. Proposed Chemical Structures of Metabolites of **1** Detected after Incubation with Rat and Human Liver Microsomes as Well as *in Vivo* in Rat Plasma



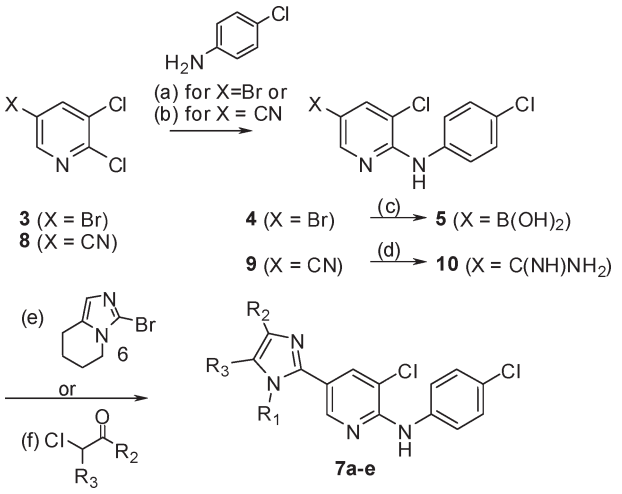
aimed at exploring bioisosteric replacements for the left-hand fragment in **1**. Compound **2** was used as a reference for the optimization of this series, which contains the central and right-hand-side fragments that led to most potent derivatives described in our preceding letter, combined with the piperidyl amide left-hand-side fragment of **1**.[16] Several 5-ring heterocyclic bioisosteres such as oxadiazole, triazole, and isoxazole were investigated and shown to be inactive. Only the imidazole system proved to be a suitable bioisosteric replacemement for the piperidyl amide, albeit with reduced potency.

The imidazole derivatives were prepared by the methods depicted in Scheme 2, whereas the benzimidazole analogues were synthesized according to Scheme 3. The synthesis started by reacting pyridine **3** with 4-chloroaniline to afford **4**, followed by boronic acid formation to give **5**. Suzuki coupling with **6** provided **7a**.[19] The synthesis of **7b**−**e** commenced with a Buchwald reaction between pyridine **8** and 4-chloroaniline, leading to **9**, followed by amination of the nitrile group to give the amidine **10**. Condensation of **10** with α-chloroketones and subsequent N-alkylation of the imidazole ring afforded **7b**−**e**.

The preparation of the benzimidazole derivatives started with the 2-chloronicotinic acid **11** or ester **12**, which were reacted with an aromatic amine to provided **13a** and **14**. In the case of **14**, the ester group was first hydrolyzed to **13b**, and the carboxylic acid intermediates were then engaged in condensation reactions with phenylenediamine reagents to give rise to the benzimidazole compounds **15a**−**e**, **16** and **17**. For compounds **16** and **17** (W = Cl), final N-alkylation, followed by separation of the regioisomers, led to **15f**−**i**.

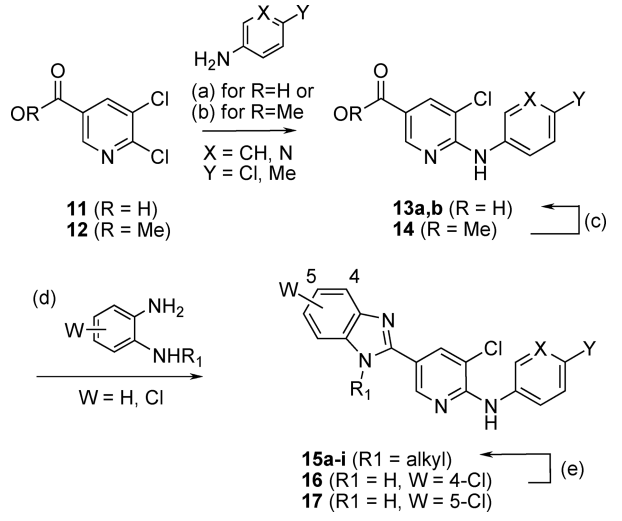
The bicyclic derivative **7a**, which can be seen as a cyclized version of compound **2**, served as a lead for our optimization. The SAR evaluation around the imidazole moiety started with

Scheme 2[a]



[a] Reagents and conditions: for X = Br, (a) NaH, THF, 70 °C, 14 h; (c) BuLi, $B(OiPr)_3$, THF, −78 °C to rt, 3 h; (e) $Pd(PPh_3)_4$, Na_2CO_3, C_6H_6, MeOH, 120 °C, 40 min; for X = CN, (b) $Pd(OAc)_2$, *rac*-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (*rac*-BINAP), K_2CO_3, toluene, 100 °C, 16 h; (d) NaOMe, NH_4Cl, MeOH, 65 °C, 2 h; (f) (i) NH_4OH, THF, H_2O, 80 °C, 5 h−18 h; (ii) R_1I, K_2CO_3, DMF, rt, 18 h.

Scheme 3[a]



[a] Reagents and conditions: for R = H, (a) AcOH, 150 °C, 75 min; for R = Me, (b) $Pd(dba)_3$, *rac*-BINAP, K_2CO_3, toluene, 160 °C, 2 h; (c) 1 M NaOH, MeOH, rt, 1 h; (d) Polyphosphoric acid (PPA), 180-210 °C, 0.1−18 h; (e) R_1I, NaH, DMF, rt, o/n.

structurally simpler derivative, where the fused 6-membered ring of **7a** was opened. The 1,5-dimethylimidazole derivative **7b** displayed only marginal activity, whereas the 1,4-dimethyl analogue **7c** exhibited micromolar potency. Exploration of the *N*-alkyl substituent permitted identification of the potent *n*-propyl derivative **7d**. Further exploration of the 4- and 5-positions led to compound **7e**, and subsequent bridging of those two positions into a benzimidazole moiety resulted in **15a**, the most active derivatives of this subset (Table 1).

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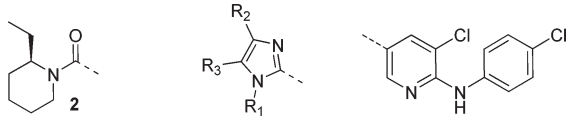
Table 1. Bioisosteric Replacement of the Piperidyl Amide Fragment: *In Vitro* hmGluR5 Antagonism



compd	R_1	R_3	R_2	IC_{50} [μM][a]
2				0.064
7a	$-(CH_2)_4-$		H	1.2
7b	Me	Me	H	48% @ 10
7c	Me	H	Me	2.9
7d	Pr	H	Me	0.23
7e	Pr	Me	Me	0.25
15a	Pr	−phenyl−[b]		0.18

[a] hmGluR5 Ca^{2+} flux[7] using glutamate as agonist; data are geometric means of $n \geq 2$. [b] Benzimidazole.

Having identified the benzimidazole moiety as a suitable bioisostere for the former piperidyl amide, the 4-chloroaniline right-hand fragment was exchanged for the less lipophilic and more soluble 3-amino-6-methylpyridyl group as in **1**, leading to compound **15b**.

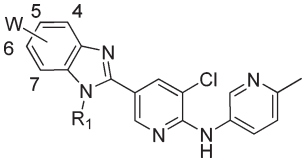
Further exploration of the SAR around the benzimidazole moiety revealed that structural variation of the N-substituent was tolerated with minimal impact on the potency, but significant difference in terms of human microsomal stability (**15c−e**). Subsequently, a "chloro screen" on the benzimidazole moiety (**15f−i**) permitted identification of the 7-chlorobenzimidazole **15i** as the most potent analogue of this series (Table 2). Due to its improved potency, **15i** was selected for further profiling *in vitro* and *in vivo* despite a similarly high intrinsic clearance in liver microsomes compared to original compound **1**.

In vitro radioligand displacement assays utilizing [^{3}H]-ABP688[20] showed that **15i** is a high-affinity ligand at the previously characterized allosteric binding site located in the membrane-spanning region of mGlu5 receptors[21] with a K_i at the human recombinant receptor of 2.4 nM.[22] In addition, **15i** showed a high degree of selectivity in functional assays over representatives of group I, II and III metabotropic glutamate receptor subtypes (IC_{50} > 10 μM for hmGluR1, -2, -7) and ionotropic glutamate receptors (IC_{50} > 10 μM).[23]

Single-dose pharmacokinetic studies of **15i** in rats showed that this compound is superior to **1** in terms of lower plasma clearance, prolonged mean residence time and apparent elimination half-life. This could not be expected based on the similar intrinsic clearance of both compounds in liver microsomes, but might be explained by the higher plasma protein binding of **15i**,[24] resulting in a protective effect *in vivo*. Brain penetration and bioavailability were similar (Table 3).

As in a preceding study[16] with **1**, we assessed efficacy and potency of **15i** *in vivo* by determining the compound's effect on the expression of fear-potentiated startle (FPS) in rats.[25,26] In the present study, rats were orally treated with 0.3, 1, and 3 mg/kg of **15i,** or vehicle 60 min prior to the behavioral test (group size n = 10). The FPS response was reduced in a dose-dependent manner; the effects caused by 1 and 3 mg/kg were statistically significant vs the vehicle control (Figure 2). The

Table 2. Optimization of Benzimidazole Fragment: *In Vitro* hmGluR5 Antagonism



compd	R_1	4-W	5-W	6-W	7-W	IC_{50} [μM][a]	HLM[b]	RLM[b]
15b	Pr	H	H	H	H	0.10	83	158
15c	Et	H	H	H	H	0.11	120	118
15d	Bu	H	H	H	H	0.36	347	279
15e	*i*Bu	H	H	H	H	0.11	55	826
15f	Pr	Cl	H	H	H	0.20	74	69
15g	Pr	H	Cl	H	H	> 0.5	400	34
15h	Pr	H	H	Cl	H	0.13	56	33
15i	Pr	H	H	H	Cl	0.024	83	112

[a] hmGluR5 Ca^{2+} flux[7] using glutamate as agonist; data are geometric means of $n \geq 2$. [b] HLM: human liver microsomes. RLM: rat liver microsomes. CL_{int} (intrinsic clearance) reported in μL/min/mg protein.

Table 3. Pharmacokinetic Parameters[a] of **15i**[b] and **1**[c]

	15i	**1**
plasmaCL (mL/min/kg)	15	37
MRT (h)	2.2	0.9
$t_{1/2}$ (h)	1.9	0.9
T_{max} (h)	0.5	0.25
C_{max}[d] (pmol/mL/μmol/kg)	60	41
AUC_{po}[d] (pmol h/mL/μmol/kg)	449	241
F (%)	41	54
brain/plasma-AUCpo ratio	1.67	1.36

[a] n = 3 Sprague−Dawley rats/group. [b] 3.6 μmol/kg po, 2.4 μmol/kg iv. [c] 30 μmol/kg po, 10 μmol/kg iv. [d] Dose-normalized value.

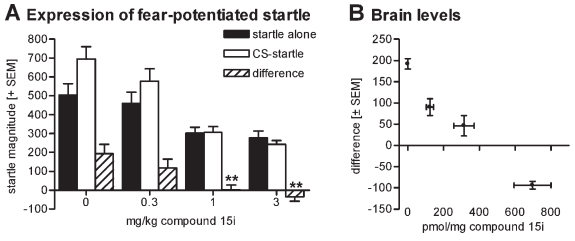


Figure 2. (A) Expression of fear-potentiated and baseline startle response (0.3−3 mg/kg compound **15i**). Mean startle magnitudes and SEMs of startle stimulus alone and CS-startle stimulus trials, respectively, as well as the difference. Multifactorial ANOVA: interaction treatment × trial type: F3,36 = 7.49, $p < 0.001$, $p = 0.001$; $^{**}p < 0.01$ (Dunnett post hoc test vs 0 mg/kg). (B) Brain concentration (mean ± SEM) of compound **15i** in the different treatment groups as well as the mean percent startle difference (±SEM). Compound **15i** brain levels are negatively correlated with the expression of fear-potentiated startle. For experimental details see the Supporting Information.

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minimal effective dose of **15i** is thus considered to be 1 mg/kg po in this paradigm, whereas we found 3 mg/kg for **1**.[25,26]

The amount of FPS reduction induced by **15i** was positively correlated with the compound's plasma and brain concentrations, measured in a subset (n = 4) of the animals that underwent the FPS test (samples collected at 90 min postdose). Both plasma and brain concentrations were proportional to the applied doses (0.3 to 3 mg/kg); the brain/plasma ratio varied between 1.2 and 2.0.

In conclusion, biotransformation investigations with compound **1** identified structural liabilities that guided a focused chemical optimization effort resulting in **15i**, a selective, orally bioavailable mGluR5 antagonist of similar potency and affinity *in vitro* and superior metabolic stability compared to **1**. *In vivo* characterization of **15i** in the FPS model revealed a robust anxiolytic-like effect with a minimal effective dose of 1 mg/kg po and positive correlation with brain and plasma exposures. In view of this promising profile, compound **15i** was considered for further development.

SUPPORTING INFORMATION AVAILABLE Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author: *E-mail: ralf.glatthar@novartis.com.

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(18) Abundances of components are not corrected for different ionization efficiencies in electrospray

(19) Compound **6** was prepared by bromination of commercially available 5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine. See Supporting Information for experimental details.

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(22) A similar binding affinity (K_i: 30 nM) was observed in membranes prepared from cells expressing rat brain tissue (cortex/hippocampus).
(23) Selectivity to ionotropic glutamate receptors was assessed with radioligand displacement assays in rat brain membranes at the AMPA (glutamate binding site), kainate (glutamate binding site) and NMDA (glutamate and glycine binding sites) subtypes.
(24) Rat plasma protein binding of compounds **15i** and **1** were assessed by equilibrium dialysis. **15i** showed a free fraction of 1.7% whereas **1** showed a free fraction of 12.7%.
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