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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

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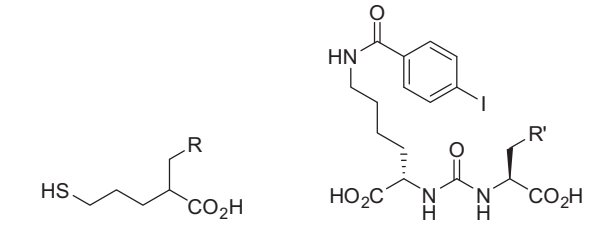
ABSTRACT

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).

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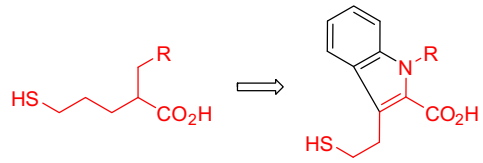
GCPII inhibitors containing a bioisostere for the P1′ glutamate using a potent urea-based GCPII inhibitor, DCIBzL (K_i = 0.01 nM), as a template.[8] From this extensive SAR study, they identified several glutamate-free inhibitors with K_i values below 20 nM, including P1′ allyl substituted analog. Although 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.

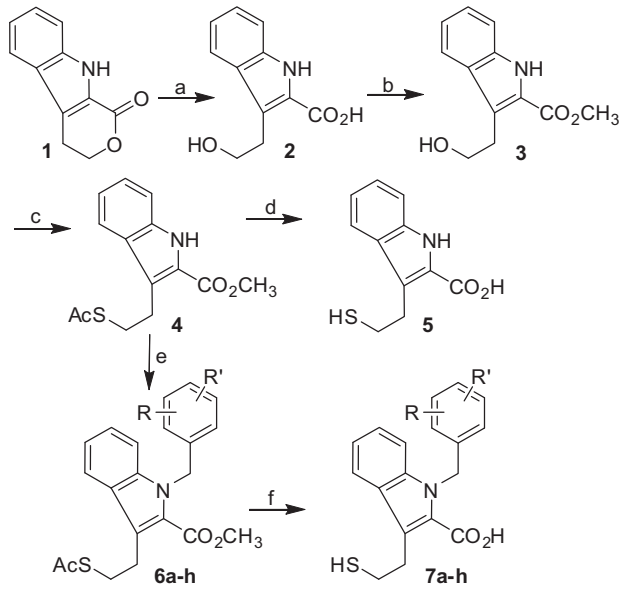


Introduction of indole into GCPII inhibitors

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**.

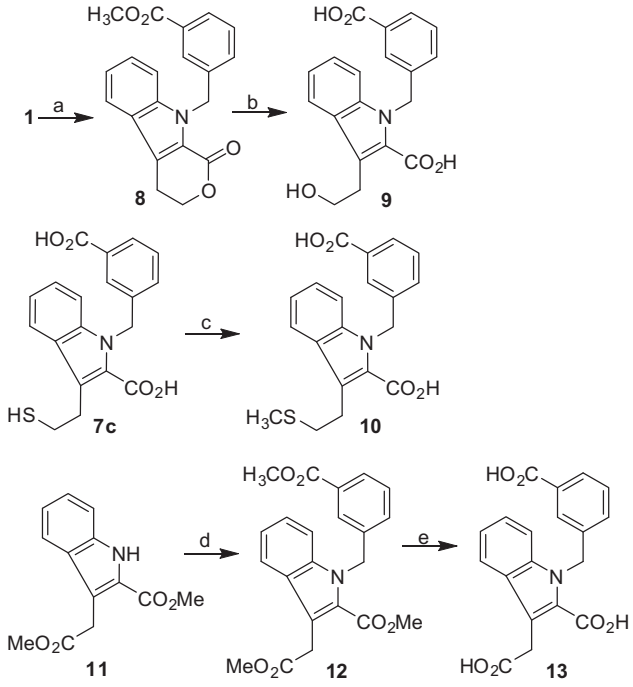
In order to assess the significance of a thiol group as a zinc-binding group, we synthesized a few analogs in which the thiol of compound **7c** is replaced with other functional groups. As outlined in Scheme 2, N-alkylation of **1** with methyl 3-(bromomethyl)benzoate followed by hydrolysis gave **9**. 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

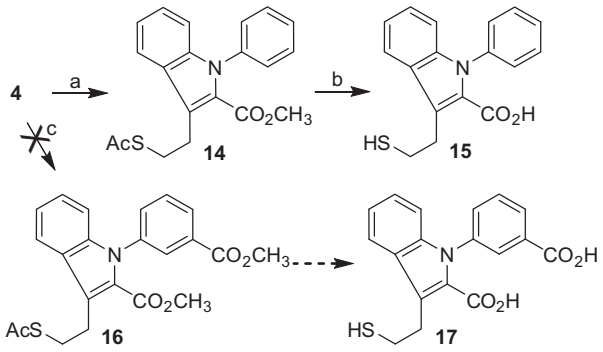


6a (R = H, R' = H); **7a** (R = R" = H)
6b (R = 2-CO_2CH_3, R' = H); **7b** (R = 2-CO_2H, R' = H)
6c (R = 3-CO_2CH_3, R' = H); **7c** (R = 3-CO_2H, R' = H)
6d (R = 4-CO_2CH_3, R' = H); **7d** (R = 4-CO_2H, R' = H)
6e (R = 2-Br, R' = 5-CO_2CH_3); **7e** (R = 2-Br, R' = 5-CO_2H)
6f (R =3-*tert*-Bu, R'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

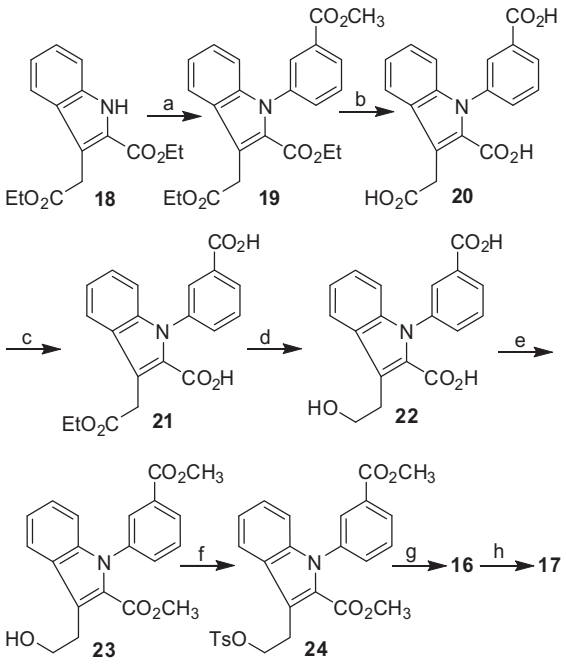
Scheme 1. Reagents and conditions: (a) 3 N KOH–THF, rt, 4.5 h; (b) concd H_2SO_4, MeOH, reflux, overnight; (c) (i) PPh_3, DIAD, THF, 0 °C, 30 min; (ii) **3** and AcSH, THF, 0 °C, 2 h, 63% from **1**; (d) degassed 0.5 N KOH, THF, rt, 20 h, 68% (e) (i) NaH, DMF, −15 °C, 15 min; (ii) $ArCH_2Br$, −15 °C,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



Scheme 3. Reagents and conditions: (a) $Cu(OAc)_2$, phenylboronic acid, pyridine, dichloromethane, rt, 2 days, 33%; (b) 0.6 N KOH–dioxane, rt, overnight, 93%; (c) $Cu(OAc)_2$, (3-methoxycarbonylphenyl)boronic acid, pyridine, dichloromethane, rt.



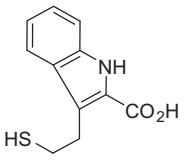
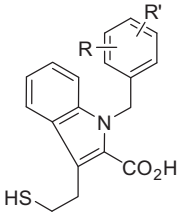
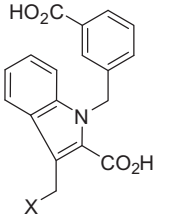
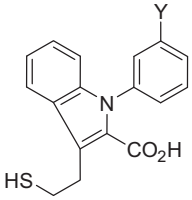
Scheme 4. (a) methyl 3-bromobenzoate, K_2CO_3, CuBr, *N*-methylpyrrolidine, 170 °C, overnight, 73%; (b) 3.2 N NaOH, THF–MeOH, rt, overnight; (c) concd H_2SO_4, ethanol, rt, 2.5 h, 94% from **19**; (d) $LiBH_4$, DME (containing 3% MeOH), reflux, 3 h; (e) CH_2N_2, MeOH, rt, 2 h; (f) TsCl, triethylamine, dichloromethane, rt, 48 h, 63% from **21**; (g) AcSK, DMF, rt, overnight; (h) 0.5 N KOH–THF, rt, 24 h; 85% from **24**.

give *N*-3-carboxyphenyl derivative **19**. Hydrolysis of all the ester groups followed by re-esterification of the aliphatic carboxyl group gave **21**. Reduction of the ester group, followed by re-esterification of the remaining carboxyl groups afforded **23**. The hydroxyl group was then converted to a tosylate ester and substituted by potassium thioacetate to afford **16**. Hydrolysis of all the ester groups gave the desired final product **17**.

Inhibitory potencies of the indole-based compounds were evaluated using *N*-acetyl-L-aspartyl-[^{3}H]-L-glutamate as a substrate and a purified recombinant GCP II.[12] The results are summarized in Table 1.

N-Unsubstituted indole derivative **5** inhibited GCPII with an IC_{50} value of 1.2 μM. *N*-Benzyl derivative **7a** exhibited 10-fold increase in IC_{50} value. However, incorporation of a carboxyl group into the benzene ring dramatically enhanced the inhibitory potency as shown by compounds **7a–h**. The significant improvement in potency by the addition of a carboxyl group suggests that these

Table 1
Inhibition of GCPII by indole-based compounds

Compd	Structure	IC_{50} (nM)[a]
5		1200 ± 300
7a ((R = R′ = H)		12,000 ± 5000
7b (R = 2-CO_2H, R′ = H)		22 ± 21
7c (R = 3-CO_2H, R′ = H)		22 ± 10
7d (R = 4-CO_2H, R′ = H)		94 ± 12
7e (R = 2-Br, R′ = 5-CO_2H)		54 ± 9
7f (R = 3-*t*-Bu, R′ = 5-CO_2H)		34 ± 24
7g (R = 4-Br, R′ = 3-CO_2H)		140 ± 10
7h (R = 2-CO_2H, R′ = 5-OCH_3)		93 ± 41
9 (X = CH_2OH)		24,000 ± 11,000
10 (X = CH_2SCH_3)		20,000 ± 6000
13 (X = CO_2H)		1700 ± 1000
15 (Y = H)		6100 ± 1000
17 (Y = CO_2H)		22 ± 4

[a] Values are means ± SD of at least three experiments.

N-substituents bind in the S1′ pocket which consists of positively charged residues. Position of the carboxyl group (*ortho*-, *meta*-, or *para*-) had only marginal effects on the GCPII inhibitory potency. This could be explained by the rotational flexibility contained in the benzyl group coupled with the relatively mobile positively charged residues at the S1′ pocket.[13] Additional substituent to the phenyl group made little change in IC_{50} values except for *para*-bromo substitution as shown by compound **7g**.

Substitution of the thiol group of **7c** with either hydroxyl group (compound **9**) or methyl sulfide group (compound **10**) resulted in the substantial loss of inhibitory potency. Even substitution by a carboxyl group (compound **13**), which is known to serve as a zinc-binding group, led to a significant increase in IC_{50} value. These findings underscore the critical role of the thiol group of **7c** in the binding to the active site zinc atom(s) of GCPII.

Consistent with the effect of N-benzylation, *N*-phenyl substituted derivative **15** exhibited significantly lower inhibitory potency. Incorporation of a carboxyl group into the *meta*-position (compound **17**), however, resulted in increased potency comparable to those of potent *N*-benzyl series. It is worth noting that the entire skeletal frame of CMBA is embedded in compound **17** with an increasing log *D* value due to the added lipophilicity (from −2.79 for CMBA to −0.11 for compound **17** at pH 7.4).[14] Furthermore, elimination of the chiral center makes the achiral indole-based inhibitors more attractive platform from which a practical drug candidate can be developed. The nearly identical potency of CMBA (15 nM) and **17** (22 nM) demonstrates unexpectedly high degree of tolerance to the structural changes exhibited by the GCPII active site. One could take advantage of this feature and expand the structural diversity of GCPII inhibitors by exploring various conformationally constrained ring systems.

In summary, we have identified a novel series of GCPII inhibitors based on the indole scaffold. These compounds represent the first achiral inhibitors reported for GCPII and offer a substantial advantage over chiral GCPII inhibitors in terms of development feasibility. In addition, the use of synthetic intermediate **4** allows us to generate a wide variety of *N*-benzyl analogs in two steps and further optimize the structure to maximize potency. Furthermore, the breadth of synthetic methods available for 4-, 5-, 6-, and/or 7-sustituted indole-2-crabxylic acid should facilitate SAR studies with respect to the benzene ring portion. Additional binding affinity gained through this part of the molecules might allow us to explore alternative zinc groups and bioisosteres for the two carboxylic groups without significant loss of inhibitory potency. These steps may lead to the discovery of a new generation of GCPII inhibitors with much improved drug-like molecular properties.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.109.

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14. Log *D* values were calculated using ACD/PhysChem Suite, version 12.0.