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Therapeutic potential of group III metabotropic glutamate receptor ligands in pain

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ABSTRACT

Metabotropic glutamate receptors (mGluRs) modulate neurotransmission all along the pain neuraxis. While the involvement of group I and group II mGluRs in pain is well documented, information has only just started to emerge concerning the role and contribution of group III mGluRs subtypes to pain modulation. Recent data suggests that these receptors reduce symptoms in animal models of chronic pain, as well as regulate neurotransmission at different levels of ascending and descending pain pathway, suggesting that group III mGluRs may be interesting therapeutic targets for the development of analgesics.

INTRODUCTION

Pain is one of the most common symptoms in clinical medicine and represents a permanent medical problem, being an essential component in the therapeutic management of many diseases. Pain can be classified as acute when it is short lasting or chronic when it persists for a long time after the original affection. Acute pain serves the important function of protecting the integrity of the body by detecting actual or potential tissue damage. Chronic pain is among the most debilitating and costly afflictions in North America and Europe, seriously affecting the quality of life of more than 19% of adult Europeans [1-3]. Unfortunately, while acute pain can be correctly managed, chronic pain is not efficiently alleviated by current treatments [4-6]. Therefore, a better understanding of cellular and molecular pathophysiological mechanisms is essential for identifying new pharmacological targets.

Glutamate and pain

Glutamate is the main excitatory neurotransmitter of the mammalian central nervous system and is implicated in many physiological and pathological processes. Glutamate is notably the main neurotransmitter involved in pain transmission. At the synaptic level, glutamate activates two classes of receptors: ionotropic and metabotropic glutamate receptors (mGluRs). Central sensitization of the pain neuraxis is associated with hyperexcitability of the glutamatergic system and leads to the development of the evoked pain symptoms, allodynia (pain due to a stimulus that does not normally provoke pain) and hyperalgesia (increased pain from a stimulus that normally provokes pain) observed in patients with chronic pain [7]. Both iGluRs and mGluRs are involved in the induction and the maintenance of this sensitization. The blockade of increased glutamatergic activity may represent a pivotal mean to reduce chronic pain but awaits a clearer identification of adequate targets.

Metabotropic glutamate receptors

mGluRs are G-protein coupled receptors activated by glutamate, the major excitatory neurotransmitter of the central nervous system (CNS). They are involved in the modulation of synaptic activity. They are thus considered as potential therapeutic targets since less side effects are anticipated compared to essential actors of synaptic transmission.

The 8 members of this family are classified in 3 groups: Group I receptors (mGlu1 and 5) are post-synaptic and positively modulate glutamatergic transmission while group II (mGlu2 and 3) and group III receptors (mGlu4, 6, 7 and 8) are predominantly presynaptic and play an inhibitory role on neurotransmission (except for mGlu6, a post-synaptic receptor which is expressed solely in bipolar ON cells in the retina). Group III mGluRs can act either as autoreceptors on glutamatergic terminals or heteroreceptors on GABAergic terminals.

mGluRs form constitutive dimers composed of two subunits cross-linked by a disulphide bridge. Dimer formation is mandatory for the function of these receptors [8]. It has long been believed that mGluRs strictly assemble into homodimers but a recent study has shown that certain mGluR subtypes can heterodimerize *in vitro* [9]. Heterodimerization could have consequences notably in terms of pharmacological profile, signalling response and protein partners. To date, there is no clear evidence of heterodimerization of mGluRs *in vivo*. However, recently, mGlu2/4 heterodimers have been suspected of existing at corticostriatal synapses, based on the detection of a unique pharmacological profile as compared to the mGlu2 or mGlu4 homodimers [10]. Since compatible mGluR subtypes coexist in several regions of the CNS, our comprehension of the regulation of CNS function by mGluRs, including pain, may evolve rapidly in the light of heterodimerization.

The different subtypes of mGluRs are expressed all along the pain neuraxis where they modulate the perception of pain (Figure 1). In general, blocking group-I mGluRs or activating group-II mGluRs alleviates pain (see [11-13] for recent reviews).

The present review will focus on group III mGluRs for which less information is available, mainly due to the lack of selective pharmacological tools. However, the recent progress in the development of subtype selective ligands are opening the way to a better understanding of their modulatory function and therapeutic potential in pain.

GROUP III MGLUR PHARMACOLOGY

As described in Figure 1, mGlu4, 7, 8 receptors of group III are localized all along the pain neuraxis. In order to investigate if these receptors may be novel therapeutic targets to reduce pain symptoms, various ligands were used (Figure 2). However, interpretation of these experiments should take into consideration the limitations of these compounds. Most of the drugs that were evaluated are non-selective among the group III subtypes or display metabolic instability. Moreover, a 100 ratio of EC50's in cell-based assays does not ensure a selective effect *in vivo* since other factors such as bioavailability, receptor localization and drug concentration may also have an influence.

Both orthosteric ligands and allosteric modulators have been employed. These ligands are listed in this chapter, together with their effects and, when known, their limitations.

Orthosteric ligands

Since mGlu4, 7, 8 receptors are mostly presynaptic, agonists were used to evaluate if down-regulation of neurotransmitter release mediated by their activation may provide any benefit to treat

pain symptoms (Figure 2). The most widely applied drug is **L-AP4**, a selective agonist of group III mGlu receptors, which does not discriminate between mGlu4 and 8 receptors, that has been used in inflammatory and neuropathic pain models [14-24]. L-AP4 is about 10 times more potent than glutamate, however, it does not cross the blood brain barrier and needs to be injected *in situ*. **L-SOP**, a phosphate analog of L-AP4 with similar properties, has been used in early studies about the contribution of group III mGluRs in the periaqueductal gray to the regulation of nociception [25,26]. ACPT-I is a group III mGluR agonist demonstrating similar selectivity to L-AP4 but is able to be administered systemically [27,28]. The use of **ACPT-I** [27] provided successful results in rat models of inflammatory and neuropathic pain, while leaving acute pain perception unchanged in healthy rats [29]. The weaker agonist, **ACPT-III**, was also used and shown to enhance the antiallodynic action of morphine in a neuropathic pain model in rats [30]. Two compounds **(S)-DCPG** [31] and **LSP4-2022** [32] have been tested as mGlu8 and mGlu4 receptor subtype selective agonists, respectively, in inflammatory or neuropathic pain models [23,33-37]. However, a recent study suggests that care must be taken when employing higher concentrations of DCPG since non-selective effects in slices from mice lacking mGlu8 receptors have been observed with DCPG concentrations above 1 μ M, primarily mediated by mGlu2 receptors [38].

Competitive group III mGlu receptor antagonists have been used to assess the validity of agonist effects but also to verify the interaction between glutamatergic and GABAergic synapses in the spinal dorsal horn after stimulation of the nociceptive primary afferents [39]. The most potent group III antagonist is **LY341495**, however, it is even more potent at group II mGlu receptors [40]. Nevertheless, it has been used in several studies [17,18,20,22,29,39,41]. Other antagonists, such as **MAP4**, **MSOP** and **CPPG**, are more specific to group III but are weaker. Yet they were still useful tools in most pain studies (MAP4 [16,29,35,41,42], MSOP[20,23,25,34,43-46], CPPG [17-19,39])

Allosteric modulators

Positive allosteric modulators (PAMs) have been assayed for pain relief (Figure 2). **PHCCC**, the first identified mGlu4 selective PAM [47,48], revealed similar effects to ACPT-I in inflammatory or neuropathic pain models [29]. Another mGlu4 PAM, **VU155041** [49], attenuated mechanical hyperalgesia in a neuropathic pain rat model when injected intrathecally [54], but was ineffective in changing thermal nociception and ON and OFF cell activity when injected in the dorsal striatum of naive or neuropathic rats [37]. The discovery of new mGlu4 receptor PAMs [50] should allow further investigations. An mGlu8 receptor PAM, **AZ12216052** [51], behaved like (S)-DCPG, the orthosteric agonist with similar selectivity, increasing tail flick latency and OFF cell activity and decreasing ON cell activity in neuropathic rats after injection into the dorsal striatum [37]. However, AZ12216052

reduced anxiety in mGlu8 KO mice, suggesting that this PAM may also act on other receptors mGlu8 [52]. The mGlu7 agonist-PAM **AMN082** [53] has attracted much interest and was introduced in the largest evaluation of group-III PAMs' [21,23,33-35,46,54,55]. However, contradictory results were described and should be taken with precaution as AMN082 is rapidly metabolized into a monoamine transporter inhibitor [56]. A negative allosteric modulator (NAM) of mGlu7 receptor, **MMPIP** [57], was recently used in series of experiments, showing the antinociceptive effect of the blockade of this particular receptor in the ventrolateral periaqueductal gray in formalin and neuropathic pain models [58]. Interestingly, the inhibiting activity of MMPIP has been reported to be context dependent, meaning that MMPIP may not antagonize the coupling of mGlu7 to its native signaling pathways in all cellular contexts, as exemplified by its inability to block agonist-mediated responses at the Schaffer collateral-CA1 synapse, where mGlu7 has been previously shown to modulate neurotransmission [59]. This context-dependent signaling bias has to be taken into consideration when using this compound and its derivatives. In addition, the new mGlu7 NAM's **ADX71743** [60] and **XAP044** [61] should allow future investigations.

PHENOTYPES OF GROUP III MGLURS KNOCK-OUT MICE

Mice lacking mGlu4 receptors display normal spontaneous motor activity but present an impaired ability to learn and memorize [62,63]. The sensitivity to strong noxious mechanical compression is altered and the onset of the nociceptive behavior in the inflammatory phase of the formalin test is accelerated in mGlu4 KO mice as compared to their wild type littermates, whereas responses to punctate mechanical stimulation and nocifensive responses to thermal noxious stimuli are not modified [36]. To our knowledge, the sensory perception phenotypes of mGlu7 and mGlu8 KO mice have not been determined. Mice lacking mGlu7 present increased seizure susceptibility [64] together with an antidepressant and anxiolytic-like phenotype [65] while mGlu8 KO mice exhibit an anxiogenic phenotype [51,66,67]. Interestingly, subtle differences in amygdala-dependent behavior and physiology between mGlu7 and mGlu8 KO mice suggest differential roles of these receptors. Indeed, while mGlu7 KO mice display a general deficit in conditioned fear, mGlu8 KO mice show a dramatic reduction in contextual fear [68]. These results are well correlated with studies that used a pharmacological approach (see below). Since there is a strong comorbidity between chronic pain, depression and anxiety [4], targeting these receptors may be of interest in chronic pain.

LOCALIZATION AND ROLE OF GROUP III MGLURS IN THE PAIN NEURAXIS

The presence of group III mGluRs has been identified in different areas involved in pain processing (Figure 1), with the exception of mGlu6 which is exclusively expressed in the retina.

Periphery

Few studies have addressed the role of peripheral group III mGluRs in pain. Intraplantar group III mGluR agonist L-AP4 delivery attenuates the increased nociception induced by capsaicin [22,69] or bee venom [20], while administration of L-AP4 into the knee joint reduces hyperalgesia induced by carrageenan-induced arthritic pain [24]. mGlu8 has been identified in the unmyelinated axons of digital nerves [69]. To our knowledge, the expression of other subtypes of group III mGluRs that may also mediate L-AP4 action in the periphery has not been documented.

Spinal cord

The superficial laminae of the dorsal horn of the spinal cord receives sensory information from primary afferents responding to noxious and non-noxious stimuli (see [70] for a comprehensive review on pain processing in the dorsal horn). mGlu4 is present in the inner lamina II of the dorsal horn of spinal cord [71-73], more precisely, both in the presynaptic terminal of unmyelinated C-fibers and spinal neurons terminals [36]. mGlu7 is located in presynaptic terminal of sensory neurons in the laminae I and II in the dorsal horn [73] but the nature of sensory neurons expressing mGlu7 remains to be determined. mGlu8 is located in the soma of TRPV1 expressing-unmyelinated DRG neurons [69,74], but doesn't seem to be present in the spinal cord [75,76].

Interestingly, spinal group III mGluRs, and mGlu4 in particular, seem to be devoted to the modulation of hypersensitivity to applied stimuli observed in animal models of persistent or chronic pain, whilst leaving reactions to acute noxious stimuli unchanged in naive animals. Indeed, spinal group III mGluR activation by intrathecal injection of selective group III agonists (L-AP4 or ACPT-I) reduces hypersensitivity to pain in animal models of inflammatory or neuropathic pain without affecting acute pain perception in healthy animals [15,16,29,77]. Moreover, activation of spinal group III mGluRs decreases firing of spinal cord dorsal horn projection neurons [16] and their ability to control the excess of excitatory transmission is reinforced in spinal cord of inflamed or neuropathic animals [19,36].

Similar effects are obtained by the selective activation of spinal mGlu4. Indeed, intrathecal application of the selective mGlu4 agonist LSP4-2022 [36], or the selective mGlu4 PAMs PHCCC [29] and VU0155041 [55], alleviates pain hypersensitivity in animal models of chronic pain without

affecting acute pain in healthy animals. Interestingly, the systemic injection of mGlu4 agonist LSP4-2022 abolishes mechanical hypersensitivity in inflammation [36]. On the other hand, intrathecal administration of AMN082, an mGlu7 PAM, does not reduce hyperalgesia in neuropathic pain [55].

Supraspinal

At the supraspinal level, the various subtypes of group III mGluRs can be found in the different areas of the pain ascending and descending pathways (Figure 1).

The main ascending projections from dorsal horn neurons are directed toward the thalamus and the midbrain periaqueductal grey region (PAG). The thalamus is notably an essential relay and processing point of nociceptive inputs from the spinal cord to the cerebral cortex. The thalamus shows an intense labeling for almost all mGluRs, as revealed by *in situ* hybridization [78]. However, the potential role of group III mGluRs in the processing of sensory information in the thalamus remains to be clarified.

The amygdala is an important center for the processing of the emotional component associated with pain [79]. 'In arthritic rats, the group III agonist, L-AP4, inhibits the pain-related synaptic plasticity of the central nucleus of the amygdala (CeA), the output structure that projects to the hypothalamus and several brainstem regions such the PAG [80].

An important pain descending pathway originates within PAG and projects to the spinal dorsal horn via the rostral ventromedial medulla (RVM) [81]. Activation of this descending system elicits analgesia by inhibiting ascending nociceptive transmission at the spinal cord level. Stimulation of group III mGluRs in the PAG facilitates pain behavior in naïve animals and in the formalin test [26,82].

So far, preclinical studies aimed to decipher the individual roles of group III mGluRs in the supraspinal structures involved in pain processing have mainly focused on the opposing effects of mGlu7 and mGlu8 (See [13,83] for extensive and recent reviews on the subject). Indeed, while systemic administration of the mGlu7 PAM AMN082 decreases allodynia in an animal model of neuropathic pain [54], and systemic administration of the mGlu8 selective agonist (S)-3,4-DCPG reduces hyperalgesia induced by inflammatory or neuropathic pain model [34], opposing effects are observed these two receptors are observed at the level of PAG, RVM or amygdala. Blockade of mGlu7 by microinjection of the selective mGlu7 NAM MMPiP in the PAG reduces the pain related behaviors in formalin and neuropathic pain models and differentially modulates RVM ON and OFF cell activity [58], while its activation increases pain [33]. On the other hand, injection of the mGlu8 selective

agonist (S)-3,4-DCPG into the PAG alleviates pain in mice models of inflammatory and neuropathic pain [33,34]. Moreover, activation of mGlu8 in the dorsal striatum by local injection of (S)-3,4-DCPG or AZ12216052, a selective mGlu8 PAM, inhibits ON cells and stimulates OFF cells within RVM and reduces thermoceptive responses and mechanical allodynia in neuropathic pain [37]. At the level of the amygdala, activation of mGlu7 in the CeA by AMN082 aggravates sensory and emotional components in a model of arthritic inflammatory pain, while mGlu8 activation by (S)-3,4-DCPG reduces them [84,85]. Interestingly, the opposite effects of mGlu7 and mGlu8 activation might be correlated with their respective autoreceptor or heteroreceptor role on glutamatergic or GABAergic terminals in PAG [33] and their segregation in different neuronal pathways within the amygdala [68,86].

CONCLUSION

Taken together, these data suggest that group III mGluRs could be interesting therapeutic targets to alleviate chronic pain. Indeed, these receptors seem to be dedicated to the modulation of pain hypersensitivity and cognitive and emotional impairments observed in preclinical models of chronic pain. However, with the help of the novel selective pharmacological tools, further studies will be needed in order to clarify the precise roles of each subtype and their therapeutic potential.

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CONFLICT OF INTEREST

Nothing declared

ABBREVIATIONS

ACPT, (1*S*,3*R*,4*S*)-1-amino-cyclopentane-1,3,4-tricarboxylic acid; AP4, 2-amino-4-phosphonobutyric acid; DCPG, (*S*)-3,4-dicarboxyphenylglycine; CPPG, α -cyclopropyl-4-phosphonophenylglycine; LY341495 (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid; LSP4-2022 2-amino-4-(((4-(carboxymethoxy)phenyl)(hydroxy)methyl)(hydroxy)phosphoryl)butanoic acid; MAP4, (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid; MMPIP, 6-(4-methoxyphenyl)-5-methyl-3-pyridin-4-ylisoxazolo[4,5-*c*]pyridin-4(5*H*)-one; SOP, serine-*O*-phosphate; MSOP, α -methylserine-*O*-phosphate; PHCCC, *N*-Phenyl-7-(hydroxyimino)cyclopropa[*b*]chromen-1*a*-carboxamide

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Table 1. Potencies of ligands used to study group III mGluR function in preclinical models of pain.

	mGlu4	mGlu6	mGlu7	mGlu8	Ligand issues
Agonist (EC50 μM)					
Glu (**)	3.4	24.2	>500	2.4	
L-AP4 *	0.2	1	249	0.3	not selective among mGlu4/6/8 [87]
L-SOP [88] [87]	1-4	3	160-1200	2	not selective among mGlu4/6/8 [87]
ACPT-I (**)	1.7	10.6	280	5	not selective among mGlu4/6/8 [29]
(S)-DCPG [31](*)	8.8-3.2	3.6-3,2	>100	0.03-0.04	not selective at concentration >1 μ M [38]
LSP4-2022 (*)[32]	0.1	4.4	11.6	29.2	rapid clearance [32]
Antagonists (IC50 μM)					
LY341495 [88]	6.8-9.7	1.1-1.8	0.99	0.17	not selective among mGluRs [87]
MAP4 [88] [87]	90-190			25-105	weak potency [87]
CPPG [88]	12	4	17	11	weak potency [88]
PAM (EC50 μM)					
PHCCC (*)	5.1				lack of selectivity, relatively low potency and poor solubility [49]
VU155041 (*)	1.0				poor brain exposure [50]
AZ12216052 [51]	1.0				lack of selectivity described in a study [52]
AMN082 [53]					metabolic instability [56] (55)
NAM (IC50 μM)					
MMPIP [57]			0.22		context dependent inhibitor [59]
ADX71743[60]			0.06		poor solubility [60]
XAP044 [61]			3.5		recent disclosure, no hindsight [61]

EC50 or IC50 values are taken from the literature, except for * and ** which stands for EC50 values determined either by intracellular Ca release measurement* or inositol phosphate accumulation** on rat clone of group III mGluRs transiently expressed in HEK293 cells (personal data).

FIGURE LEGENDS

Figure 1. Localization and function of mGluRs in the pain neuraxis. The different subtypes of mGluRs are expressed all along the pain neuraxis where they modulate pain perception. Ascending pain pathway is in red and descending pathway in green. Up arrows mean that activation of a particular subtype is proalgesic while down arrows mean that activation is analgesic. PAG: Periaqueducal Gray; RVM: Rostral ventromedial medulla; DRG: Dorsal Root Ganglion.

Figure 2. Chemical structures of orthosteric and allosteric group III mGlu receptor ligands.

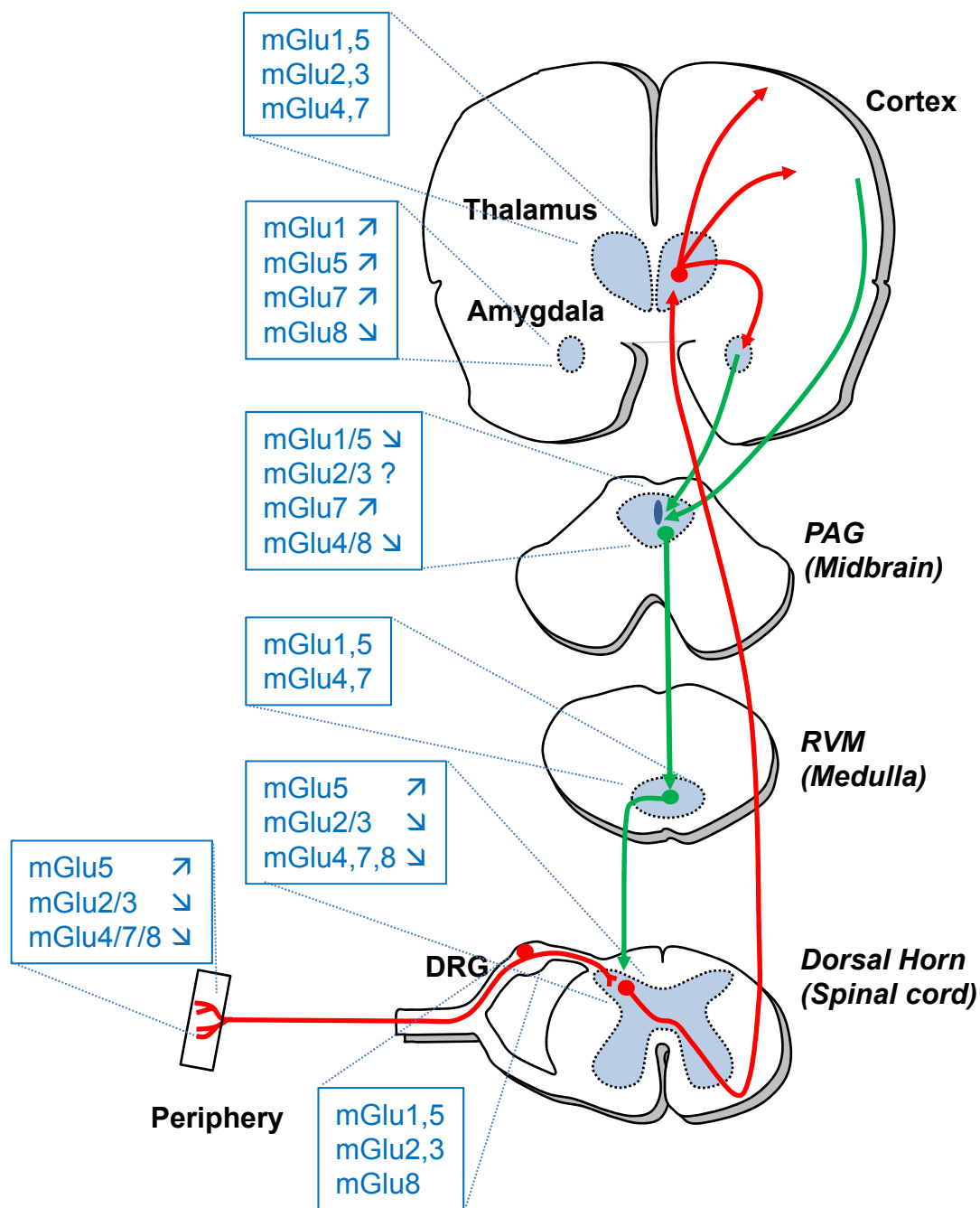
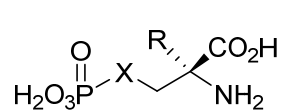
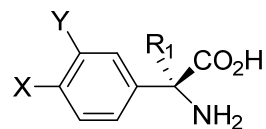


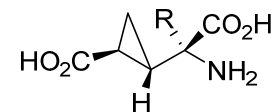
Figure 1. Localization and function of mGluRs in the pain neuraxis.



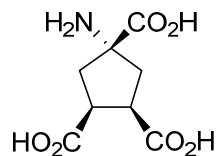
L-AP4 X = CH₂ R = H
 L-SOP X = O R = H
 MAP4 X = CH₂ R = Me
 MSOP X = O R = Me



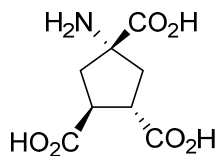
(S)-DCPG X = Y = CO₂H R₁ = H
 CPPG X = PO₃H₂ Y = H R₁ = cyclopropyl



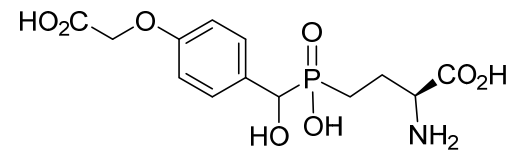
LY341495 R = 9'-xanthylmethyl



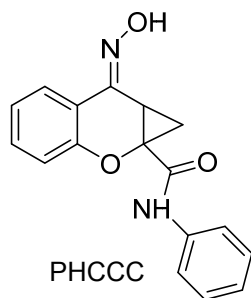
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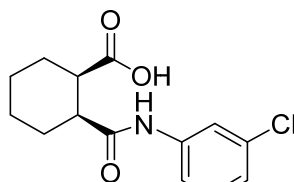
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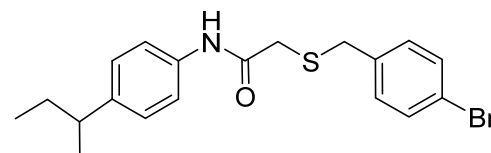
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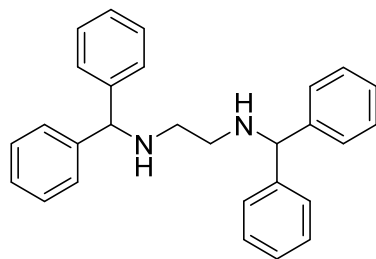
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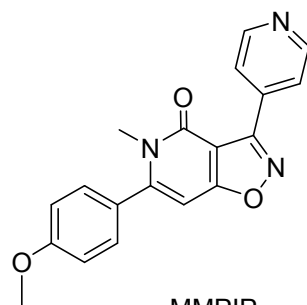
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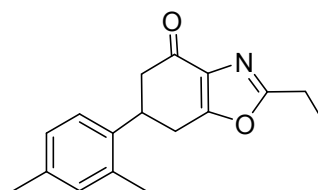
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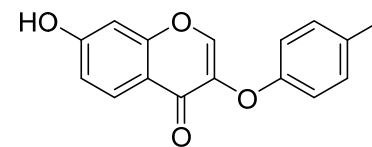
AMN082



MMPiP



ADX71743



XAP044

Figure 2. Chemical structures of orthosteric and allosteric group III mGlu receptor ligands.