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SYNTHESIS AND GCP II INHIBITORY ACTIVITY OF 4[4-(3-BROMOBENZYL)-5-HYDROXYISOXAZOL-3-YL]BENZOIC ACID HETEROCYCLIC ANALOGS

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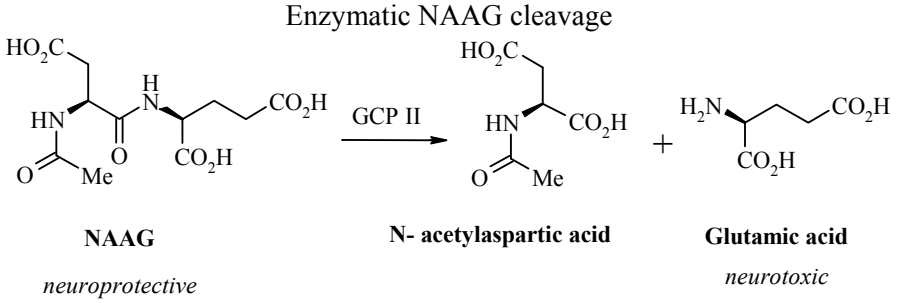
A method for the synthesis of analogs of glutamate carboxypeptidase II inhibitor 4-[4-(3-bromobenzyl)-5-hydroxyisoxazol-3-yl]benzoic acid – 4-[4-(3-bromobenzyl-5-hydroxypyrazol-3-yl]-benzoic acid and 4-[4-(3-bromobenzyl)-3-hydroxyisoxazol-5-yl]benzoic acid from 4-(2-ethoxycarbonylacetyl)benzoic acid was developed. The GCP II inhibitory activity of all the compounds synthesized was determined. Substitution of the 5-hydroxyisoxazole group by the 5-hydroxypyrazole group led toa complete loss of activity, while replacement with the 3-hydroxyylisoxazole gave the compound with slightly less inhibitoer activity comparing with the initial compound.

Keywords:, glutamatecarboxypeptidase II inhibitors, 3-hydroxyisoxazole, 5-hydroxyisoxazole, 5-hydroxypyrazole.

Glutamate carboxypeptidase II (GCP II) is an enzyme, which in the mammalian brain hydrolyses N-acetylaspartylglutamic acid (NAAG) into N-acetylaspartic and glutamic acids [1]. The substrate of the enzyme (NAAG) is an endogenous mGluR3 receptor agonist with a neuroprotective effect [2]. The product of NAAG hydrolysis – glutamic acid causes neurodegeneration at high concentrations [3]. GCP II inhibitors regulate both the concentration of NAAG and glutamic acid in the mammalian brain and are potential reagents in treating neurodegenerative diseases associated with glutamateric system dysfunction [4].



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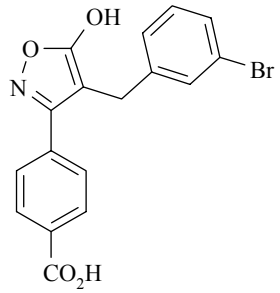
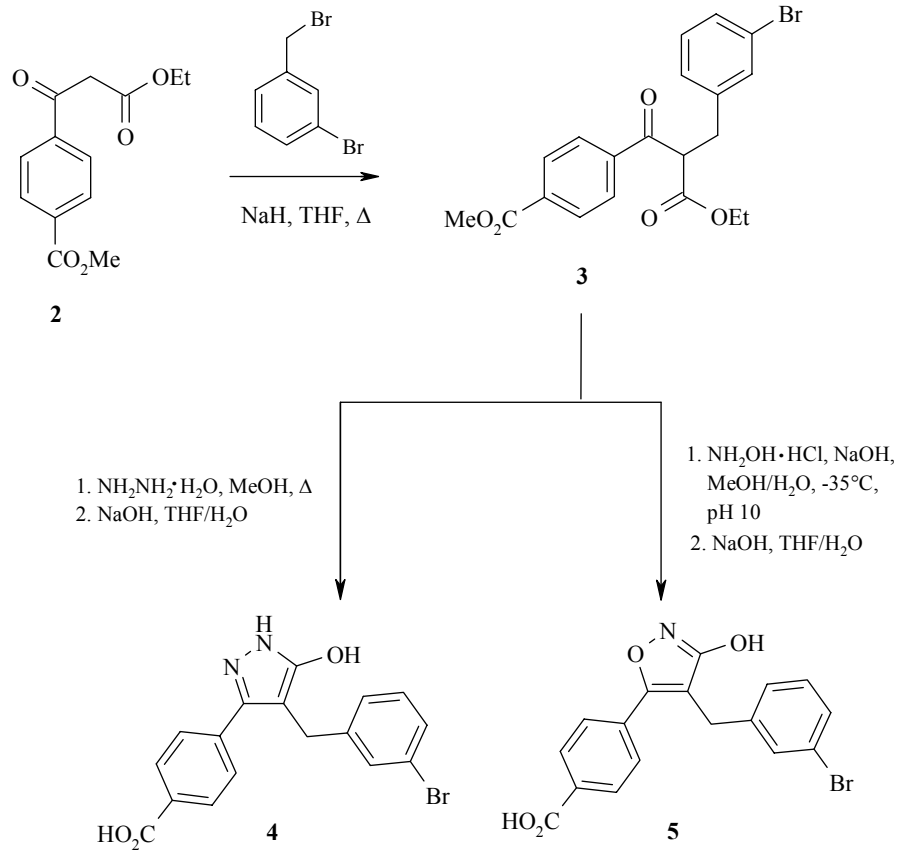
The known GCP II inhibitors are very polar derivatives of polycarboxylic acids with low bioavailability, which does prevent the development of these compounds as medicines [5-8]. GCP II inhibitors with the lowest polarity are dicarboxylic acids, but they contain readily metabolized thio- and hydroxamic acid functions [7,8]. Recently we discovered a new class of GCP II inhibitors based on 4-(5-hydroxyisoxazol-3-yl)benzoic acid, among which 4-[4-(3-bromobenzyl)-5-hydroxyisoxazol-3-yl]benzoic acid (**1)** had a particularly high activity (IC_{50} = 1μM).



1

Here we present the results of our work on the modification of the basic heterocycle in inhibitor **1**. To establish structure–activity relationship between the different analogs 5-hydroxyisoxazole group in 4-(3-bromobenzyl)-5-hydroxyisoxazole **1** was substituted by other heterocycles – 3-hydroxyisoxazole and 5-hydroxy-pyrazole.



2 **3** **4** **5**

The starting material for the preparation of the heterocycles of interest was the commercially available methyl 4-(2-ethoxycarbonylacetyl)benzoate (**2**).

β-Keto ester **2** was alkylated with 3-bromobenzyl bromide in the presence of NaH [9] to give methyl 4-[3-(3-bromophenyl)-2-ethoxycarbonylpropionyl]benzoate (**3**). To obtain the final product 5-hydroxypyrazole **4** the alkylated β-keto ester **3** was condensed with hydrazine in methanol on heating. After formation of the pyrazole ring, alkaline hydrolysis gave product **4**. To prepare 3-hydroxyisoxazole **5** compound **3** was condensed with hydroxylamine in a basic medium at low temperature. Selective formation of 3-hydroxyisoxazole **5** occurs under these conditions [10]. We noted that on heating β-keto ester **3** with hydroxylamine hydrochloride in a neutral medium the corresponding 5-hydroxyisoxazole was formed.

The tests on inhibition of the GCP II activity showed that 4-[4-(3-bromobenzyl)-5-hydroxypyrazol-3-yl]benzoic acid (**4**) is practically inactive als GCP II inhibitor (20% inhibition with c = 10 μM), while GCP II inhibitor activity of 4-[4-(3-bromobenzyl)-3-hydroxyisoxazol-5-yl]benzoic acid **(5)** is lower than that of the starting compound **1** (IC_{50} = 7 μM). Nevertheless this activity is sufficient to use 3-hydroxyisoxazole as an alternative basic heterocycle for further investigation to obtain GCP II inhibitors with nanomolar activity.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Varian Mercury BB (200 MHz) instrument in $CDCl_3$ (compound **3**) and DMSO-d_6 (compounds **4** and **5**) with TMS as internal standard. Chromato-mass spectra were recorded on a Micromass Q-Tof mass spectrometer and an Acquity UPLC System liquid chromatograph with an Acquity UPLC BEH C18 1.7 μm, 2.1×50 mm column. Melting points were measured in capillaries in an Optimelt apparatus and are uncorrected. Reactions and purity of compounds were monitored by TLC on DC Alufolien, Kieselgel 60 F_{254} (Merck) plates with 4:1 petroleum ether–ethyl acetate and 9:1 ethyl acetate–methanol systems. The plates were developed with iodine vapor, 1% ninhydrin in acetone, and UV light (254-365 nm). Kieselgel (35-70 and 60-200 μm) was used for column chromatography with petroleum ether–ethyl acetate as eluent. Solvents were purified and dried by standard methods and drying agents (CaO, NaOH, CaH_2, $CaCl_2$), distilled before using. Reagents were purchased from Acros and Aldrich.

Methyl 4-[3-(3-bromophenyl)2-ethoxycarbonylpropionyl]benzoate (3). A solution of 4-(2-ethoxycarbonylacetyl)benzoic acid **2** (1.22 g, 4.9 mmol) in dry THF (50 ml) was cooled in an ice bath and NaH (60% in mineral oil) (196 mg, 4.9 mmol) was added in small portions while stirring. The cooled mixture was stirred for 20 min while cooling in an ice bath and then for further 30 min at room temperature. Then 3-bromobenzylbromide (593 mg, 4.9 mmol) in THF (20 ml) was added dropwise and the mixture was refluxed for 10 h. The mixture was cooled to room temperature, conc. HCl (6 ml) and ice water (50 ml) were added and the mixture was stirred vigorously. The solution was extracted with ethyl acetate (3×100 ml). The organic extracts were combined, washed with water and saturated NaCl solution and dried over Na_2SO_4. The residue was chromatographed on a silica gel column with 4:1 petroleum ether–ethyl acetate eluent. The required compound (1.34 g, 65%)was obtained as colorless crystals, mp 210-212°C. The substance was a mixture of the keto and enol tautomers. ^{1}H NMR spectrum, δ, ppm (J, Hz): 1.11, 1.25, and 1.34 (total of 6H, all triplets, J = 7, $OCH_2C\underline{H}_3$, keto and enol tautomers); 3.29 (2H, d, J = 7, benzyl CH_2 of the keto tautomer); 3.94 (6H, s, OCH_3, of the keto–enol tautomers); 4.10, 4.17, and 4.28 (total of 4H, all quartets, J = 7, $OC\underline{H}_2CH_3$ of the keto and enol tautomers); 4.58 (1H, t, J = 7, CH of the keto tautomer); 5.73 (2H, s, benzyl CH_2 of the enol tautomer); 7.08-7.38 (6H, m, 2,4,5-CH Ph'); 7.81-8.16 (10H, 2,3,5,6-CH Ph, 6-CH Ph'); 12.54 (1H, s, OH of enol tautomer). Found, %: C 57.78; H 4.33. $C_{20}H_{19}BrO_5$. Calculated, %: C 57.29; H 4.57.

4-[4-(3-Bromobenzyl)-5-hydroxypyrazol-3-yl]benzoic acid (4). Hydrazine hydrate (54 mg, 1.07 mmol) was added to a solution of compound **3** (300 mg, 0.72 mmol) in MeOH (5 ml) and the mixture was

refluxed for 2 h. The mixture was cooled to room temperature, water (5 ml) was added, the mixture was stirred vigorously, and the precipitate was collected on a filter. The precipitate was washed with water and dried *in vacuo*. The yield of colorless crystals of methyl 4-[4-(3-bromobenzyl)-5-hydroxypyrazol-3-yl]benzoate was 70 mg (25%).

Methyl 4-[5-hydroxy-4-(3-bromobenzyl)pyrazol-3-yl]benzoate (66 mg, 0.17 mmol) was dissolved in 1:1 H_2O–THF (2 ml). To this NaOH (17 mg, 0.43 mmol) was added and the solution was stirred at room temperature for 12 h. The solvent was removed under reduced pressure, water (2 ml) was added to the residue and the mixture was acidified with 10% HCl. The precipitate formed was filtered off, washed with water, and dried under reduced pressure. The yield of colorless crystals of the benzoic acid **4** was 60 mg (95%); mp 225°C. ^{1}H NMR Spectrum, δ, ppm (*J*, Hz): 3.83 (2H, s, CH_2); 7.09-7.33 (3H, m, 4,5,6-CHPh'); 7.29 (1H, s, 2-CHPh'); 7.54 (2H, d, *J* = 8, 2,6-CHPh); 7.92 (2H, d, *J* = 8, 3,5-CHPh). R_f = 6.41 min (>95%) at 254 nm). Mass spectrum, *m/z* (I_{rel}, %): 373 $[M]^+$ (75), 293 (6), 217 (100), 199 (4), 173 (39), 169 (22), 143 (3). Found, %: C 53.25; H 4.33; N 6.79. $C_{17}H_{13}BrN_2O_3$. Calculated, %: C 54.71, H 3.51, N 7.51.

4-[4-(3-Bromobenzyl)-3-hydroxyisoxazol-5-yl]benzoic acid (5). $NH_2OH·HCl$ (156 mg, 2.24 mmol) and NaOH (90 mg, 2.24 mmol) were suspended in 10:1 methanol–water (5 ml) and stirred on an ice bath for 10 min. The suspension obtained and additional NaOH (45 mg, 1.12 mmol) in 10:1 methanol–water (5 ml) were added to a solution of benzoic acid methyl ester **3** (470 mg, 1.12 mmol). The mixture was stirred at -35°C for 5 h, then warmed to +5°C and stirred for 30 min. Conc. HCl (3 ml) was then added, the mixture was stirred vigorously and evaporated under reduced pressure. The residue was crystallized from water (10 ml) and then from MeCN (5 ml), dried *in vacuo* to give colorless crystals of methyl 4-[(3-bromobenzyl)-3-hydroxy-4-isoxazol-5-yl]benzoate (35 mg, 8%).

Methyl 4-[4-(3-bromobenzyl)-3-hydroxyisoxazol-5-yl)benzoate (35 mg, 0.09 mmol) was dissolved in 1:1 water–THF (2 ml). NaOH (9 mg, 0.23 mmol) was added to the solution, which was then stirred at room temperature for 12 h. The solvent was removed under reduced pressure, water (2 ml) was added to the residue and the mixture was acidified with 10% HCl. The precipitate was collected on a filter, washed with water, and dried under reduced pressure to give white crystals of compound **5**, yield 25.3 mg (75%); mp 255°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.95 (2H, s, CH_2); 7.16-7.29 (3H, m, 4,5,6-CH Ph'); 7.39 (1H, s, 2-CH Ph'); 7.78 (2H, d, *J* = 8, 2,6-CH Ph); 8.04 (2H, d, *J* =8, 3,5-CH Ph); 11.84 (1H, br.s, CO_2H). R_f 7.4 min (>99% at 254 nm). Mass spectrum, *m/z* (*I*, %): 374 $[M]^+$ (100), 353 (18), 315 (3), 251 (7), 218 (45), 174 (56), 149 (11). Found, %: C 55.81; H 3.51; N 3.52. $C_{17}H_{12}BrNO_4$. Calculated, %: C 54.57; H 3.23; N 3.74.

Glutamatecarboxypeptidase II inhibiting activity was determined by measuring the degree of tritium-labeled N-acetyl-L-aspartyl-L-glutamate hydrolysis.

Two identical experiments were carried out in parallel with an overall volume of 1 ml, each of which contained 50 mmol Tris-HCl, pH 7.4, 30 nmol of N-acetyl-L-aspartyl-L-[3,4-^{3}H]glutamate and 30-50 μg of membrane protein. The test was initiated by adding to the mixture membrane protein (with or without inhibitor) the temperature of which had been equilibrated at 37°C. The test solution was stirred intensively and it reached a temperature of 37°C in 15 min. The experiment was concluded by the addition of ice-cold sodium phosphate buffer (1.0 ml, 0.1 M, pH 7.4). Aliquots of the test solution were added to Dowex AG 1-X8400 ion-exchange resin in PP minicolumns (Millipore). Glutamate was eluted from the column with 1.0 M formate (1.8 ml). The mixture obtained was diluted with 15 ml of scintillation solution (SuperMix, Perkin-Elmer) and the radioactivity was determined with a scintillation spectrometer.

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REFERENCES

1. M. B. Robinson, R. D. Blakely, R. Couto, and J. T. Boyle, *J. Biol. Chem.,* **262**, 14498 (1987).
2 B. Wroblewska, J. T. Wroblewski, S. Pshenichkin, A. Surin, S. E. Sullivan, and J. H. Neale, *Neurochem.,* **69**, 174 (1997).
3 W. Danysz, C. G. Parsons, J. Bresink, and G. Quack. *Drug News Perspect.,* **8**, 261 (1995).
4 P. F. Jackson and B. S. Slusher. *Curr. Med. Chem.,* **8**, 949 (2001).
5 P. F. Jackon, K. L. Tays, K. M. Maclin, Y.-S. Ko, W. Li, D. Vitharana, T. Tsukamoto, D. Stoermer, X.-C. M. Lu, K. Wozniak, and B. S. Slusher. *J. Med. Chem.,* **44**, 4170 (2001).
6 A. P. Kozikowski, J. Zhang, F. Nan, P. A. Petukhov, E. Grajkowska, J. T. Wroblewski, T. Yamamoto, T. Bzdega, B. Wroblewska, and J. H. Neale. *J. Med. Chem.,* **47**, 1729 (2004).
7 P. Majer, P. F. Jackson, G. Delahanty, B. S. Grella, Y.-S. Ko, W. Li, Q. Liu, K. M. Maclin, J. Polakowa, K. A. Shaffer, D. Stoermer, D. Vitharna, E. Y. Wang, A. Zakrzewski. C. Rojas, B. S. Slusher, K. M. Wozniak, E. Burak, T. Limsakun, and T. Tsukamoto. *J. Med. Chem.,* **46**, 1989 (2003).
8 D. Stoermer, Q. Liu, M. R. Hall, J. M. Flanary, A. G. Thomas, C. Rojas, B. S. Slusher, and T. Tsukamoto. *Biorg. Med. Chem. Lett.,* **13**, 2097 (2003).
9 M. Nomura, T. Tanase, T. Ide, M.Tsunoda. M. Suzuki, H. Uchiki, K. Murakami, and H. Miyachi. *J Med. Chem.,* **46**, 3581 (2003).
10 E. Falch, L. Brehm, I. Mikkelsen, T. N. Johansen, N. Skjaebaek, B. Nielsen, T. B. Stensbol, B. Ebert, and P. Krogagaard-Larsen. *J. Med. Chem.,* **41**, 2513 (1998).