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SYNTHESIS AND ANTI-INFLAMMATORY AND ANTIULCER ACTIVITY OF A GLYCYRRHIZIC ACID CONJUGATE WITH L-PHENYLALANINE METHYL ESTER

L. A. Baltina,[1,*] T. A. Sapozhnikova,[1] S. F. Gabdrakhmanova,[1] N. S. Makara,[1] R. M. Kondratenko,[2] and F. A. Zarudii[1]

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A conjugate of glycyrrhizic acid (GA) with L-phenylalanine methyl ester was synthesized using *N*-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. Chemical modification of GA via addition of L-phenylalanine methyl ester moieties in the carbohydrate part of the glycoside was shown to form a marginally toxic compound with high anti-inflammatory activity in carrageenan- and formalin-induced mouse inflammation models and with pronounced antiulcer activity in rats. This was a significant advantage of this compound over known anti-inflammatory drugs.

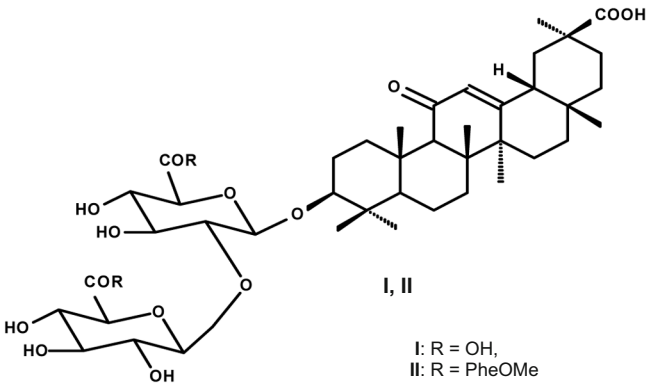
Keywords: glycyrrhizic acid, conjugate, anti-inflammatory and antiulcer activity.

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac (orthophen), indomethacin, ibuprofen, acetylsalicylic acid (aspirin), etc. are widely used in medical practice and possess several side effects that cause irritation or ulceration of the gastrointestinal tract (GIT), immunosuppressive action, and cardio- and nephrotoxicity [1 – 3]. Dyspeptic disorders occur in 30 – 40% of patients receiving NSAIDs; stomach and duodenal erosion and ulceration, 10 – 20%; and hemorrhaging and perforation, 2 – 5% [4]. Therefore, the search for new anti-inflammatory drugs with low toxicity that do not irritate stomach mucous membranes is a challenging problem for medicinal chemistry, pharmacy, and pharmacology.

The design of new drugs based on available natural compounds of plant origin (secondary metabolites) is a promising modern approach of medicinal chemistry. Despite the competition with synthetic drugs, natural compounds can lead to new promising clinical candidates and drugs with various types of action [5]. Natural compounds and their derivatives account for up to 40% of the yearly global drug circulation and are especially widely employed in oncology and metabolic and infectious diseases [5 – 7]. Bioactive secondary metabolites produced by plants are interesting as scaffolds for synthetic and semisynthetic modifications, the synthesis of combinatorial libraries of compounds, and studies of structure–activity relationships [8]. The structures of natural compounds with established biological activity can be optimized to produce new derivatives and their analogs and can generate new lead compounds of interest for preclinical studies [9].

Glycyrrhizic acid (GA, **I**) is the main triterpene glycoside of licorice roots (*Glycyrrhiza glabra* L. and *G. uralensis* Fisher), has been used since antiquity in traditional medicine of China and Eastern and Western countries, and is an available natural compound with a broad spectrum of biological and pharmacological activity (anti-inflammatory, antiulcer, immunomodulating, antioxidant, antidote, neuro- and hepatoprotective, antiviral) [10, 11]. Chemical modification of GA is a promising pathway for preparing new biologically active medicines [12, 13]. Effective anti-inflammatory, antiulcer, immunomodulating, and antiviral agents were found by us among GA derivatives [10, 12, 14]. Several GA derivatives containing ureide, carbamate, and amino-acid

[1] Ufa Institute of Chemistry, Special Structural Subdivision of Ufa Federal Research Center, Russian Academy of Sciences, 71 Prosp. Oktyabrya, Ufa, Bashkortostan, 450054 Russia.
[2] Bashkir State Medical University, Ministry of Health of the Russian Federation, 3 Lenina St., Ufa, Bashkortostan, 450008 Russia.
[*] e-mail: baltina@anrb.ru



pharmacophores were shown to be promising marginally toxic anti-inflammatory agents [10, 12].

The goals of the present work were to synthesize and study the anti-inflammatory and antiulcer activity of a GA conjugate with L-phenylalanine methyl ester (**II**) in experimental inflammation and stomach mucous membrane destruction models in mice and rats as compared to GA and the known NSAIDs diclofenac (orthophen), phenylbutazone (butadion), and acetylsalicylic acid and the antiulcer drugs carbenoxolone (disodium salt of glycyrrhetinic acid hydrogen succinate) and omeprazole.

Conjugate **II** was synthesized via the activated ester method using *N*-hydroxysuccinimide (HOSu) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (DEC) in DMF with GA/HOSu/DEC/AC ratios 1/4.3 – 5.2/3.0 – 3.5/2.5 – 3.0 mmol at 0 – 5°C for 1 h and at room temperature for 24 h in the presence of an excess of *N*-ethylmorpholine (NEM) and was isolated by column chromatography over silica gel in 57% yield. The physicochemical and spectral characteristics of **II** agreed with those published earlier and obtained from **II** synthesized using HOSu–*N*,*N*′-dicyclohexylcarbodiimide (DCC) [15].

Anti-inflammatory activity of **II** was studied using two mouse paw inflammation models, i.e., induced by carrageenan solution (1%) and formalin solution (3%). Compound **II** at doses of 25, 50, and 100 mg/kg was administered intragastrically to mice 1 h before reproducing and 1 and 2 h after reproducing acute inflammation (edema). The reference drugs were GA and the widely used anti-inflammatory medicines orthophen, butadion, and acetylsalicylic acid at therapeutic doses. Table 1 presents the test results.

Conjugate **II** at doses of 25 and 50 mg/kg had pronounced anti-inflammatory action in the carrageenan-induced model that exceeded that of GA at doses of 50 and 100 mg/kg and was analogous to that of orthophen at a therapeutic dose (8 mg/kg) (Table 1). The anti-inflammatory activity of **II** at doses of 25 and 50 mg/kg in this model was also more pronounced than those of the known drugs butadion and acetylsalicylic acid at therapeutic doses.

Conjugate **II** at a dose of 50 mg/kg in the formalin-induced inflammation model exhibited stronger anti-inflammatory activity than GA at the same dose (Table 1). The anti-inflammatory activity of **II** at a dose of 50 mg/kg was analogous to that of orthophen and exceeded those of butadion and acetylsalicylic acid.

The antiulcer activity of **II** at a dose of 100 mg/kg was studied in rats with an experimental indomethacin-induced stomach ulcer model. GA and carbenoxolone at doses of 100 mg/kg and omeprazole at a therapeutic dose (20 mg/kg) were used as the reference drugs [10, 16]. GA conjugate **II** reduced statistically significantly the degree of damage to stomach mucous membranes in the indomethacin stomach ulcer destruction model in rats and had antiulcer action analogous to that of carbenoxolone at equal doses (100 mg/kg). Table 2 presents the test results.

TABLE 1. Anti-inflammatory Activity of the Compounds in Mice (n = 7)

Compound	Dose, mg/kg	Paw edema increase, %, induced by			
		carrageenan	p	formalin	p
Conjugate **II**	100	50.0 ± 2.7	< 0.001	39.0 ± 2.8	< 0.001
Conjugate **II**	50	34.8 ± 5.4	< 0.001	35.0 ± 3.8	< 0.001
Conjugate **II**	25	35.6 ± 4.6	< 0.001	–	–
GA	100	59.1 ± 5.3	< 0.05	41.5 ± 3.9	< 0.001
GA	50	60.4 ± 1.9	< 0.05	44.3 ± 3.2	< 0.001
Orthophen	8	39.0 ± 3.2	< 0.001	35.2 ± 3.2	< 0.001
Butadion	56	40.4 ± 3.4	< 0.001	46.1 ± 2.6	< 0.05
Acetylsalicylic acid	98	49.5 ± 3.6	< 0.001	46.3 ± 2.2	< 0.001
Control		68.6 ± 2.9		70.0 ± 2.7	

$m \pm M$; n is the number of animals in a group; $p < 0.05$, statistically significant vs. the control according to Student t-criterion.

The antiulcer effect of **II** was twice that of GA and 1.5 times that of omeprazole in this rat model of experimental stomach ulcers.

Acute toxicity of **II** was studied using laboratory female white mice (18 – 20 g) with a single intragastric administration. The toxicity parameters were calculated according to Litchfield–Wilcoxon [17]. The LD_{50} of **II** was 4,000 mg/kg intragastrically. Compound **II** was classified as moderately hazardous according to the classification of GOST 12.1.007.76.

Thus, chemical modification of GA via addition of L-phenylalanine methyl ester to the carbohydrate part of the glycoside had a substantial effect on the anti-inflammatory and antiulcer properties of the derivative. Conjugate **II** was marginally toxic and possessed high anti-inflammatory activity in carrageenan- and formalin-induced inflammation models in mice. The high anti-inflammatory activity of **II** was combined with antiulcer activity, which was an advantage of this compound over known anti-inflammatory drugs and made it promising for expanded pharmacological studies as a new anti-inflammatory and antiulcer agent.

EXPERIMENTAL CHEMICAL PART

PMR and ^{13}C NMR spectra were recorded with TMS internal standard on a Bruker AMX-300 spectrometer at operating frequency 300 and 75.5 MHz. IR spectra were taken from mineral-oil mulls on a Prestige-21 IR spectrophotometer (Shimadzu). Optical activity was measured on a PerkinElmer 341 polarimeter using a 1-dm tube at 20 – 22°C (λ_{Na} 546 nm).

TLC used Sorbfil plates (Sorbpolimer, Russia). Spots of compounds were detected by H_2SO_4 (5%) in EtOH followed by heating at 120 – 130°C for 2 – 3 min. Column chromatography used silica gel (50 – 160 μm fraction; Imid OOO, Russia).

GA (96%) obtained from glycyram drug substance [18] was used in the work. *N*-Hydroxysuccinimide (Aldrich, USA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (Roth, Germany), and L-phenylalanine methyl ester hydrochloride (Reanal, Hungary) were purchased. The reference drugs for studies of anti-inflammatory activity were diclofenac drug substance (orthophen, Tatkhimfarmpreparaty, Russia), phenylbutazone (butadion, Obolenskoe Pharmaceutical Co., Russia), and acetylsalicylic acid (Medisorb, Russia) at therapeutic doses. Antiulcer activity was compared to that of carbenoxolone (disodium salt of glycyrrhetinic acid hydrogen succinate) [10, 16] and the antiulcer drug omeprazole (Omez, India).

TABLE 2. Antiulcer Activity of the Compounds in Rats ($n = 6$)

Compound	Dose, mg/kg	Average number of stomach ulcers induced by	
		indomethacin	ð
Conjugate **II**	100	5.8 ± 0.6	< 0.001
GA	100	12.1 ± 1.5	> 0.05
Carbenoxolone	100	5.8 ± 0.4	< 0.001
Omeprazole	20	9.0 ± 0.7	< 0.05
Control	-	14.0 ± 1.8	-

$m \pm M$; n is the number of animals in a group; $p < 0.05$, statistically significant vs. the control according to Student *t*-criterion.

3-*O*-{2-*O*-[*N*-(β-D-Glucopyranosyluronoyl)-L-phenylalanine methyl ester]-*N*-(β-D-glucopyranosyluronoyl)-L-phenylalanine methyl ester}-(3β,20β)-11-oxo-30-norolean-12-ene (2). A solution of GA (1.64 g, 2 mmol) in DMF (40 mL) at 0 – 5°C was treated with HOSu (1.2 g, 10.4 mmol), DEC (1.14 g, 6.0 mmol), L-phenylalanine methyl ester hydrochloride (5.0 mmol), and *N*-ethylmorpholine (10 mmol); cooled; stirred for 1 h; held at 20 – 22°C for 24 h with periodic stirring; diluted with cold H_2O; and acidified with citric acid to pH 3 – 4. The precipitate was filtered off, rinsed with H_2O, dried, and chromatographed over a column of silica gel with elution by $CHCl_3$–EtOH (300:10, 200:10, 100:10, 50:10, vol%). Yield 0.65 g (57%) (amorphous compound). R_f 0.52 (benzene–EtOH, 5:1); $[\alpha]_D^{20}$ + 60°C (c 0.05, EtOH). Lit. [15]: $[\alpha]_D^{20}$ + 62° (c 0.04, EtOH). IR spectrum, ν_{max}, cm^{-1}: 3500 – 3200 (OH, NH); 1743 (COOH); 1661 (C^{11}=O); 1528 (CONH), 1500 (Ph). PMR spectrum (CD_3OD), δ, ppm: 0.74 (3H, s, CH_3), 0.81 (3H, s, CH_3), 0.91 – 0.96 (3H, m, CH, CH_2), 1.04 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.14 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.23 – 1.30 (2H, m, CH_2), 1.41 (6H, s, $2CH_3$), 1.57 – 1.95 (6H, m, CH, CH_2), 2.40 – 2.70 (5H, m, CH, CH_2), 3.10 – 3.32 (5H, m, CH, CH_2), 3.50 – 3.40 (4H, m, CH, CH_2), 3.68, 3.70 (6H, both s, $2OCH_3$), 3.70 – 3.78 (4H, m, H4′, H5′, H4″, H5″), 4.55 – 4.82 (6H, m, H1′-H3′, H1″-H3″), 5.60 (1H, s, H12), 7.32 – 7.15 (10H, m, $2C_6H_5$). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 202.6 (C11), 180.5 (C30), 172.6 (C6″); 171.3 (C13), 171.3 (C6′), 128.9 (C12), 104.9 (C1′), 104.7 (C1″), 90.7 (C3), 81.0 (C2′), 77.9 (C5″), 77.3 (C5′), 76.0 (C3″), 75.8 (C3′), 75.4 (C2″), 73.5 (C4′), 73.4 (C4″), 63.1 (C9), 56.4 (C5), 48.2 (C18), 46.8 (C8), 44.9 (C20), 44.6 (C14), 42.5 (C19), 40.7 (C4), 40.3 (C1), 39.0 (C22), 38.1 (C10), 33.9 (C7), 33.0 (C17), 32.0 (C21), 29.2 (C29), 28.8 (C23), 28.4 (C28), 27.6, 27.4, 27.4 (C15, C16, C2), 23.9 (C27), 19.3 (C26), 18.5 (C6), 17.4 (C25), 17.1 (C24); 2PheOMe: 173.0, 172.9, 137.6, 130.5, 130.4, 129.7, 128.2, 54.9, 54.5, 53.0, 52.9, 38.4, 38.1. $C_{62}H_{84}O_{18}N_2$. Mol. mass 1145.3.

EXPERIMENTAL PHARMACOLOGICAL PART

Experiments used laboratory male white rats (200 – 220 g) and laboratory white mice of both sexes (18 – 20 g). Animals were kept under standard vivarium con-

ditions with free access to feed and water. Animal experiments were conducted in compliance with international rules (European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, No. 123, Strasbourg, 1986; Protocol of Amendment to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Strasbourg, June 22, 1998) and were approved by the Biomedical Ethics Committee, UfIC, Subdivision of UFRC, RAS.

Anti-inflammatory activity of **II** was studied using laboratory male white mice and two acute inflammation models induced by carrageenan solution (1%) and formalin solution (3%) [19, 20]. The irritants were injected into the plantar fascia of the right hind paw at a dose of 0.05 mL. The left paw acted as an untreated control. Each group included seven animals. The reference drugs were GA and the widely used anti-inflammatory drugs diclofenac drug substance (orthophen), phenylbutazone (butadion), and acetylsalicylic acid at therapeutic doses. All compounds were administered perorally 1 h before and 1 and 2 h after reproducing inflammation. Conjugate **II** was administered at doses of 25, 50, and 100 mg/kg; GA, 50 and 100; orthophen, 8; butadion, 56; and acetylsalicylic acid, 98. Control animals received diluent (distilled H_2O with polysorbate-80). Edema strength was determined after 3 h from the difference in volumes of the healthy and test paws and was compared with the results for the control group. The results were expressed in percent.

Antiulcer activity of **II** was studied in rats (six animals per group) for a stomach ulcer indomethacin model [21] as compared to GA and the known antiulcer agents carbenoxolone and omeprazole. Ulcers of stomach mucous membranes were induced by i.p. injection of indomethacin at a dose of 20 mg/kg. Rats were deprived of access to feed with unlimited access to water for 1 d before the experiment. Compound **II** and the reference drugs (GA, carbenoxolone) were administered intragastrically to animals at a dose of 100 mg/kg; omeprazole, at a dose of 20 mg/kg 1 h before reproducing stomach damage. Damage to stomach mucous membranes was assessed visually 24 h after administering the ulcer-inducing agent.

Acute toxicity of **II** was studied in laboratory female white mice (18 – 20 g) (five animals per group) with a single administration into the stomach. Doses were selected based on acute toxicity data for GA (LD_{50} = 5,000 mg/kg) [10]. Conjugate **II** was administered at doses of 1,000, 2,000, 3,000, 4,000, and 5,000 mg/kg. Animals were observed for 14 d, noting postponed deaths of animals, general condition, locomotor activity, and demand for feed and water. Toxicity parameters were calculated observing recommendations for studies of general toxicity of biologically active compounds [17, 19].

Statistical analysis used the Statistica 10.0 program. Data were given as averages (m) and their errors ($\pm M$). Intergroup differences were evaluated using the Student t-criterion. Results were considered statistically significant for $p < 0.05$.

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REFERENCES

1. E. L. Nasonov and A. E. Karateev, *Russ. Med. Zh.*, **14**, 1769 – 1773 (2006).
2. T. M. Laidlaw and K. N. Cahill, *J. Allergy Clin. Immunol. Pract.*, **5**, 537 – 545 (2017).
3. S. Harirforoosh, W. Asghar, and F. Jamali, *J. Pharm. Pharm. Sci.*, **16**, 821 – 847 (2013).
4. Ya. S. Zimmerman, *Unsolved and Debatable Issues of Modern Gastroenterology*, MEDpress-inform, Moscow (2013).
5. G. M. Cragg and D. J. Newman, *Biochim. Biophys. Acta*, **1830**, 3670 – 3695 (2013).
6. D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, **79**, 629 – 661 (2016).
7. S. R. Leicach and H. D. Chludil, *Stud. Nat. Prod. Chem.*, **42**, 267 – 304 (2014).
8. E. C. Barnes, R. Kumar, and R. A. Davis, *Nat. Prod. Rep.*, **33**, 372 – 381 (2016).
9. J. M. Rollinger, T. Langer, and H. Stuppner, *Curr. Med. Chem.*, **13**, 1491 – 1507 (2006).
10. G. A. Tolstikov, L. A. Baltina, V. P. Grankina, et al., *Licorice: Biodiversity, Chemistry and Use in Medicine* [in Russian], Akademicheskoe Izd. Geo, Novosibirsk (2007).
11. X. Wang, H. Zhang, L. Chen, et al., *J. Ethnopharmacol.*, **150**, 781 – 790 (2013).
12. L. A. Baltina, *Curr. Med. Chem.*, **10**, 155 – 171 (2003).
13. C. S. Graebin, H. Verli, and J. A. Guimaraes, *J. Braz. Chem. Soc.*, **21**, 1595 – 1615 (2010).
14. L. A. Baltina, R. M. Kondratenko, L. A. Baltina Jr., et al., *Pharm. Chem. J.*, **43**(10), 539 – 548 (2009).
15. L. A. Baltina, V. V. Zarubaev, L. A. Baltina, et al., *Bioorg. Med. Chem. Lett.*, **25**, 1742 – 1745 (2015).
16. R. S. H. Finney and A. L. Tarnoky, *J. Pharm. Pharmacol.*, **12**, 49 – 58 (1960).
17. J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 – 113 (1949).
18. R. M. Kondratenko, L. A. Baltina, S. R. Mustafina, et al., *Pharm. Chem. J.*, **35**(2), 101 – 104 (2001).
19. L. A. Baltina, N. G. Serdyuk, A. F. Ismagilova, et al., *Pharm. Chem. J.*, **31**(9), 480 – 481 (1997).
20. A. N. Mironov and N. D. Bunyatyan, *Handbook for Preclinical Drug Trials* [in Russian], Part 1, Grif i K, Moscow (2013).
21. S. Y. Lim, J. S. Byun, D. J. Kim, and M. A. Kwak, *J. Physiol. Pathol. Korean Med.*, **26**, 181 – 188 (2012).