ELSEVIER

Progress in the discovery and development of glutamate carboxypeptidase II inhibitors

Takashi Tsukamoto, Krystyna M. Wozniak and Barbara S. Slusher

MGI Pharma, Inc., 6611 Tributary Street, Baltimore, MD 21224, USA

During the past 10 years, substantial progress has been made in the discovery and development of small molecule glutamate carboxypeptidase II (GCP II) inhibitors. These inhibitors have provided the necessary tools to investigate the physiological role of GCP II as well as the potential therapeutic benefits of its inhibition in neurological disorders of glutamatergic dysregulation. This review article details key GCP II inhibitors discovered in the last decade and important findings from preclinical and clinical studies.

Glutamate plays a key role as an excitatory neurotransmitter in both the central and peripheral nervous systems. [1,2] Excess glutamate, however, is implicated in a number of neurological disorders, including stroke, spinal cord injury, amyotrophic lateral sclerosis (ALS), peripheral neuropathy, chronic pain, schizophrenia and epilepsy. Conventional therapeutic approaches for treating these diseases have focused on blocking postsynaptic glutamate receptors with small molecules. To this end, a wide variety of glutamate receptors have been evaluated as therapeutic targets for neurological disorders associated with excess glutamate toxicity. Of these glutamate receptors, the *N*-methyl-D-aspartic acid (NMDA) receptor has been most extensively studied over the past decade and several potent NMDA receptor antagonists have advanced to clinical trials with limited success [3–5].

An alternative therapeutic approach to blocking postsynaptic glutamate receptors would be the upstream reduction of presynaptic glutamate. One of the major sources of glutamate in the nervous system is believed to be from hydrolysis of the neuropeptide, *N*-acetylaspartylglutamate (NAAG), catalyzed by a membrane-bound extracellular metalloprotease, glutamate carboxypeptidase II (GCP II) [6]. In theory, inhibition of GCP II would prevent the release of glutamate in the extracellular space and, thereby, provide neuroprotective effects against excitotoxicity. Furthermore, GCP II inhibition would increase the levels of NAAG, which has been shown to act as an agonist at group II metabotropic glutamate receptors (mGluR), particularly $mGluR_3$ [7]. This would provide additional neuroprotection because activation of $mGluR_3$ appears to reduce the synaptic release of glutamate [8]. Inhibition of GCP II, therefore, has gained considerable attention as a strategy to suppress glutamate excitotoxicity via a dual mechanism of action [9].

The main scope of this article is to share our experience of working toward the development of GCP II inhibitors as therapeutic agents. Two excellent review articles have recently been published on related topics. One review focuses on the role of NAAG in various neurodegenerative disorders [10] while the other concentrates on GCP II inhibitors and their therapeutic potentials [11]. Another review article written a decade ago, amazingly, is still relevant to today's questions around NAAG and is highly recommended to those needing an introduction to the field [12]. These review articles serve as perfect complements to this article, which is written through the eyes of industrial scientists involved in many phases of drug discovery and development efforts surrounding GCP II inhibitors.

A brief historical view on GCP II

The existence of a NAAG-hydrolyzing enzyme was first suggested by Riveros and Orrego, who observed the release of glutamate in rat brain cortex membranes treated with NAAG. [13] Subsequently, Coyle's group identified and characterized this enzyme in the rat nervous system, which hydrolyzes NAAG into *N*-acetylaspartate and glutamate (Figure 1) [14,15]. Because of this initially identified substrate specificity, the enzyme was termed *N*-acetylated-alpha-linked acidic dipeptidase (NAALADase).

There are other names used for this protein and this is truly a reflection of its diverse functions in different tissues and organs. It

Corresponding author: Tsukamoto, T. (takashi.tsukamoto@mgipharma.com)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2007.07.010

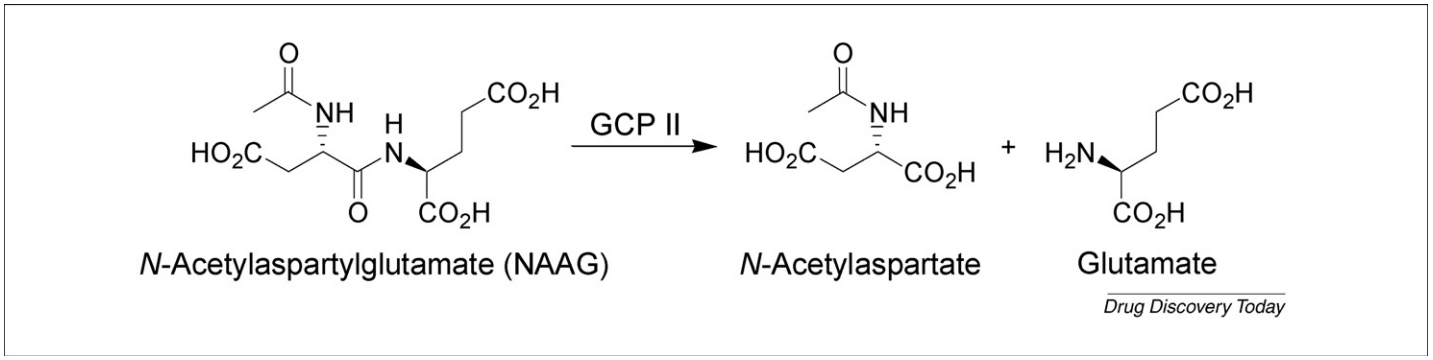

FIGURE 1

Hydrolysis of NAAG catalyzed by GCP II. GCP II inhibition may provide neuroprotective effects via a dual mechanism involving both a reduction of glutamate and an elevation of NAAG.

started to come together when Coyle's group identified prostate-specific membrane antigen (PSMA), an investigational prostatic carcinoma marker, as a NAALADase protein [16]. Subsequently, Pinto's group found that PSMA hydrolyzes the γ-glutamyl linkage of folate polyglutamate [17], which may be associated with its role in the prostate. On the other hand, Halstead's group, who had been independently investigating brush border folyl-γ-glutamate carboxypeptidase (FGCP), found that the hydrolase is derived from the same gene as NAALADase and PSMA [18].

In view of the enzyme's substrate specificity for peptides with a glutamate residue at the C-terminus, the enzyme is now listed as GCP II in the MEROPS database (see http://merops.sanger.ac.uk). Some also use the term NAAG peptidases (NPs) [10,11], referring not only to GCP II, but also GCP III, a homologous enzyme identified during the characterization of GCP II null mutant mice [19,20].

Early GCP II inhibitors

Early efforts in identifying GCP II inhibitors were directed toward peptide analogs of NAAG. This was a fairly successful approach, with meaningful outcome, since NAAG itself has a remarkably low K_m value (87–540 nM) for GCP II.

Coyle's group investigated the effect of various peptides and amino acids on GCP II-catalyzed hydrolysis of [^{3}H]-NAAG [14]. Most known GCP II inhibitors with IC_{50} values in the nanomolar range are peptides containing a glutamate residue at the C-terminus. One exception to this is quisqualic acid, which has an IC_{50} of 480 nM. Serval's group found that *N*-acetyl-L-aspartyl-β-linked-L-glutamate (β-NAAG) was a non-hydrolyzable competitive inhibitor of GCP II with a K_i value of 0.70 μM [21]. His group extended their structure–activity relationship (SAR) studies to other *N*-acylated L-glutamate analogs and identified additional GCP II inhibitors with K_i values in the low micromolar range [22]. Johnson's group synthesized a series of dipeptides containing a terminal glutamate and evaluated them for their ability to inhibit GCP II [23]. *N*-Fumaryl-L-glutamate was the most potent GCP II inhibitor identified with an IC_{50} value of 800 nM. The newly discovered folylpolyglutamate hydrolase activity of GCP II prompted Coyle's group to perform a very systematic assessment of various gamma-glutamyl peptides in the GCP II assay [24]. They identified (*N*-acyl-gamma-glutamyl)glutamate as the minimal fragment required for potent binding to GCP II.

Although these peptide-based GCP II inhibitors have not been utilized in assessing the therapeutic utility of GCP II inhibition, they have established a foundation of knowledge in substrate/inhibitor specificity of the enzyme. C-terminal glutamate residues are essential for the strong affinity to the enzyme. Requirements for the P1 position are not as stringent as those of P1' position although an additional carboxylate group at the P1 side chain appears to significantly improve the affinity. These findings have provided the fundamental basis upon which a new class of GCP II inhibitors has been rationally designed.

Discovery of 2-PMPA

Research on the therapeutic utility of GCP II inhibition reached a major turning point in 1996, when the first potent inhibitor of GCP II, 2-(phosphonomethyl)pentanedioic acid (2-PMPA, Figure 2) was identified [25]. 2-PMPA is one of the most potent known competitive inhibitors with a K_i value of 0.2 nM in a GCP II assay [26]. The high potency of 2-PMPA can be attributed to the strong chelation of the phosphonate group to an active site zinc ion, as well as the interaction of the glutarate (pentanedioic acid) portion of the inhibitor with the C-terminal glutamate recognition site of GCP II.

Unlike the peptide-based GCP II inhibitors identified earlier, 2-PMPA has no cleavable peptide bond and is significantly more potent in inhibiting GCP II. More importantly, although 2-PMPA can be considered an analog of glutamate, it has shown exquisite selectivity for GCP II over various receptors, transporters, and enzymes associated with glutamate [27]. This selectivity, coupled with its low molecular weight and high aqueous solubility/stability, permitted 2-PMPA to serve as a pharmacological tool to study the mechanism and physiological role of GCP II, as well as the potential therapeutic effects of its inhibition.

The first to report the therapeutic utility of GCP II inhibition and its mechanistic basis, using 2-PMPA in ischemia models was Slusher *et al.* [27] who demonstrated neuroprotection in both an *in vitro* rat tissue culture model of cerebral ischemia and an *in vivo* middle cerebral artery occlusion (MCAO) model of stroke in rats (Figure 3). In parallel to these studies, the extracellular levels of NAAG and glutamate were measured in the caudate nucleus of the brain using microdialysis probes. Consistent with GCP II inhibition, it was demonstrated that 2-PMPA selectively increases NAAG levels and attenuates the ischemia-induced rise in extracellular glutamate.

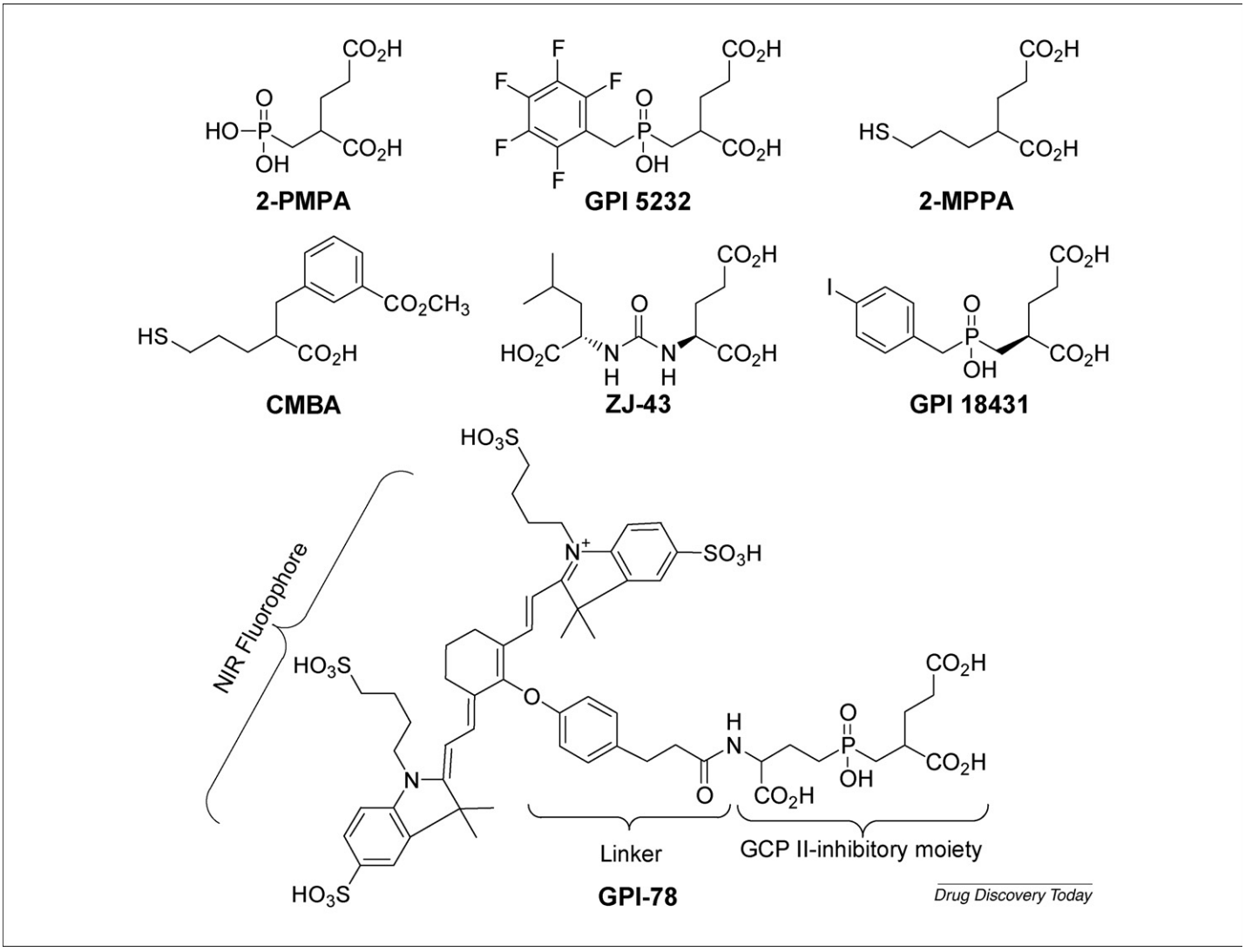

FIGURE 2

Representative GCP II inhibitors. 2-PMPA is a phosphonate-based GCP II inhibitor that has been used in a number of studies to investigate the physiological role of GCP II and the therapeutic benefits of GCP II inhibition. GPI 5232 is a phosphinate-based GCP II inhibitor. Its (*S*)-enantiomer was the first clinical candidate to emerge from our internal discovery research. 2-MPPA is the first reported orally available GCP II inhibitor and the first inhibitor that has been tested in human. CMBA is one of the first P1′-modified GCP II inhibitors that exhibit potency superior to that of the base compound. ZJ43 is a urea-based GCP II inhibitor currently under preclinical research and development (see http://www.acentadiscovery.com). GPI 18431 is an optically pure phosphinate-based GCP II inhibitor. The iodine-containing inhibitor was specifically designed for our crystallographic studies in order to obtain accurate phase information. GPI-78 is a high-affinity NIR fluorescent contrast agent for imaging of PSMA. The molecule consists of a tetra-sulfonated heptamethine indocyanine- NIR fluorophore conjugated to a phosphinate-based GCP II inhibitor through a linker.

Subsequent to these findings, 2-PMPA has been tested in multiple *in vitro* and *in vivo* models of neurological disorders associated with glutamate excitotoxicity. 2-PMPA afforded robust neuroprotection from injuries induced by hypoxia in neuron-enriched primary cultures derived from rat embryo E15 cerebellum [28]. 2-PMPA exhibited significant neuroprotective effects in animal models of ischemic disease, such as neonatal hypoxia-ischemia [29], brain asphyxia [30], retinal ischemia [31] and ischemic spinal cord injury [32]. In addition to ischemia, 2-PMPA was found to be effective in reducing allodynia [33,34], formalin-induced agitation behavior [35], seizure [36,37], morphine tolerance [38] and cocaine-associated sensitization and place preference [39,40]. 2-PMPA also had significant neuroprotective effects in an *in vitro* model of chronic glutamate-mediated motor neuron degeneration, suggesting therapeutic utility of GCP II inhibition in ALS [41]. In addition, 2-PMPA was found to be protective against glucose-induced programmed cell death (PCD) and neurite degeneration of dorsal root ganglion (DRG) neurons in a cell culture model of diabetic neuropathy [42].

2-PMPA has also served as a critical tool in various mechanistic studies pertaining to the physiological roles of GCP II and its substrate NAAG. Our group reported that 2-PMPA-mediated neuroprotection in an *in vitro* model of metabolic inhibition requires glial cells, where GCP II is primarily expressed. We also found that the neuroprotective effect of 2-PMPA against *in vitro* and *in vivo* ischemic injury involves the release of transforming growth factors, TGF-βs [43]. Using 2-PMPA, Lieberman's group conducted a series of experiments in crayfish nervous tissues to explore the possible role of NAAG as a neuron-to-glia and neuron-to-neuron signaling agent and its ability to regulate GCP II [44–46]. Further-

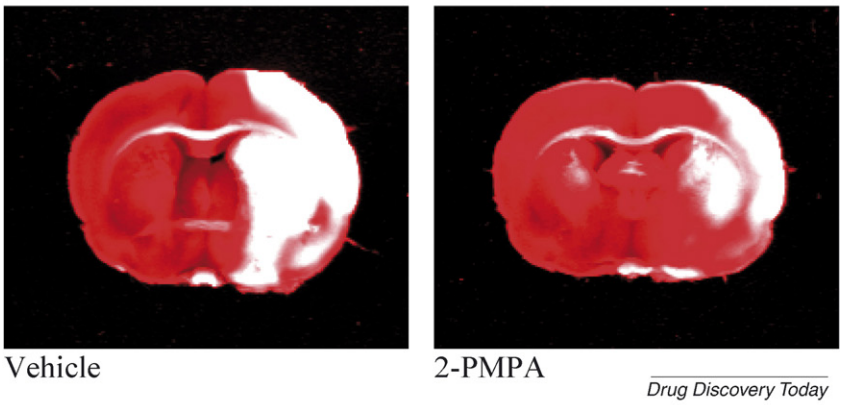

FIGURE 3

Representative forebrain images of TTC-stained brain slices from rats treated with vehicle or 2-PMPA (100 mg/kg i.p. bolus followed by 20 mg/kg/hour i.v. for four hours) at 60 min after the onset of MCAO. Viable tissue appears red as a result of the reduction of the dye by functional mitochondrial enzymes while infarcted tissue appears white. Reproduced from Ref. [27].

more, *in vivo* electrophysiological studies have shown that 2-PMPA produced a dose-dependent inhibition of electrical-evoked responses of rat dorsal horn neurons in normal and carrageenan-inflamed animals [47]. In another electrophysiological study, exogenous NAAG was found to modulate non-quantal secretion of acetylcholine from the presynaptic terminal of the neuromuscular synapse via activation of postsynaptic NMDA receptors and synthesis of nitric oxide (NO) in muscle fibers [48]. This effect was completely prevented in the presence of 2-PMPA, suggesting that glutamate, produced by GCP II-catalyzed hydrolysis of the exogenous NAAG, is playing a major role in this process.

In more recent efforts to establish mechanistic evidence to support the therapeutic utility of GCP II inhibition, mice were generated in which the *Folh1* gene encoding GCP II was disrupted (*Folh1*−/− mice) [19]. We found that *Folh1*−/− mice were less susceptible to ischemic stroke and sciatic nerve crush injury, compared to wild-type littermates, supporting the hypothesis that modulation of GCP II activity represents an attractive approach to neuroprotection [49].

While these studies have provided a compelling therapeutic rationale for GCP II inhibition, the poor pharmacokinetic profile of 2-PMPA, due to the highly polar nature of the molecule, limited its practical value as a therapeutic drug. This prompted us to launch a new project aimed at identifying potent GCP II inhibitors with an improved pharmacokinetic profile through extensive SAR studies using 2-PMPA as a template. This activity created a need for an efficient method to screen a large number of compounds for their ability to inhibit GCP II. After securing a supply of human recombinant GCP II, [50] we developed a microplate-based radioactive assay that is rapid and requires a minimal amount of radioactive material [26].

GPI 5232

SAR studies on 2-PMPA analogs focused on structural modification to the glutarate portion (P1′ side chain) of 2-PMPA and incorporation of an alkyl group into the phosphorous atom [51]. Analysis of the data revealed a well-defined structural requirement for potent GCP II inhibition. Alteration of the P1′ side chain greatly reduces GCP II inhibitory potency, while the replacement of the phosphonate group of 2-PMPA with an alkylphosphinate moiety provided a series of reasonably potent GCP II inhibitors.

Among these phosphinate-based compounds, GPI 5232 (Figure 2) showed significant *in vivo* efficacy in the rat MCAO model of stroke. Most notably, this compound provided significant neuroprotection, even when administered 120 min after the onset of MCAO, whereas 2-PMPA was only effective upto 90 min post occlusion [52]. The results indicated that GPI 5232 may extend the therapeutic time window in stroke patients.

At that time, GCP II inhibitors were also being examined for their ability to act as potential analgesic agents for neuropathic pain, based on the preclinical findings with 2-PMPA [34]. GPI 5232 was examined for its antinociceptive effects, using the rat chronic constrictive injury (CCI) model of neuropathic pain. Daily intraperitoneal administration of GPI 5232 (10 mg/kg) significantly attenuated the CCI-induced thermal hyperalgesic state, relative to the vehicle-treated control [51]. Furthermore, it was demonstrated that treatment of type 1 diabetic BB/Wor rats with GPI 5232 (10 mg/kg/day, i.p.) from onset of diabetes for six months had beneficial effects on hyperalgesia, nerve function and structural degenerative changes in diabetic polyneuropathy [53].

GPI 5232 has been used to explore the therapeutic potential of GCP II inhibition in psychiatric disorders where dysregulation of glutamate has been observed. Acute exposure to GPI 5232 (30 mg/kg, i.p.), administered 30 min before a social interaction test, inhibited isolation-induced aggressive behavior in SJL mice that had been individually housed long term [54].

The high aqueous solubility and solution stability of GPI 5232 provided ideal chemical properties for the development of an intravenous formulation of GPI 5232. In preparation for the clinical development of GPI 5232 injection for future stroke clinical trials, both enantiomers of GPI 5232 were prepared. It was found that the (*S*)-enantiomer was primarily responsible for GCP II inhibition In concordance with its GCP II inhibitory properties, the (*S*)-enantiomer also provided superior neuroprotection in the rat MCAO model of stroke [55].

In 1999, the clinical development of GPI 5232 for stroke was stopped due to the high cost and complexity associated with clinical trials for stroke (at that time, a number of experimental drugs targeting NMDA receptors had failed to show efficacy in clinical trials [3]) and the greater opportunity for GCP II inhibitors in treating chronic diseases such as diabetic neuropathy. A corporate decision was made to refocus the program on the treatment of chronic neurological disorders. Although GPI 5232 was efficacious in animal models of chronic neurological disorders by parenteral administration, it exhibited negligible oral bioavailability. It therefore became essential to identify an orally active GCP II inhibitor.

2-MPPA

It was assumed that the highly polar phosphonate and phosphinate groups were the primary cause of poor oral bioavailability of the phosphorous-based GCP II inhibitors. This assumption prompted us to reduce the polarity of the compounds by substituting the phosphorous group with the less polar thiol group. These efforts led to the discovery of a thiol-based GCP II inhibitor, 2-(3-mercaptopropyl)pentanedioic acid (2-MPPA, also known as GPI 5693, Figure 2), with an IC_{50} value of 90 nM [56]. Most importantly, 2-MPPA was found to be orally bioavailable in rats (%F = 71) and exhibited efficacy in an animal model of neuropathic pain following oral administration.

The efficacy of the orally active GCP II inhibitor, 2-MPPA, was assessed in various animal models of chronic neurological disorders by oral administration. Treatment of G93A familial amyotrophic lateral sclerosis (FALS) transgenic mice with 2-MPPA by daily oral administration resulted in a delayed onset of neurological symptoms, reduced motor-neuron death and prolongation in median survival [57].

The therapeutic effects of long-term oral administration of 2-MPPA on established painful and sensory neuropathy in the spontaneously diabetic BB/Wor rat was examined and it was found that oral 2-MPPA significantly improved hyperalgesia, nerve conduction velocity, and underlying myelinated fiber atrophy [58].

Furthermore, oral administration of 2-MPPA blocked the acquisition and expression of the conditioned place preference (CPP) response to cocaine in male rats, suggesting that GCP II inhibition may be of use in treating cue-induced craving in cocaine addicts [40]. In addition, the treatment of C57/B1 mice with oral 2-MPPA prevented the development of morphine tolerance without affecting acute morphine antinociception, implying the utility of GCP II inhibitors in patients receiving long-term morphine treatment [59].

The functional role of 2-MPPA with respect to its neuroprotective effects has been investigated in hippocampal mossy fiber-CA3 pyramidal cell synapses. 2-MPPA suppresses mossy fiber-CA3 synaptic neurotransmission by increasing NAAG, which activates presynaptic mGluR3 and inhibits glutamate release. This mechanistic study indicated that the efficacy of 2-MPPA in various preclinical models of neurological disorders is attributable to the presynaptic regulation of glutamatergic neurotransmission [60].

In these preclinical studies, 2-MPPA was provided as a racemic mixture. Before advancing to clinical development, both enantiomers of 2-MPPA were prepared, with the expectation that only one of them would be a potent GCP II inhibitor [55]. Contrary to expectations, both enantiomers of 2-MPPA inhibited GCP II with nearly equal potency. Subsequently, both enantiomers were tested for their antinociceptive effects, using the rat CCI model, where both of them exhibited a similar efficacy profile. Based on these efficacy results, coupled with indistinguishable preclinical safety profiles of the two enantiomers, racemic 2-MPPA was advanced to clinical development for the treatment of painful diabetic neuropathy.

In 2001, a double-blind, placebo-controlled clinical study with oral 2-MPPA was initiated in healthy subjects, which represented the first administration of a GCP II inhibitor in humans [61]. The main aim of this exploratory study was to assess the single-dose pharmacokinetics and to evaluate safety and tolerability including CNS effects. Overall, 2-MPPA was safe and generally well-tolerated at plasma exposures that were effective in animal model of neuropathic pain. More importantly, no clinically significant adverse CNS effects, normally associated with glutamate receptor antagonism were observed. These findings warrant a multiple dose study in healthy subjects to further investigate safety and pharmacokinetics of 2-MPPA after repeated oral dosing.

Other classes of GCP II inhibitors

Since the discovery of 2-PMPA, tremendous efforts have been made to identify new classes of GCP II inhibitors. Subsequent to the discovery of 2-MPPA, we have channeled our medicinal chemistry efforts into two directions.

In one approach, we have investigated the effect of P1′ side chain modification on GCP II inhibitory potency and found that several more lipophilic 2-MPPA analogs, including 3-(2-carboxy-5-mercaptopentyl)-benzoic acid (CMBA, Figure 2), inhibit GCP II in a more potent manner than 2-MPPA [62]. These compounds have also shown improved potency in the rat chronic constriction injury (CCI) model of neuropathic pain by oral administration. These findings represent the first successful attempt to improve the potency by modifying the glutarate moiety of GCP II inhibitors.

In another approach, we have explored other zinc-binding groups in an attempt to diversify the structures of our GCP II inhibitors. To this end, a series of hydroxamate-containing compounds were synthesized and evaluated for their ability to inhibit GCP II [63]. The most potent compound within this series inhibited GCP II with an IC_{50} value of 220 nM.

The most notable work in identifying alternative zinc-binding group involves a series of urea-based GCP II inhibitors reported by Kozikowski's group [64–66]. Among them, ZJ-43 (Figure 2) has demonstrated efficacy in various animal models of neurological disorders including schizophrenia [66], traumatic brain injury (TBI) [67], neuropathic pain [68] and inflammatory peripheral pain [69]. In addition, *in vivo* microdialysis revealed that ZJ-43 reduces the TBI-induced rise in dialysate glutamate levels and sustains increased levels of NAAG over a longer period of time, compared to a vehicle control [70]. These findings are consistent with earlier reports [27] on microdialysis studies using 2-PMPA and provide further mechanistic evidence that GCP II inhibitors produce neuroprotection by regulating levels of glutamate and NAAG.

Structural studies of GCP II

Efforts on the structural analysis of GCP II have made steady progress over the past decade, edging closer toward applying structure-based rational drug design to the discovery of new GCP II inhibitors.

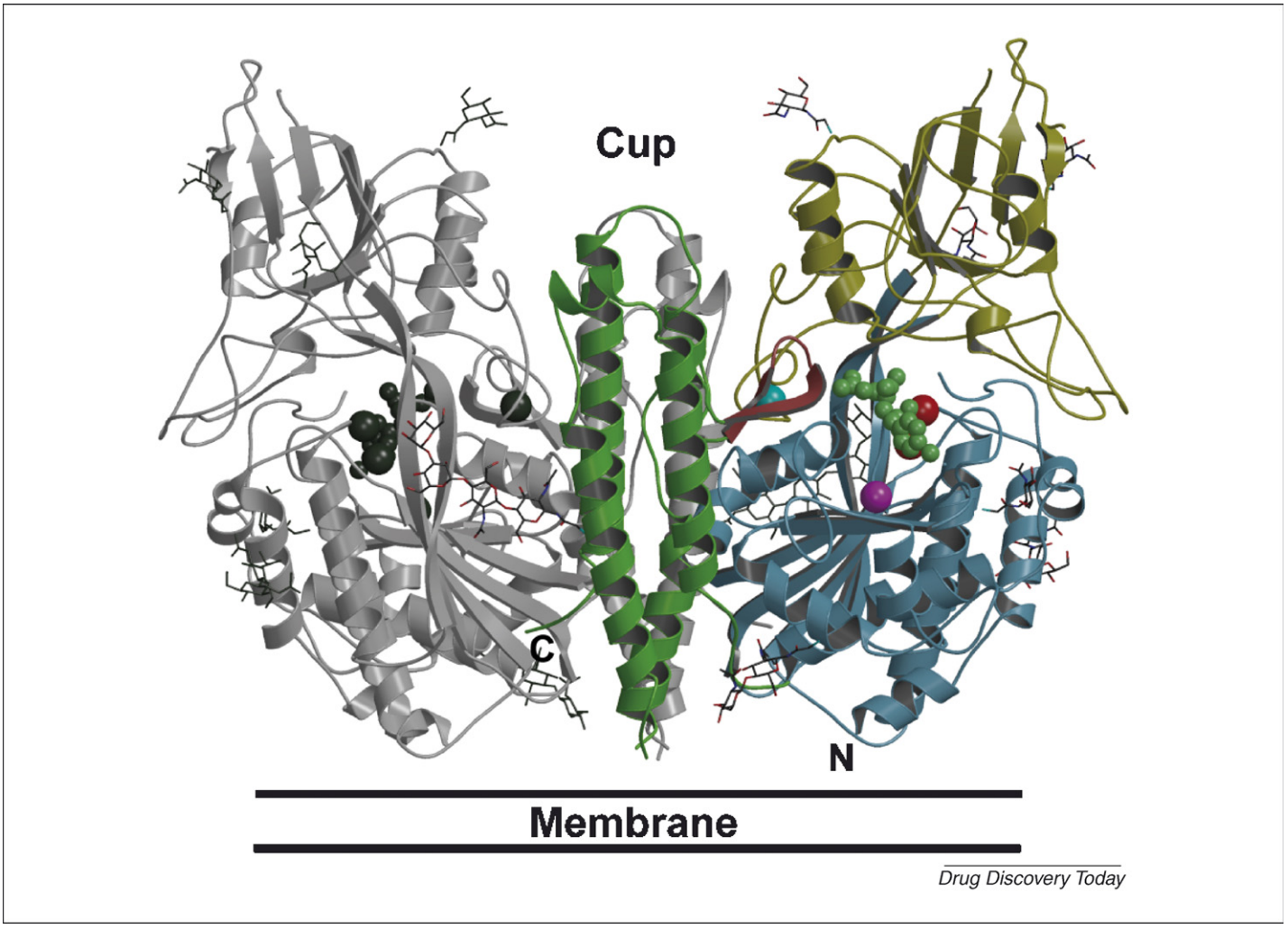

FIGURE 4

Three-dimensional structure of the GCP II dimer in complex with GPI 18431 (PDB code: 2C6C). One subunit is shown in a gray scale, while the other is color coded to highlight three distinct domains: the protease domain (blue), the apical domain (olive), and the C-terminal domain (green). The active site zinc ions are shown as red spheres, the calcium ion near the monomer–monomer interface as a blue sphere, and the chloride ion as a purple sphere. GPI 18431 is shown as green spheres. The 'glutarate sensor' (the ß15/ß16 hairpin) is shown in brown. Reproduced from Ref. [78].

The first breakthrough in this area was made in 1997 by Rawlings and Barrett [71]. They successfully assigned the catalytic domain of GCP II to peptidase family M28, which contains co-catalytic zinc metallopeptidases. Moreover, through sequence alignment with the known structure of an aminopeptidase in family M28, they correctly predicted that the ligands of two catalytic site zinc ions are His377, Asp387, Glu425, Asp453, and His553. They also identified Glu424 as a catalytic base and several basic residues, Arg463, Lys499, Arg536, and Lys545, potentially forming the specific pocket geared towards substrates bearing multiple negative charges such as NAAG.

Coyle's group performed well-thought-out site-directed mutagenesis of the key residues in GCP II [72]. Kinetic profiles of mutant enzymes are in good agreement with the predicted assignment of these residues by Barrett and Rawlings [71].

Following these findings, an attempt to construct a three dimensional model of GCP II was made by us [73] and Kozikowski's group [74], using a homology modeling approach. Although inspiring in many respects, the utility of these models in rationally designing GCP II inhibitors has been limited, due to some factors that were not taken into account during the modeling process. One of them is the effect of glycosylation on the structure of GCP II and its enzymatic activity. As a type II membrane protein, GCPII is heavily glycosylated. The removal of oligosaccharide moieties is known to diminish its enzymatic activity, presumably due to improper protein folding [75,76]. This poses questions concerning the validity of these predictive models, which were constructed in the form of apoprotein.

The first crystal structure of GCP II was solved in 2005 by Bjorkman's group at 3.5-Å resolution, providing a major step toward the understanding of the true structural features of this enzyme and mechanistic implications [77]. Subsequently, we reported crystal structures of the extracellular domain of GCP II in complex with glutamate, inorganic phosphate, and the GCP II inhibitor, GPI 18431 (Figure 2), at 2.0, 2.4, and 2.2 Å resolution, respectively [78]. GCPII folds into three distinct domains: the protease domain (domain I), the apical domain (domain II), and the C-terminal domain (domain III) (Figure 4). The three crystal structures correspond to different states of the active site and indicate an induced-fit mechanism of substrate recognition by GCP II, in which the ß15/ß16 hairpin (residues 692–704) serves as a

'glutarate sensor'. Very recently, three more crystal structures of GCP II were determined in ligand-free form [79] as well as complex with quisqualate and 2-PMPA [80]. This series of crystal structures should provide a further important basis for the rational structure-based design of new GCP II inhibitors.

GCP II inhibitors in oncology

There are two areas where GCP II inhibitors offer promise in the field of oncology. The first is to prevent and/or treat chemotherapy-induced neuropathy. The second is to utilize GCP II inhibitors as ligands to PSMA, which is highly expressed in prostate cancer and human solid tumor neovasculature.

Chemotherapy-induced neuropathy is a dose-limiting toxicity for many important chemotherapeutic agents, including platins (cisplatin and oxaliplatin) and taxanes (paclitaxel and docetaxel). Although the mechanism by which chemotherapeutic agents induce neuropathy is not well-understood, recent evidence suggests that impaired glutamate clearance due to the decreased expression of glutamate transporters in the dorsal horn plays a key role in taxol-induced hyperalgesia [81,82]. Consistent with these findings, it has been shown that 2-MPPA significantly prevents the deficit in sensory nerve conduction velocity induced by taxol (Figure 5A) [83]. In subsequent studies in xenograft models, we have also shown that co-administration of 2-MPPA does not reduce the anti-tumor effects of taxol (Figure 5B). This observation indicates that 2-MPPA attenuates neurotoxic effects of taxol without compromising its antitumor activity.

Prostate-specific membrane antigen (PSMA) is a type 2 integral membrane glycoprotein expressed in prostatic epithelial cells and is upregulated throughout the course of prostate cancer progression. PSMA is also present at high levels on newly formed blood vessels (neovasculature) of many types of solid tumors. Because of these properties, PSMA has been the target for diagnostic and therapeutic applications in prostate cancer including tumor imaging and immunotherapy, as is evident from the use of monoclonal antibodies specific for PSMA for the detection of occult prostate cancer [CYT-356 (Prosta-Scint)] and for immunotherapy for prostate cancer [10–13].

The fact that PSMA is structurally identical to GCP II and retains its enzymatic function has provided an intriguing opportunity for GCP II inhibitors to extend their utility as substitutes for anti-PSMA antibodies.

Tenniswood's group was the first to demonstrate such potential utility with their phosphinate-based GCP II inhibitors containing a fluorescent group [84]. These compounds bind stably to the membrane of viable LNCaP cells (PSMA-expressing), but not to DU-145 cells (PSMA-non-expressing) as monitored by fluorescent microscopy, demonstrating potential use of fluorescent-labeled GCP II inhibitors as diagnostic markers in prostate cancer.

Pomper's group have used urea-based GCP II inhibitors labeled with either [^{11}C] or [^{125}I] and performed imaging of PSMA-positive and PSMA-negative lesions in xenograft models of prostate cancer using positron emission tomography (PET) and single photon emission computed tomography (SPECT), respectively [85,86]. An [^{125}I]-labeled GCP II inhibitor has also been recently used to measure GCP II levels in the rodent brain [87].

In our efforts to develop a novel near-infrared (NIR) fluorescent contrast agent specific for PSMA, we chose a phosphinate analog of gamma-glutamylglutamate as a modular ligand to the active site of PSMA [88]. The selection is based on the findings that its free amino group can be used to attach a variety of functional units (such as fluorescent dyes) without a loss of affinity for PSMA [89]. Using a tetra-sulfonated heptamethine indocyanine NIR fluorescent derivative GPI-78 (Figure 2), we demonstrated sensitive and specific *in vitro* imaging of endogenous and ectopically expressed PSMA in human cells as well as *in vivo* imaging of xenograft tumors (Figure 6) [90]. Our studies also shed light onto the challenges that could limit the utility of GPI-78 as well as other GCP II inhibitor-

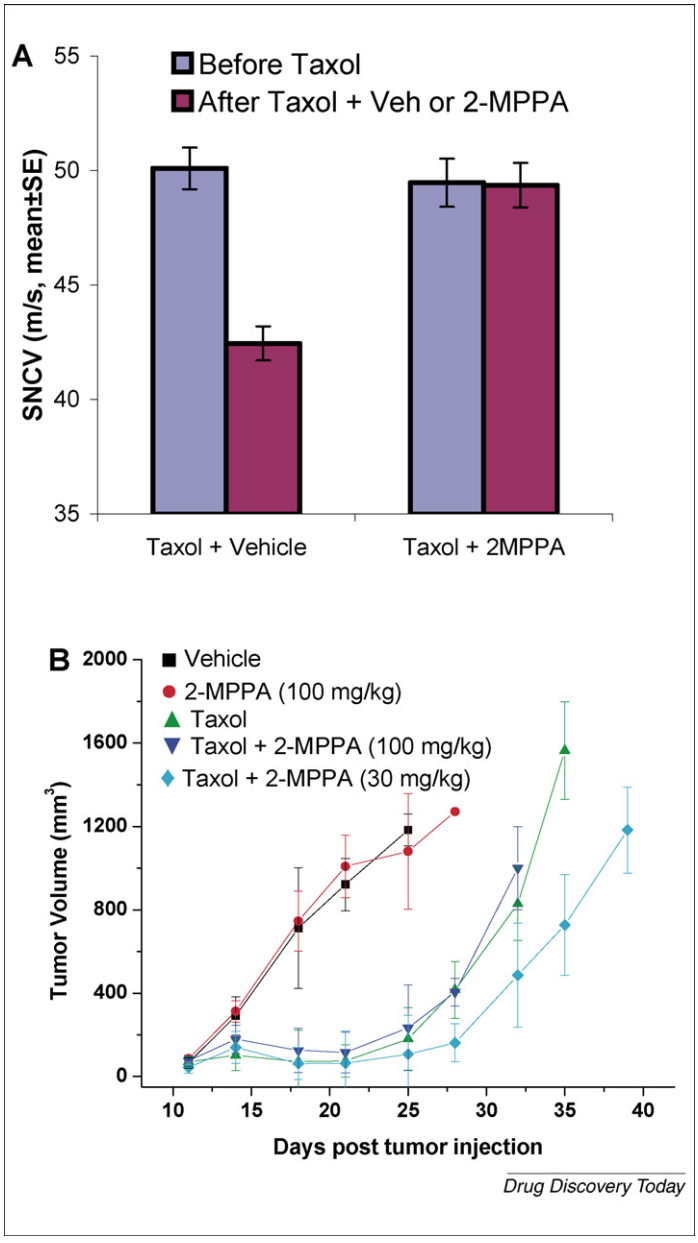

FIGURE 5

Effects of 2-MPPA on taxol neuropathy and antitumor efficacy in rodent models. (A) 2-MPPA (30 mg/kg/day p.o.) prevents the deficits in sensory nerve conduction velocity (SNCV) caused by Taxol (25 mg/kg, 4 doses in 2 weeks). SNCVs were measured at baseline (before the first taxol treatment) and 14 days after treatment initiation. $N = 12$ per group. (B) Taxol efficacy (15 mg/kg i.v. qdx5) in an ovarian xenograft model (OVCAR-3) is not altered by administration of 2-MPPA (30 and 100 mg/kg/day p.o.). 2-MPPA (100 mg/kg/day p.o.) alone had no therapeutic effects in this model.

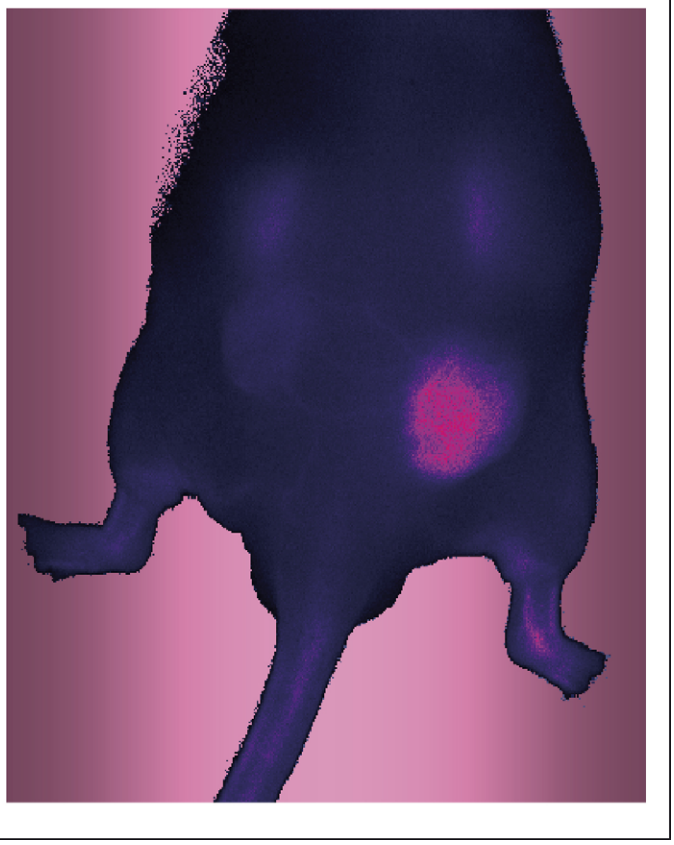

FIGURE 6

Representative *in vivo* image of human prostate cancer xenograft tumors in athymic nu/nu mice after intravenous injection of GPI-78. On the right flank was a PSMA-positive LNCaP tumor. On the left flank was a PSMA-negative TsuPR1 tumor. PSMA-positive tumor exhibited appreciable NIR fluorescence signal above background due to the binding of GPI-78. Reproduced, with permission, from the front cover of *Molecular Imaging* 2005, Vol. 4, Issue 4.

based PSMA ligands reported to date. First, the hydrodynamic diameters of GPI-78 might be too small for effective imaging. That is, renal clearance is so rapid that tumor contact time is minimal. Another problem is competition with endogenous phosphate, which is known to bind to the active site of GCP II. These problems could be overcome by combining a larger NIR fluorescent carrier molecule with multivalent GCP II inhibitor.

These studies also suggest that conjugation of radiotherapeutic or chemotherapeutic agents to GCP II inhibitors may provide a drug delivery system targeting PSMA-expressing tumors. Such an attempt was first made by Kozikowski's group, who synthesized the urea-based GCP II inhibitor-doxorubicin conjugate for targeting the prostate cancer cells [91]. Although the conjugate retained potent GCP II inhibitory activity, it did not exhibit antitumor activity in either PSMA-positive C4-2 or PSMA-negative PC3 prostate cancer cells. They speculated that the conjugate is not undergoing the appropriate enzymatic processing required to release the active component in the cytoplasm.

It is not yet known whether inhibition of the carboxypeptidase activity of PSMA alone has therapeutic implications in prostate cancer and/or angiogenesis. New studies on the physiological role of PSMA, however, have the potential to provide insight into this long-standing question.

Bacich's group proposed that PSMA gives prostate cancer cells a growth advantage in the tumor microenvironment, possibly through its ability to hydrolyze the gamma-glutamyl tail of folate polyglutamates released by surrounding dead and dying cells, resulting in folate that can be taken up by cells for replication [92]. Indeed, 2-PMPA was found to reduce the enhanced proliferation of LNCaP cells cultured in the presence of penta-γ-glutamated folate. Thus, GCP II inhibition may represent a novel approach for slowing prostate cancer growth.

Shapiro's group found that GCP II plays an important role in angiogenesis as a cell-surface carboxypeptidase by modulating integrin signal transduction [93]. Their findings that 2-PMPA abrogates angiogenesis could have implications for new antiangiogenesis therapy based on GCP II inhibitors.

Conclusions

The potential therapeutic opportunity offered by GCP II inhibitors is enormous and diverse. Numerous preclinical studies cited within this review have shown that GCP II inhibitors offer clinical promise in various therapeutic areas in neurology as well as oncology. In addition, the first study in human showed that 2-MPPA was safe and generally well tolerated in human at plasma exposures that were effective in various animal models of neurological disorders. We are currently pursuing applications for GCP II inhibitors in the area of chemotherapy-induced neuropathy (see http://www.mgipharma.com). If successful development is achieved, however, one could envision the therapeutic utility of GCP II inhibition being extended to other diseases associated with glutamate excitotoxicity.

Acknowledgements

We would like to thank former and current colleagues of Guilford Pharmaceuticals and MGI Pharma for their contributions at various stages of this program. As evident from the number of collaborative publications cited herein, the majority of the studies referred to as 'ours' represent collaborative efforts with academic organizations. We owe a tremendous debt to our academic collaborators who have kindly shared their expertise with us as we have explored various therapeutic benefits of GCP II inhibitors.

References

1 Watkins, J.C. (2000) l-glutamate as a central neurotransmitter: looking back. *Biochem. Soc. Trans.* 28, 297–309

2 Carpenter, K.J. and Dickenson, A.H. (2001) Amino acids are still as exciting as ever. *Curr. Opin. Pharmacol.* 1, 57–61

3 Ikonomidou, C. and Turski, L. (2002) Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? *Lancet Neurol.* 1, 383–386

4 Albensi, B.C. *et al.* (2004) Why do many NMDA antagonists fail, while others are safe and effective at blocking excitotoxicity associated with dementia and acute injury? *Am. J. Alzheimers Dis. Other Demen.* 19, 269–274

5 Wood, P.L. (2005) The NMDA receptor complex: a long and winding road to therapeutics. *IDrugs* 8, 229–235

6 Carter, R.E. and Coyle, J.T. (1998) Glutamate Carboxypeptidase II. In *Handbook of Proteolytic Enzymes* (Barrett, A.J. *et al.*, eds), pp. 1434–1437, Academic
7 Wroblewska, B. *et al.* (1997) N-acetylaspartylglutamate selectively activates mGluR3 receptors in transfected cells. *J. Neurochem.* 69, 174–181
8 Kamiya, H. *et al.* (1996) Activation of metabotropic glutamate receptor type 2/3 suppresses transmission at rat hippocampal mossy fibre synapses. *J. Physiol.* 493 (Pt 2), 447–455
9 Whelan, J. (2000) NAALADase inhibitors: A novel approach to glutamate regulation. *Drug Discov. Today* 5, 171–172
10 Neale, J.H. *et al.* (2005) The neurotransmitter N-acetylaspartylglutamate in models of pain, ALS, diabetic neuropathy. CNS injury and schizophrenia. *Trends Pharmacol. Sci.* 26, 477–484
11 Zhou, J. *et al.* (2005) NAAG peptidase inhibitors and their potential for diagnosis and therapy. *Nat. Rev. Drug Discov.* 4, 1015–1026
12 Coyle, J.T. (1997) The nagging question of the function of *N*-acetylaspartylglutamate. *Neurobiol. Dis.* 4, 231–238
13 Riveros, N. and Orrego, F. (1984) A study of possible excitatory effects of *N*-acetylaspartylglutamate in different *in vivo* and *in vitro* brain preparations. *Brain Res.* 299, 393–395
14 Robinson, M.B. *et al.* (1987) Hydrolysis of the brain dipeptide *N*-acetyl-L-aspartyl-L-glutamate. Identification and characterization of a novel *N*-acetylated alpha-linked acidic dipeptidase activity from rat brain. *J. Biol. Chem.* 262, 14498–14506
15 Slusher, B.S. *et al.* (1990) Rat brain *N*-acetylated alpha-linked acidic dipeptidase activity. Purification and immunologic characterization. *J. Biol. Chem.* 265, 21297–21301
16 Carter, R.E. *et al.* (1996) Prostate-specific membrane antigen is a hydrolase with substrate and pharmacologic characteristics of a neuropeptidase. *Proc. Natl. Acad. Sci. U. S. A.* 93, 749–753
17 Pinto, J.T. *et al.* (1996) Prostate-specific membrane antigen: a novel folate hydrolase in human prostatic carcinoma cells. *Clin. Cancer Res.* 2, 1445–1451
18 Halsted, C.H. *et al.* (1998) Folylpoly-gamma-glutamate carboxypeptidase from pig jejunum. Molecular characterization and relation to glutamate carboxypeptidase II. *J. Biol. Chem.* 273, 20417–20424
19 Bacich, D.J. *et al.* (2002) Deletion of the glutamate carboxypeptidase II gene in mice reveals a second enzyme activity that hydrolyzes *N*-acetylaspartylglutamate. *J. Neurochem.* 83, 20–29
20 Bzdega, T. *et al.* (2004) The cloning and characterization of a second brain enzyme with NAAG peptidase activity. *J. Neurochem.* 89, 627–635
21 Serval, V. *et al.* (1990) Competitive inhibition of N-acetylated-alpha-linked acidic dipeptidase activity by *N*-acetyl-L-aspartyl-beta-linked L-glutamate. *J. Neurochem.* 55, 39–46
22 Serval, V. *et al.* (1992) *In vitro* and *in vivo* inhibition of *N*-acetyl-L-aspartyl-L-glutamate catabolism by *N*-acylated L-glutamate analogs. *J. Pharmacol. Exp. Ther.* 260, 1093–1100
23 Subasinghe, N. *et al.* (1990) Synthesis of acyclic and dehydroaspartic acid analogues of Ac-Asp-Glu-OH and their inhibition of rat brain *N*-acetylated alpha-linked acidic dipeptidase (NAALA dipeptidase). *J. Med. Chem.* 33, 2734–2744
24 Luthi-Carter, R. *et al.* (1998) Hydrolysis of the neuropeptide *N*-acetylaspartylglutamate (NAAG) by cloned human glutamate carboxypeptidase II. *Brain Res.* 795, 341–348
25 Jackson, P.F. *et al.* (1996) Design, synthesis, and biological activity of a potent inhibitor of the neuropeptidase *N*-acetylated alpha-linked acidic dipeptidase. *J. Med. Chem.* 39, 619–622
26 Rojas, C. *et al.* (2002) Kinetics and inhibition of glutamate carboxypeptidase II using a microplate assay. *Anal. Biochem.* 310, 50–54
27 Slusher, B.S. *et al.* (1999) Selective inhibition of NAALADase, which converts NAAG to glutamate, reduces ischemic brain injury. *Nat. Med.* 5, 1396–1402
28 Tortella, F.C. *et al.* (2000) Neuroprotection produced by the NAALADase inhibitor 2-PMPA in rat cerebellar neurons. *Eur. J. Pharmacol.* 402, 31–37
29 Cai, Z. *et al.* (2002) Neuroprotective effects of *N*-acetylaspartylglutamate in a neonatal rat model of hypoxia-ischemia. *Eur. J. Pharmacol.* 437, 139–145
30 Cai, Y. *et al.* (2006) Complex character analysis of heart rate variability following brain asphyxia. *Med. Eng. Phys.* 28, 297–303
31 Harada, C. *et al.* (2000) *N*-acetylated-alpha-linked-acidic dipeptidase inhibitor has a neuroprotective effect on mouse retinal ganglion cells after pressure-induced ischemia. *Neurosci. Lett.* 292, 134–136
32 Long, J.B. *et al.* (2005) Inhibition of glutamate carboxypeptidase II (NAALADase) protects against dynorphin A-induced ischemic spinal cord injury in rats. *Eur. J. Pharmacol.* 508, 115–122
33 Yamamoto, T. *et al.* (2001) Spinal *N*-acetyl-alpha-linked acidic dipeptidase (NAALADase) inhibition attenuates mechanical allodynia induced by paw carrageenan injection in the rat. *Brain Res.* 909, 138–144
34 Chen, S.R. *et al.* (2002) Effect of 2-(phosphono-methyl)-pentanedioic acid on allodynia and afferent ectopic discharges in a rat model of neuropathic pain. *J. Pharmacol. Exp. Ther.* 300, 662–667
35 Yamamoto, T. *et al.* (2001) Inhibition of spinal *N*-acetylated-alpha-linked acidic dipeptidase produces an antinociceptive effect in the rat formalin test. *Neuroscience* 102, 473–479
36 Witkin, J.M. *et al.* (2002) NAALADase (GCP II) inhibition prevents cocaine-kindled seizures. *Neuropharmacology* 43, 348–356
37 Luszczki, J.J. *et al.* (2006) 2-phosphonomethyl-pentanedioic acid (glutamate carboxypeptidase II inhibitor) increases threshold for electroconvulsions and enhances the antiseizure action of valproate against maximal electroshock-induced seizures in mice. *Eur. J. Pharmacol.* 531, 66–73
38 Popik, P. *et al.* (2003) Morphine tolerance and reward but not expression of morphine dependence are inhibited by the selective glutamate carboxypeptidase II (GCP II, NAALADase) inhibitor, 2-PMPA. *Neuropsychopharmacology* 28, 457–467
39 Shippenberg, T.S. *et al.* (2000) Modulation of behavioral sensitization to cocaine by NAALADase inhibition. *Synapse* 38, 161–166
40 Slusher, B.S. *et al.* (2001) Expression and acquisition of the conditioned place preference response to cocaine in rats is blocked by selective inhibitors of the enzyme *N*-acetylated-alpha-linked-acidic dipeptidase (NAALADASE). *Synapse* 41, 22–28
41 Thomas, A.G. *et al.* (2003) NAALADase inhibition protects motor neurons against chronic glutamate toxicity. *Eur. J. Pharmacol.* 471, 177–184
42 Berent-Spillson, A. *et al.* (2004) Protection against glucose-induced neuronal death by NAAG and GCP II inhibition is regulated by mGluR3. *J. Neurochem.* 89, 90–99
43 Thomas, A.G. *et al.* (2001) Neuroprotection mediated by glutamate carboxypeptidase II (NAALADase) inhibition requires TGF-beta. *Eur. J. Pharmacol.* 430, 33–40
44 Gafurov, B. *et al.* (2001) *N*-acetylaspartylglutamate (NAAG) is the probable mediator of axon-to-glia signaling in the crayfish medial giant nerve fiber. *Neuroscience* 106, 227–235
45 Urazaev, A.K. *et al.* (2001) Mechanisms for clearance of released *N*-acetylaspartylglutamate in crayfish nerve fibers: implications for axon-glia signaling. *Neuroscience* 107, 697–703
46 Urazaev, A.K. *et al.* (2005) Regulation of glutamate carboxypeptidase II hydrolysis of *N*-acetylaspartylglutamate (NAAG) in crayfish nervous tissue is mediated by glial glutamate and acetylcholine receptors. *J. Neurochem.* 93, 605–610
47 Carpenter, K.J. *et al.* (2003) Effects of GCP-II inhibition on responses of dorsal horn neurons after inflammation and neuropathy: an electrophysiological study in the rat. *Neuropeptides* 37, 298–306
48 Malomouzh, A.I. *et al.* (2005) Effect of *N*-acetylaspartylglutamate (NAAG) on non-quantal and spontaneous quantal release of acetylcholine at the neuromuscular synapse of rat. *J. Neurochem.* 94, 257–267
49 Bacich, D.J. *et al.* (2005) Mice lacking glutamate carboxypeptidase II are protected from peripheral neuropathy and ischemic brain injury. *J. Neurochem.* 95, 314–323
50 Barinka, C. *et al.* (2002) Substrate specificity, inhibition and enzymological analysis of recombinant human glutamate carboxypeptidase II. *J. Neurochem.* 80, 477–487
51 Jackson, P.F. *et al.* (2001) Design and pharmacological activity of phosphinic acid based NAALADase inhibitors. *J. Med. Chem.* 44, 4170–4175
52 Williams, A.J. *et al.* (2001) Electroencephalogram analysis and neuroprotective profile of the *N*-acetylated-alpha-linked acidic dipeptidase inhibitor, GPI5232, in normal and brain-injured rats. *J. Pharmacol. Exp. Ther.* 299, 48–57
53 Zhang, W. *et al.* (2002) GCPII (NAALADase) inhibition prevents long-term diabetic neuropathy in type 1 diabetic BB/Wor rats. *J. Neurol. Sci.* 194, 21–28
54 Lumley, L.A. *et al.* (2004) Reduced isolation-induced aggressiveness in mice following NAALADase inhibition. *Psychopharmacology (Berl.)* 171, 375–381
55 Tsukamoto, T. *et al.* (2005) Enantiospecificity of glutamate carboxypeptidase II inhibition. *J. Med. Chem.* 48, 2319–2324
56 Majer, P. *et al.* (2003) Synthesis and biological evaluation of thiol-based inhibitors of glutamate carboxypeptidase II: discovery of an orally active GCP II inhibitor. *J. Med. Chem.* 46, 1989–1996
57 Ghadge, G.D. *et al.* (2003) Glutamate carboxypeptidase II inhibition protects motor neurons from death in familial amyotrophic lateral sclerosis models. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9554–9559
58 Zhang, W. *et al.* (2006) The preventive and therapeutic effects of GCPII (NAALADase) inhibition on painful and sensory diabetic neuropathy. *J. Neurol. Sci.*
59 Kozela, E. *et al.* (2005) 2-MPPA, a selective glutamate carboxypeptidase II inhibitor, attenuates morphine tolerance but not dependence in C57/Bl mice. *Psychopharmacology (Berl.)* 183, 275–284
60 Sanabria, E.R. *et al.* (2004) GCP II (NAALADase) inhibition suppresses mossy fiber-CA3 synaptic neurotransmission by a presynaptic mechanism. *J. Neurophysiol.* 91, 182–193

61 van der Post, J.P. *et al.* (2005) The central nervous system effects, pharmacokinetics and safety of the NAALADase-inhibitor GPI 5693. *Br. J. Clin. Pharmacol.* 60, 128–136

62 Majer, P. *et al.* (2006) Structural Optimization of Thiol-Based Inhibitors of Glutamate Carboxypeptidase II by Modification of the P1′ Side Chain. *J. Med. Chem.* 49, 2876–2885

63 Stoermer, D. *et al.* (2003) Synthesis and biological evaluation of hydroxamate-Based inhibitors of glutamate carboxypeptidase II. *Bioorg. Med. Chem. Lett.* 13, 2097–2100

64 Kozikowski, A.P. *et al.* (2001) Design of remarkably simple, yet potent urea-based inhibitors of glutamate carboxypeptidase II (NAALADase). *J. Med. Chem.* 44, 298–301

65 Kozikowski, A.P. *et al.* (2004) Synthesis of urea-based inhibitors as active site probes of glutamate carboxypeptidase II: efficacy as analgesic agents. *J. Med. Chem.* 47, 1729–1738

66 Olszewski, R.T. *et al.* (2004) NAAG peptidase inhibition reduces locomotor activity and some stereotypes in the PCP model of schizophrenia via group II mGluR. *J. Neurochem.* 89, 876–885

67 Zhong, C. *et al.* (2005) NAAG peptidase inhibitor reduces acute neuronal degeneration and astrocyte damage following lateral fluid percussion TBI in rats. *J. Neurotrauma.* 22, 266–276

68 Yamamoto, T. *et al.* (2004) Antinociceptive effects of *N*-acetylaspartylglutamate (NAAG) peptidase inhibitors ZJ-11, ZJ-17 and ZJ-43 in the rat formalin test and in the rat neuropathic pain model. *Eur. J. Neurosci.* 20, 483–494

69 Yamamoto, T. *et al.* (2007) Local administration of *N*-acetylaspartylglutamate (NAAG) peptidase inhibitors is analgesic in peripheral pain in rats. *Eur. J. Neurosci.* 25, 147–158

70 Zhong, C. *et al.* (2006) NAAG peptidase inhibitor increases dialysate NAAG and reduces glutamate, aspartate and GABA levels in the dorsal hippocampus following fluid percussion injury in the rat. *J. Neurochem.* 97, 1015–1025

71 Rawlings, N.D. and Barrett, A.J. (1997) Structure of membrane glutamate carboxypeptidase. *Biochim. Biophys. Acta* 1339, 247–252

72 Speno, H.S. *et al.* (1999) Site-directed mutagenesis of predicted active site residues in glutamate carboxypeptidase II. *Mol. Pharmacol.* 55, 179–185

73 Stoermer, D. *et al.* (2001) Glutamate carboxypeptidase II: Homology model and docking studies. In *222nd ACS National Meeting*, Chicago, IL, Abstract INOR 195.

74 Rong, S.B. *et al.* (2002) Molecular modeling of the interactions of glutamate carboxypeptidase II with its potent NAAG-based inhibitors. *J. Med. Chem.* 45, 4140–4152

75 Ghosh, A. and Heston, W.D. (2003) Effect of carbohydrate moieties on the folate hydrolysis activity of the prostate specific membrane antigen. *Prostate* 57, 140–151

76 Barinka, C. *et al.* (2004) Identification of the *N*-glycosylation sites on glutamate carboxypeptidase II necessary for proteolytic activity. *Protein Sci.* 13, 1627–1635

77 Davis, M.I. *et al.* (2005) Crystal structure of prostate-specific membrane antigen, a tumor marker and peptidase. *Proc. Natl. Acad. Sci. U. S. A.* 102, 5981–5986

78 Mesters, J.R. *et al.* (2006) Structure of glutamate carboxypeptidase II, a drug target in neuronal damage and prostate cancer. *EMBO J.* 22, 1375–1384

79 Barinka, C. *et al.* (2007) A high-resolution structure of ligand-free human glutamate carboxypeptidase II. *Acta Crystallograph. Sect. F. Struct. Biol. Cryst. Commun.* 63, 150–153

80 Mesters, J.R. *et al.* (2007) Human glutamate carboxypeptidase II inhibition: structures of GCPII in complex with two potent inhibitors, quisqualate and 2-PMPA. *Acta Crystallogr. D. Biol. Crystallogr.* 63, 508–513

81 Weng, H.R. *et al.* (2005) Spinal glial glutamate transporters downregulate in rats with taxol-induced hyperalgesia. *Neurosci. Lett.* 386, 18–22

82 Cata, J.P. *et al.* (2006) Altered discharges of spinal wide dynamic range neurons and downregulation of glutamate transporter expression in rats with paclitaxel-induced hyperalgesia. *Neuroscience* 138, 329–338

83 Tang, Z. *et al.* (2006) 2-MPPA, a selective inhibitor of GCPII (NAALADase/PSMA), attenuates chemotherapy-induced neuropathy in mice. In *97th AACR Annual Meeting*, Washington, DC, Abstract No. 1972.

84 Tang, H. *et al.* (2003) Prostate targeting ligands based on *N*-acetylated alpha-linked acidic dipeptidase. *Biochem. Biophys. Res. Commun.* 307, 8–14

85 Pomper, M.G. *et al.* (2002) 11C-MCG: synthesis, uptake selectivity, and primate PET of a probe for glutamate carboxypeptidase II (NAALADase). *Mol. Imag.* 1, 96–101

86 Foss, C.A. *et al.* (2005) Radiolabeled small-molecule ligands for prostate-specific membrane antigen: *in vivo* imaging in experimental models of prostate cancer. *Clin. Cancer Res.* 11, 4022–4028

87 Guilarte, T.R. *et al.* (2005) Glutamate carboxypeptidase II levels in rodent brain using [^{125}I]DCIT quantitative autoradiography. *Neurosci. Lett.* 387, 141–144

88 Valiaeva, N. *et al.* (2001) Phosphinic acid pseudopeptides analogous to glutamyl-gamma-glutamate: synthesis and coupling to pteroyl azides leads to potent inhibitors of folylpoly-gamma-glutamate synthetase. *J. Org. Chem.* 66, 5146–5154

89 Tsukamoto, T. *et al.* (2002) Phosphonate and phosphinate analogues of *N*-acylated gamma-glutamylglutamate. potent inhibitors of glutamate carboxypeptidase II. *Bioorg. Med. Chem. Lett.* 12, 2189–2192

90 Humblet, V. *et al.* (2005) High-affinity near-infrared fluorescent small-molecule contrast agents for in vivo imaging of prostate-specific membrane antigen. *Mol. Imag.* 4, 448–462

91 Jayaprakash, S. *et al.* (2006) Design and synthesis of a PSMA inhibitor-doxorubicin conjugate for targeted prostate cancer therapy. *Chem. Med. Chem.* 1, 299–302

92 Yao, V. and Bacich, D.J. (2006) Prostate specific membrane antigen (PSMA) expression gives prostate cancer cells a growth advantage in a physiologically relevant folate environment *in vitro*. *Prostate* 66, 867–875

93 Conway, R.E. *et al.* (2006) Prostate-specific membrane antigen regulates angiogenesis by modulating integrin signal transduction. *Mol. Cell Biol.* 26, 5310–5324