Bioorganic & Medicinal Chemistry Letters 20 (2010) 7222–7225

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The discovery and structure–activity relationships of indole-based inhibitors of glutamate carboxypeptidase II

Brian Grella[a], Jessica Adams[a], James F. Berry[b], Greg Delahanty[a,b], Dana V. Ferraris[a,b], Pavel Majer[a], Chiyou Ni[a], Krupa Shukla[b,c], Scott A. Shuler[b], Barbara S. Slusher[a,b,c], Marigo Stathis[b], Takashi Tsukamoto[a,b,c,*]

[a] *Eisai Research Institute, Baltimore, MD 21224, USA*
[b] *Brain Science Institute, Johns Hopkins University, Baltimore, MD 21205, USA*
[c] *Department of Neurology, Johns Hopkins University, Baltimore, MD 21205, USA*

ARTICLE INFO

Article history:
Received 25 August 2010
Revised 19 October 2010
Accepted 21 October 2010
Available online 26 October 2010

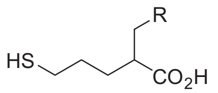
Keywords:
Glutamate carboxypeptidase II
Inhibitor
Indole
Metallopeptidase
Thiol

ABSTRACT

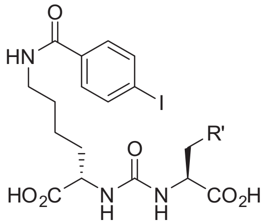
A series of *N*-substituted 3-(2-mercaptoethyl)-1*H*-indole-2-carboxylic acids were synthesized as inhibitors of glutamate carboxypeptidase II (GCPII). Those containing carboxybenzyl or carboxyphenyl groups at the N-position exhibited potent inhibitory activity against GCPII. These indole-based compounds represent the first example of achiral GCPII inhibitors and demonstrate greater tolerance of the GCPII active site for ligands with significant structural difference from the endogenous substrate, *N*-acetyl-aspartylglutamate.

Glutamate carboxypeptidase II (GCP II, EC 3.4.17.21) catalyzes the extracellular hydrolysis of the neuropeptide *N*-acetyl-aspartylglutamate (NAAG) to *N*-acetyl-aspartate and glutamate. Since the hydrolysis of NAAG is believed to be one of the major sources of glutamate in the nervous system, inhibition of GCP II has gained considerable attention as a strategy to suppress glutamate excitotoxicity leading to neurological disorders. Thus, substantial efforts have been made to identify potent and selective GCP II inhibitors.[1] Some of these inhibitors have shown efficacy in a variety of animal models of neurological diseases associated with glutamate excitotoxicity including stroke,[2] amyotrophic lateral sclerosis (ALS),[3] neuropathic pain,[4] and diabetic neuropathy.[5] Furthermore, an orally available GCPII inhibitor, 2-(3-mercaptopropyl) pentanedioic acid (2-MPPA), was tested for its safety in clinical studies and was generally well-tolerated at plasma exposures equivalent to those exhibiting efficacy in animal models of neuropathic pain.[6]

The preclinical efficacy of GCPII inhibitors in multiple animal models prompted us and other groups to explore new GCPII inhibitors with improved drug-like properties.



2-MPPA (R = CH_2CO_2H)
CMBA (R = 3-Carboxyphenyl)



DCIBzL (R' = CH_2CO_2H)
P1'-Allyl analog (R' = $CH{=}CH_2$)

Like 2-MPPA, most of the earlier series of GCPII inhibitors incorporate a glutarate moiety in the P1′ position to take advantage of the glutamate recognition site of GCPII. Increasing efforts are currently being devoted for designing P1′ substituents capable of improving potency and drug-like properties. For example, our group investigated the effect of P1′ side chain modification on GCP II inhibitory potency using 2-MPPA (IC_{50} = 90 nM) as a template and found that several more lipophilic analogs, including 3-(2-carboxy-5-mercaptopentyl)-benzoic acid (CMBA), inhibit GCP II in a more potent manner (IC_{50} = 15 nM) than 2-MPPA.[7] These compounds have also shown improved in vivo potency in the rat chronic constriction injury (CCI) model of neuropathic pain by oral administration. Pomper's group synthesized a variety of

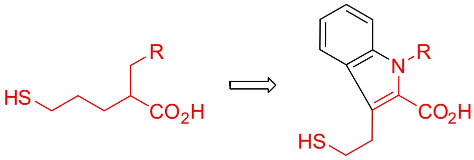
---

[*] Corresponding author. Tel.: +1 410 614 0982; fax: +1 410 614 0659.
E-mail address: ttsukamoto@jhmi.edu (T. Tsukamoto).

0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2010.10.109

GCPII inhibitors containing a bioisostere for the P1′ glutamate using a potent urea-based GCPII inhibitor, DCIBzL (K_i = 0.01 nM), as a template.[8] From this extensive SAR study, they identified several glutamate-free inhibitors with K_i values below 20 nM, including P1′ allyl substituted analog. Although the lack of a carboxyl group on the P1′ side chain resulted in the reduction of potency, the X-ray crystal structure of GCPII in complex with the allyl analog indicates that the allyl side chain minimizes the effect by contributing to the binding primarily via non-polar interactions with the side chains of Phe209, Leu261, and Leu428.[8]

These findings prompted us to explore more drastic structural changes at the P1′ position of GCPII inhibitors in an attempt to further improve their drug-like molecular properties. Our molecular design strategy lies in introducing an aromatic ring system as a core backbone, which allows multiple substitutions without generating any chiral centers. To this end, we chose indole-2-carboxylic acid as a core ring system and incorporated 2-sulfanylethyl group into the 3-position. The new scaffold possesses a reduced number of rotatable bonds and increased lipophilicity compared to CMBA. In addition, various P1′ side chains can be readily explored as substituents at the 1-postion to establish structure–activity relationships (SAR) in this series. In this Letter, we describe the design, synthesis, and biological evaluation of *N*-substituted 3-(2-sulfanylethyl)-1*H*-indole-2-carboxylic acid, representing the first achiral GCPII inhibitors with IC_{50} values in the nanomolar range.

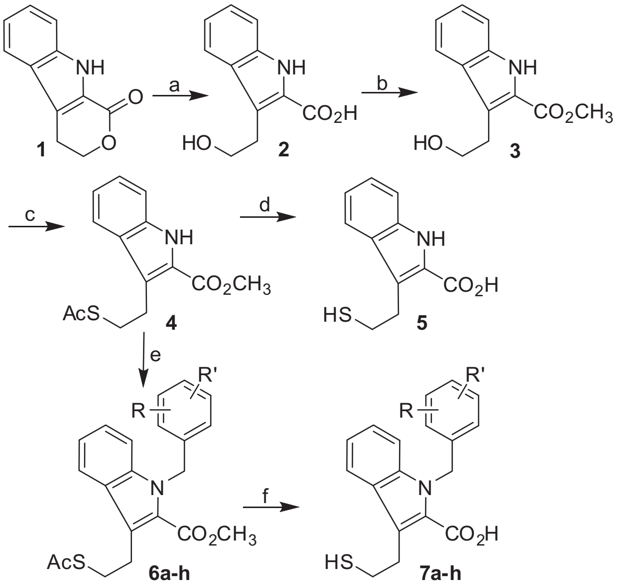


Introduction of indole into GCPII inhibitors

As shown in Scheme 1, a majority of compounds were synthesized using 3,4-dihydropyrano[3,4-*b*]indol-1(9*H*)-one **1** as a starting material. The lactone was opened under basic conditions to provide 3-(2-hydroxyethyl)-1*H*-indole-2-carboxylic acid **2**, which was subsequently converted to the corresponding methyl ester **3**. Mitsunobu reaction with thioacetic acid afforded the thioester **4**. The compound **4** was either hydrolyzed to give 3-(2-mercaptoethyl)-1*H*-indole-2-carboxylic acid **5** or alkylated at its N-position with various benzyl bromides to provide **6a–h**. Base-mediated hydrolysis of **6a–h** gave *N*-substituted 3-(2-mercaptoethyl)-1*H*-indole-2-carboxylic acids **7a–h**.

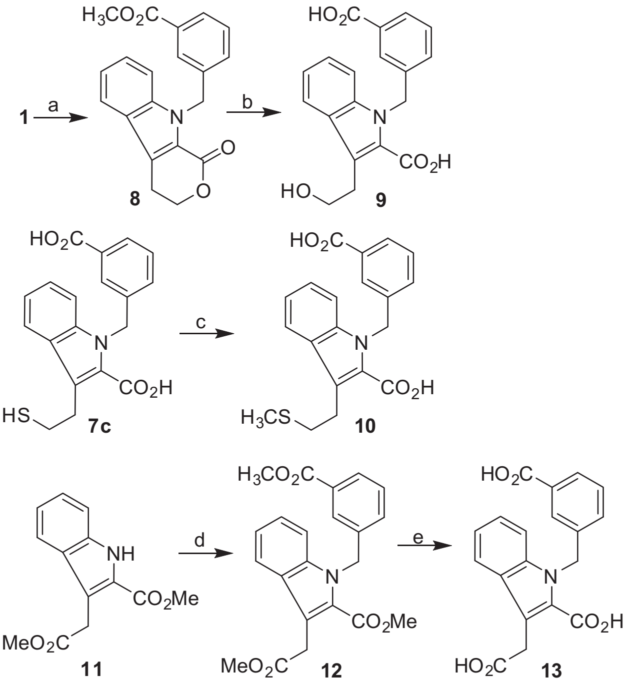
In order to assess the significance of a thiol group as a zinc-binding group, we synthesized a few analogs in which the thiol of compound **7c** is replaced with other functional groups. As outlined in Scheme 2, N-alkylation of **1** with methyl 3-(bromomethyl)benzoate followed by hydrolysis gave **9**. Compound **7c** was methylated at the sulfhydryl group using dimethyl sulfate to give the *S*-methyl derivative **10**. N-Alkylation of methyl 3-(2-methoxy-2-oxoethyl)-1*H*-indole-2-carboxylate **11**[9] with methyl 3-(bromomethyl)benzoate followed by hydrolysis gave **13**.

As illustrated in Scheme 3, N-phenylation of **4** was carried out by $Cu(OAc)_2$ mediated coupling of phenylboronic acid to **4**.[10] The poor yield (33%) of *N*-phenyl derivative **14** is presumably due to the existence of a sulfur atom in the substrate which could poison the catalysis. Base-mediated hydrolysis of **14** afforded **15** in 93% yield. A similar coupling approach failed to produce *N*-3-carboxyphenyl derivative **16**, which would ultimately lead to **17**. Thus, we redesigned our synthetic path to **17** as outlined in Scheme 4. Ethyl 3-(2-ethoxy-2-oxoethyl)-1*H*-indole-2-carboxylate **18**[11] was first coupled with methyl 3-bromobenzoate at its N-position to



6a (R = H, R' = H); **7a** (R = R" = H)
6b (R = 2-CO_2CH_3, R' = H); **7b** (R = 2-CO_2H, R' = H)
6c (R = 3-CO_2CH_3, R' = H); **7c** (R = 3-CO_2H, R' = H)
6d (R = 4-CO_2CH_3, R' = H); **7d** (R = 4-CO_2H, R' = H)
6e (R = 2-Br, R' = 5-CO_2CH_3); **7e** (R = 2-Br, R' = 5-CO_2H)
6f (R =3-*tert* -Bu, R' = 5-CO_2CH_3); **7f** (R = 3-*tert*-Bu, R' = 5-CO_2H)
6g (R = 4-Br, R' = 3-CO_2CH_3); **7g** (R = 4-Br, R' = 3-CO_2H)
6h (R = 2-CO_2CH_3, R' = OCH_3); **7h** (R = 2-CO_2H, R' = OCH_3)

Scheme 1. Reagents and conditions: (a) 3 N KOH–THF, rt, 4.5 h; (b) concd H_2SO_4, MeOH, reflux, overnight; (c) (i) PPh_3, DIAD, THF, 0 °C, 30 min; (ii) **3** and AcSH, THF, 0 °C, 2 h, 63% from **1**; (d) degassed 0.5 N KOH, THF, rt, 20 h, 68% (e) (i) NaH, DMF, −15 °C, 15 min; (ii) $ArCH_2Br$, −15 °C, then rt, overnight; (f) degassed 1 N KOH–THF, rt, 24 h; 88% for **7c** from **4**.



Scheme 2. Reagents and conditions: (a) (i) NaH, DMF, −15 °C, 15 min; (ii) methyl 3-(bromomethyl)benzoate, −15 °C, then rt, overnight, 85%; (b) NaOH, THF–MeOH, rt, overnight, 90%; (c) dimethyl sulfate, 3 N NaOH, 50 °C, 1 h, 25%; (d) (i) NaH, DMF, −15 °C, 15 min; (ii) methyl 3-(bromomethyl)benzoate, −15 °C, then rt, overnight, 70%; (e) NaOH, THF–MeOH, rt, overnight, 84%.