Synthesis and analgesic activity evaluation of derivatives of 2-[(1,4-dioxo-1-amino-4-arylbutyl-2-en-2-yl)amino]-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic acid

Alena I. Siutkina ab, Ramiz R. Makhmudov ac, Daria A. Shipilovskikh d

a: Perm State University, 614068 Bukireva st., 15, Perm, Russia
b: Perm State Pharmaceutical Academy, 614990 Ekaterininskaya st., 101, Perm, Russia
c: Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 614045 Monastyrskaya st., 82, Perm, Russia
d: Perm National Research Polytechnic University, 614077 Komsomolsky Prospekt, 29, Perm, Russia

* Corresponding author: syutkina.alyona@yandex.ru

This article belongs to the MOSM2021 Special Issue.

© 2021, The Authors. This article is published in open access form under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

Abstract

The synthesis of new derivatives of 2-[(1,4-dioxo-1-amino-4-arylbutyl-2-en-2-yl)amino]-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic acid is described. Starting 2-[(5-aryl-2-oxofuran-3(2H)-ylidene)amino]thiophene-3-carboxylic acids were obtained by intramolecular cyclisation of substituted 4-aryl-4-oxo-2-thienylaminobut-2-enolic acids in acetic anhydride. New derivatives of 2-[(1,4-dioxo-1-amino-4-arylbutyl-2-en-2-yl)amino]-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic acids were obtained via decyclization reaction of 2-[(5-aryl-2-oxofuran-3(2H)-ylidene)amino]thiophene-3-carboxylic acids. The structure of the compounds obtained was confirmed by the $^1$H and $^{13}$C NMR spectroscopy, IR spectrometry and elemental analysis methods. Analgesic activity of new compounds has been studied by the “hot plate” method on outbred white mice of both sexes with intraperitoneal injection. It was found that derivatives of 2-[(1,4-dioxo-1-amino-4-arylbutyl-2-en-2-yl)amino]-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic acid possess analgesic effect exceeding the effect of the comparison drug metamizole.

Keywords

analgesic activity
Gewald reaction
2,4-dioxobutanoic acids
3-[(thiophen-2-yl)iminofuran-2(3H)-one]

Received: 01.11.2021
Revised: 19.11.2021
Accepted: 19.11.2021
Available online: 22.11.2021

1. Introduction

The Gewald aminothiophene fragment is a promising pharmacophore group, since it was found in both natural and synthetic physiologically active compounds [1–6]. The synthesis of substituted Gewald aminothiophenes can be carried out using the Gewald reaction [7–9]. In turn, 3-imino(hydrazono)-3H-furan-2-ones have high reactivity, leading to the production of compounds of various structures [10–17]. Decyclization reactions of 3-imino-3H-furan-2-ones lead to the production of 2,4-dioxobutanoic acid derivatives, for which antiviral [18–22], analgesic [23], anti-inflammatory [24], antimicrobial [25] activity was determined.

It was previously shown that 3-imino(hydrazono)-3H-furan-2-ones can be decyclized under the action of aliphatic, aromatic, and heterocyclic amines to form amides of 4-aryl(tet-butyl)-4-oxo-2-amino(hydrazono)-2-eno acids [26, 27]. In this paper, synthesis and analogic activity of new 2-[(1,4-dioxo-1-amino-4-arylbutyl-2-en-2-yl)amino]-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic acid derivatives is discussed.

2. Experimental

IR spectra were recorded on an FSM-1202 instrument from liquid paraffin. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance III instrument (400 and 100 MHz) from CDCl$_3$ and DMSO-d$_6$ solutions relative to residual signals of the non-deuterated solvent. Elemental analysis was performed on a Leco CHNS-932 instrument. Reaction progress and individuality of obtained compounds was monitored by TLC on Sorbfil plates, eluting with a diethyl ether–benzene–acetone system (10:9:1); detecting in UV light and iodine vapor. Melting points were determined on an SMP40 instrument.
Starting substituted 4-aryl-4-oxo-2-thienylaminobut-2-enoic acids 1a,b and substituted 3-thielenylmino-3H-furan-2-ones 2a,b were obtained according to the procedure described in [28–30]. All data correspond to the previously obtained ones.

2.1. General procedure for the synthesis of N-substituted amides of 4-aryl-4-oxo-2-[(3-thiophen-2-yl)amino]-but-2-enoic acids 3a-e

A mixture of 0.001 mol of compound 2a-e and 0.001 mol of the corresponding amine in anhydrous toluene (20 mL) was stirred at 50 °C for 2 h. After cooling, the precipitate was filtered off and recrystallized.

2.2. Ethyl 2-((4-methylpyrimidin-2-yl)amino)-1,4-dioxo-4-phenylbut-2-en-2-yl)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (3a)

Yield 0.36 g (74%), orange crystals, mp 172–173 °C (isopropanol). IR spectrum, ν, cm⁻¹: 1671 (CONH, CONH₂), 1738, (COOEt), 3186, 3351 (NH). ¹H NMR spectrum (CDCl₃), δ, ppm: 1.31 t (3H, CH₃CH₂O, Jₘₙ = 7.2 Hz), 1.76 m (4H, 2CH₂), 2.40 s (3H, CH₃), 2.57 m (2H, CH₂), 2.80 m (2H, CH₄), 4.37 q (2H, CH₂CH₂O, Jₘₙ = 7.1 Hz), 6.19 s (1H, C=CH₂), 6.83 m (2H, Hₐ₉om), 7.22 m (3H, Hₐ₉om), 7.45 m (2H, Hₐ₉om), 8.43 s (1H, NH), 10.21 s (1H, NH). Found, %: C 63.60; H 5.37; N 11.43; S 6.52. Calcd.: C 63.66; H 5.34; N 11.42; S 6.54.

2.3. Ethyl 2-((1-(5-bromopyridin-2-yl)amino)-1,4-dioxo-4-phenylbut-2-en-2-yl)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (3b)

Yield 0.40 g (73%), orange crystals, mp 188–189 °C (isopropanol). IR spectrum, ν, cm⁻¹: 1667 (CONH, CONH₂), 1708, (COOEt), 3323, 3416 (NH). ¹H NMR spectrum (CDCl₃), δ, ppm: 1.40 t (3H, CH₃CH₂O, Jₘₙ = 6.9 Hz), 1.76 m (4H, 2CH₂), 2.58 m (2H, CH₂), 2.77 m (2H, CH₃), 4.36 q (2H, CH₂CH₂O, Jₘₙ = 6.9 Hz), 6.17 s (1H, C=CH₂), 6.71 s (1H, NH), 7.27 m (3H, Hₐ₉om), 7.38 m (2H, Hₐ₉om), 7.83 m (1H, Hₐ₉om), 8.13 m (1H, Hₐ₉om), 8.37 m (1H, Hₐ₉om), 10.49 s (1H, NH). Found, %: C 56.37; H 4.33; N 7.56; S 5.74. Calcd.: C 56.32; H 4.36; N 7.58; S 5.78.

2.4. Ethyl 2-((1,4-dioxo-4-phenyl-1-(thiazol-2-ylamino)-but-2-en-2-yl)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (3c)

Yield 0.41 g (85%), yellow crystals, mp 200–201 °C (isopropanol). IR spectrum, ν, cm⁻¹: 1663 (CONH, CONH₂), 1718, (COOEt), 3234, 3439 (NH). ¹H NMR spectrum (DMSO-d₆), δ, ppm: 1.38 t (3H, CH₃CH₂O, Jₘₙ = 7.2 Hz), 1.74 m (4H, 2CH₂), 2.63 m (2H, CH₂), 2.75 m (2H, CH₃), 4.35 q (2H, CH₂CH₂O, Jₘₙ = 7.2 Hz), 6.25 s (1H, C=CH₂), 7.38 m (5H, Hₐ₉om), 7.62 m (2H, Hₐ₉om), 8.12 s (1H, NH), 10.44 s (1H, NH). Found, %: C 59.83; H 4.85; N 8.72; S 13.36. Calcd.: C 59.86; H 4.81; N 8.73; S 13.31.

2.5. 2-((1-((4-Bromophenyl)amino)-4-(4-methoxyphenyl)-1,4-dioxobut-2-en-2-yl)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (3d)

Yield 0.47 g (84%), orange crystals, mp 193–195 °C (isopropanol). IR spectrum, ν, cm⁻¹: 1666, 1686 (CONH, CONH₂), 3294 (NH, NH₂). ¹H NMR spectrum (DMSO-d₆), δ, ppm: 1.69 m (4H, CH₂), 2.50 (2H, CH₂), 2.85 s (2H, CH₃), 3.84 s (3H, OCH₃), 6.37 s (1H, C=CH₂), 7.04 m (2H, Hₐ₉om), 7.47 m (2H, NH; 4H, Hₐ₉om), 8.02 m (2H, Hₐ₉om), 11.17 s (1H, NH), 12.67 s (1H, NH). Found, %: C 56.30, H 4.35, N 7.53. S 5.77. Calcd.: C 56.32, H 4.36, N 7.58, S 5.78.

2.6. 2-((4-(4-methoxyphenyl)-1-morpholino-1,4-dioxobut-2-en-2-yl)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (3e)

Yield 0.41 g (87%), yellow crystals, mp 146–148 °C (isopropanol). IR spectrum, ν, cm⁻¹: 1658 (CONH, CONH₂), 3169, 3344 (NH, NH₂). ¹H NMR spectrum (DMSO-d₆), δ, ppm: 1.73 m (4H, 2CH₂), 2.62 m (4H, 2CH₂), 3.48 m (8H, 4CH₂), 3.83 s (3H, OCH₃), 6.14 s (1H, C=CH₂), 7.00 m (2H, Hₐ₉om), 7.40 br s (1H, NH), 7.49 br s (1H, NH), 7.95 m (2H, Hₐ₉om), 12.94 s (1H, NH). Found, %: C 61.35, H 5.82, N 8.96, S 6.80. Calcd.: C 61.39, H 5.80, N 8.95, S 6.83.

Evaluation of analgesic activity was carried out in the Perm State National Research University, the Research Laboratory of Biologically Active Substances. Analgesic activity was determined on outbred white mice of both sexes weighing 18–22 g using the “hot plate” method [31]. The studied compounds were administered intraperitoneally in the form of a suspension in a 2% starch solution at a dose of 50 mg/kg 30 min before the animals were placed on a metal plate heated to 53.5 °C [32]. Studies were performed 30, 60, 90, 120 min after administration of the compound.

The indicator of the change in pain sensitivity was the length of time the animals stay on the hot plate until a defensive pain reflex occurs – licking the hind legs or trying to tear off all four paws from the surface of the plate. The time of onset of this reflex from the beginning of the placement of the animal on the plate was measured in seconds (latent period). The maximum duration of the latent period is the interval of 40 s. In the experiment we used animals with the initial time of the onset of the defensive reflex no more than 15 s. Each compound was tested on 6 animals. The results were evaluated by increasing the time of the onset of the defensive reflex compared with the initial data. The control group of animals was injected with 2% starch mucus. Metamizole sodium (Farmkhimkomplekt LLC) at a dose of 93 mg/kg (ED₅₀) was used as a comparison compound.

Statistical processing of experimental data was carried out using Student's confidence criteria. The effect was
considered significant at \( p<0.05 \) [33]. The studies were carried out in accordance with all applicable international, national and institutional guidelines for the care and use of animals.

3. Results and discussion

Starting furanones \( 2a,b \) were obtained by known literature method via intramolecular cyclization of 4-aryl-4-oxo-2-thienylaminobut-2-enolic acids \( 1a,b \) in acetic anhydride. The reaction of 3-thienyllimino-3H-furan-2-ones \( 2a,b \) with alkyl-, aryl-, hetarylamines in inert aprotic solvent proceeded with the formation of \( N \)-substituted amides of but-2-enic acids \( 3a-e \) (Scheme 1). As a result, it was found that the attack of the amino group was directed at the carbon atom of the lactone carbonyl moiety of compounds \( 2a,b \) and led to the products of the furanone cycle decyclization. The ester and amide groups under the conditions of the reaction did not participate in interaction with amines, which does not contradict the literature data.

The mechanism of the decyclization reaction of 5-aryl-2,3-dihydro-2,3-furandiones under the action of nucleophilic reagents was published based on the detailed large-scale study of kinetic data [34–36] as well as quantum chemical calculations [37]. Assuming the similarity of these structures with the iminofuranones discussed in the current paper, we can assume the validity of this mechanism for the transformations 3-thienyllimino-3H-furan-2-ones \( 2a,b \) under the action of amines as nucleophiles (NuH) described here (Scheme 2).

If the solvent cannot be a donor or acceptor of an electron pair (aprotic nonpolar or weakly polar solvents), a non-catalytic reaction occurs. The use of a nonpolar solvent contributes to the displacement of the equilibrium from the transition state \( TS1 \) to the intermediate \( I \) that leads to the limiting stage of the process with the formation of the transition state \( TS2 \).

Compounds \( 3a-e \) are crystalline substances of orange or yellow color, obtained with yields up to 87%.

We have studied the \(^1H\) NMR spectra of compounds \( 3a,b \) in DMSO-\( d_6 \) and \( 3c-e \) CDCl\(_3\). It was established that compounds \( 3a-e \) are characterized by a proton singlet of the NH group involved in a strong intramolecular hydrogen bond at 10.44–12.94 ppm, proton signals of the NHCO group at 6.71, 8.12–11.17 ppm and a proton singlet of the CH group at 6.14–6.37 ppm.

Some of the compounds obtained were examined for analgesic activity. It is shown in Table 1 that all the studied compounds have a pronounced analgesic effect, surpassing the effect of the comparison drug metamizole.

| Compound | Dosage, mg/kg | The latent period of the defensive reflex (120 min), s |
|----------|--------------|--------------------------------------------------|
| 3a       | 50           | 21.20±1.24                                      |
| 3b       | 50           | 22.40±1.83                                      |
| 3c       | 50           | 21.00±1.46                                      |
| Metamizole | 93 (ED\(_{50}\)) | 16.60±1.00                                      |
| Control  | -            | 10.30±0.60                                      |

4. Conclusions

New derivatives of 2-[(1,4-dioxo-1-amino-4-aryl)butyl-2-en-2-yl]amino]-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic acid were obtained with 73–87% yields by the decyclization reaction of 2-[(5-aryl-2-oxofuran-3(2H)-yldene)amino]thiophene-3-carboxylic acids under the action of aliphatic, aromatic and heterocyclic amines. It was found that the obtained compounds exhibited significant analgesic activity, reliably exceeding the effect of a referral drug.
**Scheme 2** The transformations 3-thienylimino-3H-furan-2-ones 2a,b under the action of amines as nucleophiles (NuH)

**Acknowledgements**

The research was supported by the Perm Research and Education Centre for Rational Use of Subsoil, 2021.

**References**

1. Thomas J, Jecic A, Vanstreels E, van Berckelaer L, Romagnoli R, Dehaen W, Liekens S, Balzarini J. Pronounced anti-proliferative activity and tumor cell selectivity of 5-alkyl-2-amino-3-methylcarboxylate thiophenes. Eur J Med Chem. 2017;132:219–235. doi: 10.1016/j.ejmech.2017.03.044

2. Regal MKA, Shaban SS, El-Metwally SA. Facile Synthesis and Antimicrobial Activity of 5-Amino-3-methyl-1-phenyl-1H-thieno[3,2-c]pyrazole-6-carbonitrile and Their Derivatives. J Heterocyclic Chem. 2018;56(1):226–233. doi: 10.1002/jhet.3399

3. Vasileva AYu, Vaganov VYu, Shiplovskikh SA, Rubtsov AE. Chemistry of Iminofurans: XV. Decyclization of Ethyl 2-[5-Aryl-2-oxofuran-3(2H)-ylidenemino]-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylates by the Action of Secondary Amines. Russ J Org Chem. 2018;54(4):582–587. doi: 10.1134/s1070428018040115

4. Barakvar SB, Sachin D, Wagh MA, Nawale LU, Choudhari AS, Bhansali S, Sarkar D, Sanjayan GJ. Design and Synthesis of 2-Amino-thiophene-proline-conjugates and Their Anti-tubercular Activity against Mycobacterium Tuberculosis H37Ra. Chem Select. 2019;4(9):2851–2857. doi: 10.1002/slct.201803370

5. Putrnan D, Poojary B, Purushotham N, Harikrishna N, Nayak SG, Kamat V. Synthesis of novel Schiff bases using 2-Amino-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile and 1,3-Disubstituted pyrazole-4-carboxaldehydes derivatives and their antimicrobial activity. Heliyon. 2019;5(8):e02233. doi: 10.1016/j.heliyon.2019.e02233

6. Rossetti A, Bono N, Candidi G, Meneghetti F, Roda G, Sacchetti A. Synthesis and Antimicrobial Evaluation of Novel Chiral 2-Amino-4,5,6,7-tetrahydrothieno[2,3-c]pyridine Derivatives. Chem Biodivers. 2019;16(6):e1900097. doi: 10.1002/cbdv.201900097

7. Zhdankin VV, Puterova Z, Krutošíková A, Végh D. Gewald reaction: synthesis, properties and applications of substituted 2-aminothiophenes. Arkov. 2010;2010(1):209–246. doi: 10.3908/ark.5550190.0011.105

8. Javadi F, Tayeeb R. Preparation and characterization of ZnO/nanoclinoptilolite as a new nanocomposite and studying its catalytic performance in the synthesis of 2-aminothiophenes via Gewald reaction. Microporous Mesoporous Mats. 2016;231:100–109. doi: 10.1016/j.micromeso.2016.05.025

9. Akbarzadeh A, Dekamin MG. A facile and environmentally benign polyethylene glycol 600-mediated method for the synthesis of densely functionalized 2-aminothiophene derivatives under ultrasound. Green Chem Lett Rev. 2017;10(4):315–323. doi: 10.1080/17518253.2017.1380234

10. Kizimova IA, Igidov NM, Kiselev MA, Dmitriev MV, Chashchina SV, Siutkina AI. Synthesis of new 2-aminopyrrole de-
11. Kizimova IA, Igidov NM, Kiselev MA, Syutkina AI, Ivanov DV. Reactions of N–[2-Oxo-5-8-furan–3(2H)–ylidene] with Primary and Secondary Alcohols. Russ J Gen Chem. 2020;90(5):815–821. doi:10.1134/S1070363220050096

12. Siutkina AI, Igidov NM, Kizimova IA. Synthesis and Properties of Alkyl 2–[2-(Diarylmethylene)hydrazinyl]–5,5-dimethyl-4-oxo–2-enoates. Russ J Org Chem. 2020;56(4):649–653. doi:10.1134/S1070428020040132

13. Zykova SS, Kizimova IA, Syutkina AI, Toksaraev Yu, Igidov NM, Ibisov DF, Boichuk SV, Dunaev PD, Galemibkova AR. Synthesis and Cytostatic Activity of (E)-Ethyl-2-Amino-5-(3,3-Dimethyl-4-Oxobutyliden)-4-Oxo-1-(2-Phenylaminobenzamido)-4,5-Dihydro-1-H-pyrrol-3-Carboxylate. Pharm Chem J. 2020;53(10):895–898. doi:10.1007/s11894-020-02086-4

14. Shipilovskikh SA, Rubtsov AE. Recyclization of 3-(Thiophen-2-ylamino)-3H-furan-2-ones on the Action of Cyanocarboxyl Derivatives. Russ J Gen Chem. 2020;5:809–814. doi:10.1134/S1070363220020084

15. Shipilovskikh SA, Rubtsov AE. One-Pot Synthesis of Thieno[3,2-e]pyrrolo[1,2-ae]pyrimidine Derivative Scaffold: A Valuable Source of PARP-1 Inhibitors. J Org Chem. 2019;84(24):15788–15796. doi:10.1021/acs.joc.9b00711

16. Shipilovskikh SA, Rubtsov AE. Decyclization of 2-[5-(4-chlorophenyl)-2-oxoxuran-3(2H)-ylideneamino]-4,5,6,7-tetrahydrobenzo[1]thiophene-3-carboxamide upon treatment with aliphatic alcohols. Russ Chem Bull. 2014;63(9):2205–2207. doi:10.1007/s11172-014-0722-4

17. Shipilovskikh SA, Rubtsov AE. Aminofuran chemistry. Decyclization of ethyl 2-[2-oxo-5-phenylfuran-3(2H)-ylideneamino]-4,5,6,7-tetrahydrobenzo[1]thiophene-3-carboxylate under the action of aliphatic amines. Russ J Org Chem. 2014;50(2):298–300. doi:10.1134/S1070363214020286

18. Baughmann BM, Jake SP, DuBois RM, Boyd VA, White SW, Webb TR. Identification of influenza endonuclease inhibitors using a novel fluorescence polarization assay. ACS Chem Biol. 2012;7(3):526–534. doi:10.1021/cb2004307

19. de Melo EB, Ferreira MM. Four-dimensional structure-activity relationship model to predict HIV-1 Integrase strand transfer inhibition using LIGTA-QSAR methodology. J Chem Inf Model. 2012;52(7):1722–1732. doi:10.1021/ci200708a

20. Deore RR, Chen GS, Chen CS, Chang PT, Chuang MH, Chien TR, Wang HC, Chen JW. 2-Hydroxy-1-oxo-1,2-dihydrosoquinoline-3-carboxylic acid with inbuilt beta-N-hydroxy-gamma-keto-acid pharmacophore as HCV NS5B Polymerase inhibitors. Curr Med Chem. 2012;19(4):613–624. doi:10.2174/09298671279881813

21. Kowalinski E, Zubieta C, Wolkerstorfer A, Zubieta C, Wolkerstorfer A, Szolar OH, Ruigrok A. Synthesis of phenanthrene beta-ymerase inhibitors. Curr Med Chem. 2012;19(4):613–626. doi:10.1016/j.bmc.2013.09.009

22. Denisova EI, Shipilovskikh SA, Makhmudov RR, Rubtsov AE. Search of analgesic activity of N-substituted 2–[(3-R-4,5,6,7-tetrahydrobenzo[1]thiophen-2-yl)-amino]-4-oxo-4-phenylbut-2-enamides. AIP Conf Proc. 2020;2280(1):040013. doi:10.1063/5.0018815