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Non-competitive inhibitors of metabotropic glutamate receptor 5 (mGluR5)

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Abstract—Based on a pharmacophore alignment on known non-competitive mGluR5 inhibitors applying 4SCan® technology, a new lead series was identified and further structurally investigated. K_i's as low as around 100 nM were achieved.

Activation of metabotropic glutamate receptors (mGluRs) modulates neuroplasticity and neuronal excitability, rendering these receptors intriguing therapeutic targets for certain neurological and psychiatric disorders, such as ischemia, schizophrenia, or chronic neurodegenerative diseases. Interference with this process has a potential for analgesic or anxiolytic treatment.[1–5] mGluRs belong to type C superfamily of G-protein-coupled receptors and are further organized into groups according to sequence homology, signal transduction mechanism, and agonist selectivity.[3,6] Group I receptors, that is, mGluR1 and mGluR5, are mainly excitatory and preferentially activate phosphoinositide-specific phospholipase C. Receptors of groups II and III (mGluRs 2, 3, and 4, 6–8, respectively), are negatively coupled to adenylate cyclase.[1–3,5,7] Antagonists for group I or agonists for group II/III mGluRs possess potential as neuroprotective agents. Especially for mGluR5, an important role in nociception was proven, making this receptor highly interesting for the treatment of neuropathic/chronic pain.[1,3,4]

Binding of glutamate to the extracellular binding site of mGluRs stabilizes an active conformation within a complex equilibrium of different active/inactive conformations of the receptor, resulting in G-protein activation. Non-competitive inhibitors bind to the transmembrane domain and seem to interrupt the transfer of conformational changes from the amino-terminal domain through the transmembrane region toward G-protein activation.[3,4,7–10] In contrast to the glutamate binding site, which is naturally highly conserved throughout all groups of mGluRs, it was anticipated to achieve higher subtype selectivity addressing the transmembrane domain with a non-competitive inhibitor rather than with a competitive one for the active site. As crystal structure data was only available for a truncated extracellular domain, including the active site,[8] a usually preferable in silico docking approach could not be applied to the discovery of new structural types of non-competitive inhibitors. With two known non-competitive inhibitors for mGluR5 at hand, MMPEP (**1**) from Novartis and **2** from NPS Pharmaceuticals (Fig. 1), displaying IC_{50} values of 10 and 74 nM at mGluR5, respectively,[11,12] a pharmacophore alignment on these compounds was performed using 4SCan®[13] with a virtual library of around 3.3 Mio commercially available compounds. This procedure resulted in the selection of 188 compounds for an initial and further 123 for a follow-up screen in a binding assay on mGluR5,[14] which directly led to the identification of a lead structure (Fig. 1), with representatives possessing K_i's below 200 nM and an excellent selectivity profile: Compounds **A3/B5** and **B3** (Tables 1 and 2) were virtually inactive at mGluR1, all mGluRs of group II and III, 5-HT_{1A}, μ opioid and dopamine D2 receptors, with K_i's above 10 μM.

Keywords: Metabotropic glutamate receptor; Molecular modeling; Non-competitive antagonist; Neuroplasticity.
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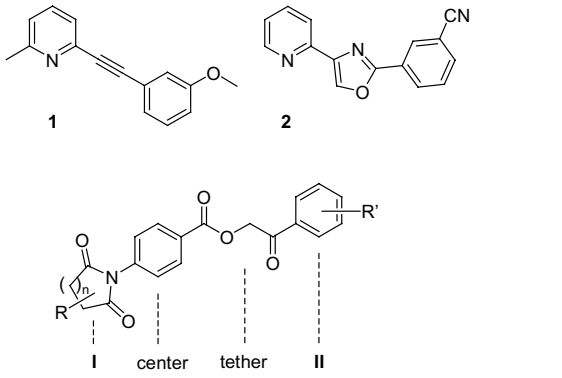
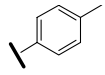
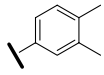
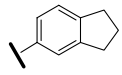
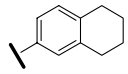
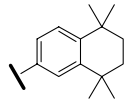
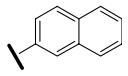
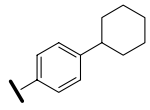
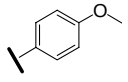
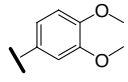
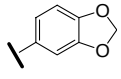
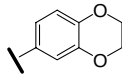
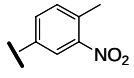
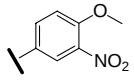
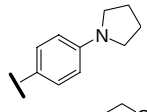
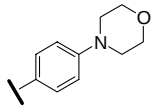


Figure 1. Known non-competitive inhibitors **1** and **2** for mGluR5 and the identified new lead structure.

Based on this structure, a synthetic route was established, allowing for a simple derivatization of portions I and II (Fig. 1) of the molecule in a highly convergent manner. Starting either with 4-aminobenzoic acid (**3a**) or its sodium salt **3b** enables the initial attachment of the anhydride- (I) or phenacyl-portion (II), respectively, and subsequent systematic SAR exploration on the remaining side of the molecule (Scheme 1). Whereas carboxylate **3b** reacted directly with corresponding phenacylbromides **6a**[15] in DMF to give keto-ester **7**, KF as weak base was required for this step to take place using a carboxylic acid **5**.[16] Imide formation with differently substituted anhydrides **4** was achieved either neatly (melting point of the anhydride <150 °C), or under microwave irradiation. For substrates with melting points between 150 and 190 °C, a smooth reaction proceeding was achieved by addition of a few drops DMF to the neat reaction mixture to give a highly concentrated solution/melt at 160 °C. For substrates with a mono-cyclic six-membered or a heteroaromatic anhydride, conversion usually stopped at the stage of the corresponding amide, lacking the final condensation to the imide unit. In those cases, EDC in DMF was necessary for imide closure, additional catalytic DMAP was added to conversions proceeding too sluggishly.[17]

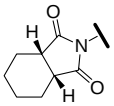
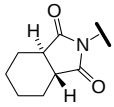
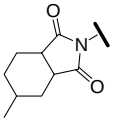
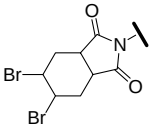
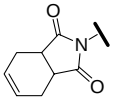
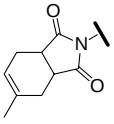
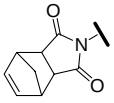
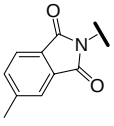
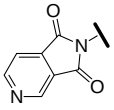
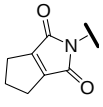
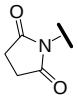
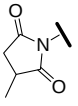
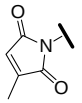
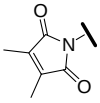
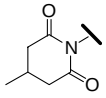
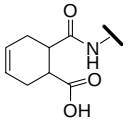
For both portions I and II of the substrate, around 30 derivatives were synthesized. *meta*-Connectivity at the central ring of these keto-esters proved to be detrimental to the activity. As becomes evident from Table 1 for part II, activity is clearly improving going from a plane phenyl ring (**A1**) via mono-substitution (**A2** and **A9**) toward 3,4-disubstitution (**A3**, **A10**). Mono-substitution in either 3- or 4-position (Cl, Br, NO_2, CN, CF_3, and piperidine tested in addition to **A2** and **A9**) resulted in low activities in general, with two exceptions being pyrrolidine- and morpholine-substituted derivatives **A15** and **A16**, which showed activity in the low micromolar range. Connecting the 3- and 4-substituents by ring closure, resulting in a more rigid framework, retained activity in case of an additional six-membered ring (**A5**, **A12** compared to **A3**, **A10**), but led to a drop in activity with five-membered rings (slightly for **A4**, drastically for **A11**). Comparing the upper and lower halves of Table 1, the more lipophilic derivatives clearly afforded lower

Table 1. Keto-ester derivatives based on structure **A** (Scheme 1) and their inhibitory activity against mGluR5

Compound	Structure **A**	K_i [nM] (inhib.@ 10 μM)
A1		— (18%)
A2		9475
A3		172
A4		401
A5		171
A6		— (32%)
A7		303
A8		18,022
A9		— (39%)
A10		1604
A11		— (21%)
A12		2037
A13		3786
A14		1683
A15		1088
A16		3359

K_i's. In order to explore the spatial nature of this hydrophobic pocket, portion II was further extended either with sterically demanding substituents (**A6**, **A8**, and with portion II being adamantyl) or flat groups (**A7**, or portion II being a *p*-biphenyl). Only rather flat structures

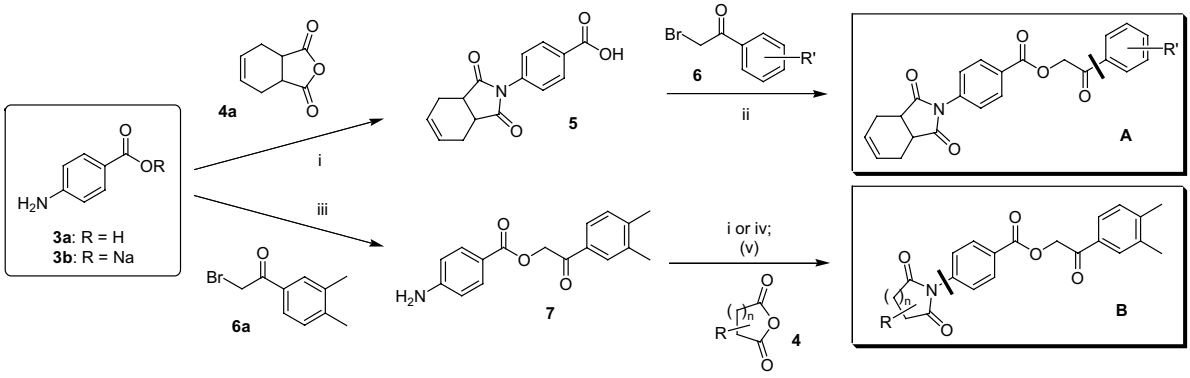
Table 2. Keto-ester derivatives based on structure **B** (Scheme 1) and their inhibitory activity against mGluR5

Compound	Structure **B**	K_i [nM] (inhib.@ 10 μM)
B1		190
B2		150
B3		113
B4		98
B5		172
B6		216
B7		1012
B8		— (28%)
B9		— (33%)
B10		— (10%)
B11		506
B12		411
B13		— (38%)
B14		4795
B15		6426
B16		3267
B17		1836
B18		— (41%)
B19		— (34%)

seem to fit nicely, with naphthalene (**A7**) or tetrahydronaphthalene (**A5**) already filling the space available. More steric bulk led to a dramatic decrease of activity. A different disubstitution pattern (e.g., 2,5-dimethyl) was not tolerated at all. The same applied to the incorporation of heteroaromatics in portion II of the molecule.

As to part I of the molecule, hexahydro- and tetrahydrophthalimide portions were most favorable (Table 2). The imide portion and the second cycle were usually *cis*-anellated, the only exception being compound **B2** (racemate). Adding a further substituent to the hexahydrophthalic moiety resulted in a slight increase in activity (**B3**, **B4**), but structurally, an additional stereocenter was introduced, whose relative configuration was investigated for **B3**. Here, an almost 1:1 ratio for *exo*- versus *endo*-methyl was determined by spin analysis of its ^{1}H NMR spectrum. Installing a double bond at that part eliminated the problem of testing mixtures of diastereomers and kept the biological activity almost within the same range (**B5**, **B6**). Increasing steric demand (norbornene unit in **B7**), or switching to phthalic portions (as e.g., in **B8**) or analogous heteroaromatics (like e.g., in **B9**) was detrimental to activity, as was an endocyclic central double bond as in **B10** and its six-ring homologue (28% inhibition at 10 μM).

Mono-cyclic imides, however, were tolerated, leading to only a slight decrease in activity of about factor 3, as long as five-membered imides were incorporated without an endocyclic double bond (cf. **B11**, **B12** vs **B14**, **B15**). The latter seems to be a general conclusion when including the poor results obtained for **B10** (and its



Scheme 1. Strategy for synthesizing a library of keto-esters. Reagents and conditions: (i) DMF, molecular sieves 4 Å, microwave, 160 °C, 90 min; (ii) KF, DMF, 65 °C, 3 h; (iii) DMF, rt, 2 h; (iv) neat, 160 °C, 1 h; (v) EDC, optional: DMAP, DMF, rt, 20 h.

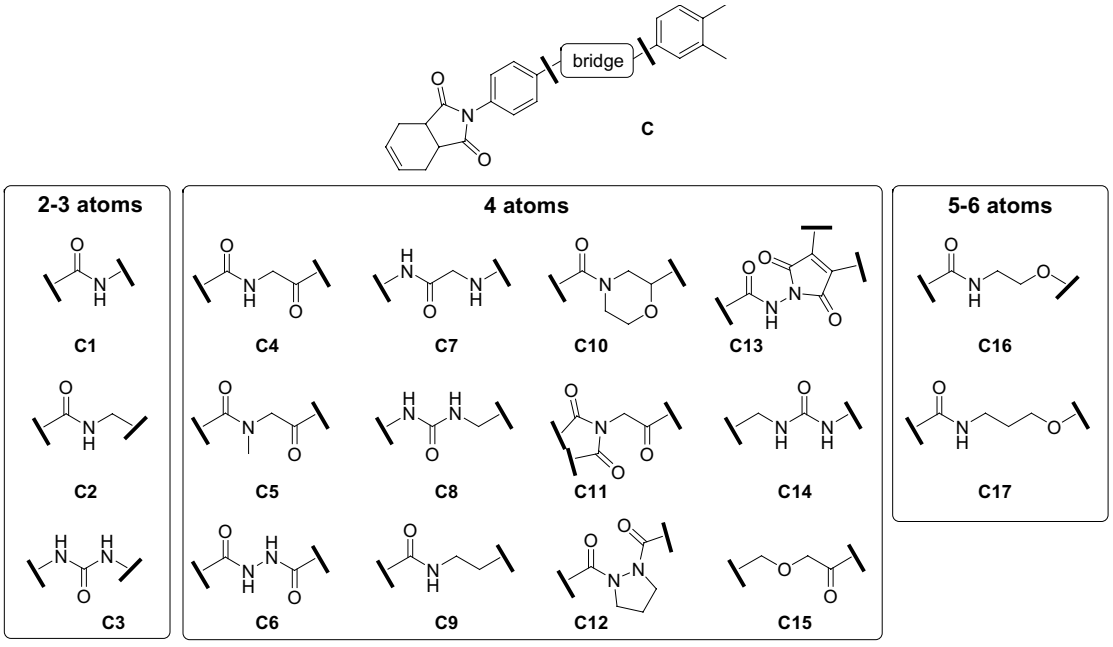


Figure 2. Replacement of the keto-ester bridge by several alternatives, activity on mGluR5 in general below 35% inhibition at 10 μM.

six-ring homologue) and phthalic derivatives **B8** and **B9**. Six-membered imides still displayed some activity in the low micromolar range at best (**B16**, **B17** vs **B18**). Two carbonyl functionalities adjacent to the nitrogen proved to be essential (**B11** vs **B13**), as did the imide structure in general as compared to an amide (**B5** vs **B19**).

For a proof of concept, the effect of these keto-esters in a cell assay had to be investigated, in which the intracellular Ca^{2+} release was monitored, stimulated by the full agonist quisqualic acid.[18] And indeed, keto-esters showed non-competitive antagonistic activity. However, these compounds displayed some instability in human plasma, with the keto-ester bridge and the imide portion being vulnerable to hydrolysis. Inconveniently, stability in portion I of the compounds increased for those structures leading to a decrease in activity, with lactam **B13** being most stable. Its half-life due to hydrolysis of only the ester bridge was determined to be 8–17 h in dependence on human plasma samples. Most unstable proved to be the saturated five-membered imides **B3** and **B11** with $t_{1/2}$ around 1–2 h.[19]

Addressing the instability at the ester bridge, a variety of different tether units were synthesized (Fig. 2). Having started with the obvious, a replacement of the ester unit by an amide (**C4**, **C5**), almost no activity was detectable. Upon attaining a few equally poor results, also with shortened (**C1**–**C3**) or lengthened (**C16**, **C17**) tethers, semi-empirical calculations of partial charge distributions were performed for the parent keto-ester **A3/B5**, several compounds with new bridging units already tested (**C3**–**C7**, **C9**, **C10**, **C12**) and for numerous proposed structures as one possibility to gain some insight into reasons for the loss in activity when changing just an atom within the tether and to prioritize new syntheses. By comparison of the charge distribution maxima/minima throughout the whole molecule to that of keto-ester **A3/B5**, compound **C11** displayed high similarities and was therefore most promising. However, not even this

prediction allowed for an identification of a compound with an activity above 35% inhibition at 10 μM.

In summary, a highly potent class of new mGluR5 inhibitors was identified using 4SCan® in silico technology, with activities down to K_i's of around 100 nM. Numerous derivatives synthesized allowed for a conclusive SAR study, offering a few possibilities for substitution at either portion I or II of the molecule, retaining adequate activity but providing different influences on physicochemical properties. A chemical stabilization of these substrates failed so far but is still under investigation.

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References and notes

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13. For a pharmacophore alignment, compounds of a virtual library are partitioned into fragments, an anchor fragment is selected and aligned on the parent structure, for example, MMPEP (**1**), and adjusted in a way that similar electrostatic and hydrophobic interaction possibilities become superimposed (unlike a structural alignment, in which similar *parts* of the molecules are superimposed). Next fragment is then connected to the first one and again adjusted for maximal interaction identities based on the position chosen for the first fragment. Several permutations of starting fragments are evaluated, resulting in a pharmacophore alignment of maximal similarities in interaction possibilities. For details, see: Seifert, M. H. J.; Wolf, K.; Vitt, D. *Biosilico* **2003**, *1*, 143.
14. Affinity of compounds at mGlu5 receptors was evaluated utilizing rat brain membranes and the ligand [^{3}H]MMPEP according to: Mutel, V.; Ellis, G. J.; Adam, G.; Chaboz, S.; Nilly, A.; Messer, J.; Blueuel, Z.; Metzler, V.; Malherbe, P.; Schlaeger, E.-J.; Roughley, B. S.; Faull, R. L. M.; Richards, G. *J. Neurochem.* **2000**, *75*, 2590.
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17. Products showed >95% purity as determined by LC–MS and NMR spectroscopy.
18. Functional effects of the compounds were evaluated in a FLIPR assay on CHO cells transfected with mGlu5 receptors.
19. All compounds tested were completely stable in physiological buffer (PBS) for at least 24 h.